Understanding respiratory illness in an HIV positive population with a high uptake of antiretroviral therapy

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Declaration

I, James Brown, declare that the work presented in this thesis is my own.

Where information has been derived from other sources, I confirm that this has been indicated in the thesis.

Abstract

The availability of effective antiretroviral therapy (ART) means that HIV infection is now a manageable chronic condition. However, although AIDS-related conditions are now rare in people living with HIV (PLWH) using ART, this population may be at greater risk of some non-AIDS conditions (such as cardiovascular disease) than the general population. The *respiratory* health of PLWH using ART has been less well explored. This thesis provides novel insights into respiratory illness among PLWH in a population with a high uptake of antiretroviral therapy.

I have summarised existing evidence in a narrative literature review of respiratory illness in PLWH and systematic review of studies comparing respiratory symptoms in people with and without HIV. I have evaluated the prevalence of respiratory illness and carriage of respiratory bacterial and viral pathogens in cross-sectional data. I used molecular microbiology techniques to explore the carriage of pathogenic respiratory bacteria in PLWH. I have completed a 12-month prospective study of a cohort of HIV positive and negative participants to determine the frequency of acute respiratory illness and factors associated with illness incidence and severity in this population.

I found that respiratory symptoms are more common among HIV positive than negative people despite ART and that this difference is only partly explained by established risk factors such as tobacco smoking. In the population studied, the frequency of objective respiratory impairment as measured by spirometry was lower than that reported in many other HIV positive populations. I found no difference in the frequency of acute respiratory illnesses between HIV positive and negative individuals, however PLWH reported more severe symptoms and were more likely to seek healthcare when these illnesses occurred. In collaboration with colleagues, I have assessed interventions to improve or maintain respiratory health among PLWH. We evaluated the uptake of influenza immunisation and referral to smoking cessation services and identified barriers to these cost-effective interventions.

In summary, PLWH remain at greater risk of respiratory illness than the general population despite ART. In part this is due to greater exposure to known risk factors such as tobacco smoking, but even after adjustment for these, an independent effect of HIV status remains. We need a better understanding of the causes of this and interventions to improve the respiratory health of this population.

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List of abbreviations used in this thesis

AIDS	Acquired Immunodeficiency Syndrome
ALIVE	AIDS Linked to Intravenous Experience (study)
ART	Antiretroviral Therapy
BLAST	Basic Local Alignment Search Tool
BMI	Body Mass Index
CD4	Cluster of Differentiation 4-bearing T lymphocyte
CMV	Cytomegalovirus
CCR5	C-C chemokine receptor type 5
CXCR4	C-X-C chemokine receptor type 4
COPD	Chronic Obstructive Pulmonary Disease
CGPS	Copenhagen General Population Study
DLCO	Diffusing Capacity for Carbon Monoxide
DNA	Deoxyribonucleic acid
EQ5D	Euro-Qol 5 Dimension (score)
EXHALE	Examinations of HIV Associated Lung Emphysema (study)
FEV1	Forced Expiratory Volume in one second
FVC	Forced Vital Capacity
GP	General Practitioner
HIV	Human Immunodeficiency Virus
IAC	Internal Amplification Control
ITU	Intensive Therapy Unit
LMIC	Low and Middle-Income Countries
MACS	Multicentre AIDS Cohort Study
MRC	Medical Research Council
MSM	Men who have sex with men
OR	Odds Ratio
PCP	*Pneumocystis jirovicii* pneumonia
PCR	Polymerase Chain Reaction
PHQ-9	Patient Health Questionnaire 9
PLWH	People Living with HIV
RNA	Ribonucleic acid
SGRQ	St George's Respiratory Questionnaire
START	Strategic Timing of Antiretroviral Therapy (study)
TB	Tuberculosis

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Impact statement

The work presented in this thesis aims to benefit people living with HIV, their care providers, policy makers and researchers working in this field. It contributes to the growing evidence regarding the respiratory health of people living with HIV and how this has changed with the advent of antiretroviral therapy. As people living with HIV age, the comorbidities associated with ageing will become more prevalent. Evidence presented here can be used to guide the provision of care in the future, given that HIV is increasingly managed as a chronic manageable condition.

People living with HIV will be a key audience for this research – there is a large and often well-informed audience of HIV positive people who wish to know about novel work into HIV and associated co-morbidities. The dissemination of such findings is often mediated through community information and advocacy groups such as HIV i-base and NAM. These have already been involved in the information exchange of outputs of this research and we will continue to work with them.

The data presented here contributes to work provided by other researchers in this field, my novel findings should stimulate new avenues for productive further investigation. A key example of this is the consistent demonstration of greater respiratory symptoms among people living with HIV, which appears to persist (albeit at a reduced level) in those using antiretroviral therapy. Understanding why this is the case, and how this can be mitigated, offers the potential to improve the quality of life of this population in the long term.

Although the primary data presented in this thesis derives from people living with HIV in the UK, findings from this work will be of relevance to populations elsewhere. An encouraging aspect of HIV care over recent years has been the growing proportion HIV positive people globally who are able to access antiretroviral therapy. Although significant challenges remain in sustaining this and ensuring that populations achieve consistent access and levels of response similar to those found in resource rich settings, there is likely to be growing numbers of people living with HIV as a stable chronic condition in many settings including low and middle-income countries. Research such as that presented in this thesis, which aims to understand how other comorbid conditions might differ in people with HIV compared to those without, will be of relevance to populations in these settings.

Introduction and overview of thesis

Overview of thesis and statement of hypothesis

Respiratory illness has been a key manifestation of HIV infection since the Acquired Immunodeficiency Syndrome (AIDS) was first recognised (1). However, the successful provision of antiretroviral therapy (ART) has transformed the epidemiology of respiratory illness among people living with HIV: In the UK, 90% of HIV positive individuals who attend HIV care are now using ART and 92% have a CD4 count above 350 cells/µL(2). This means that for most individuals accessing HIV care, opportunistic respiratory infections such as *Pneumocystis jirovecii* pneumonia (PCP) are now rare (3).

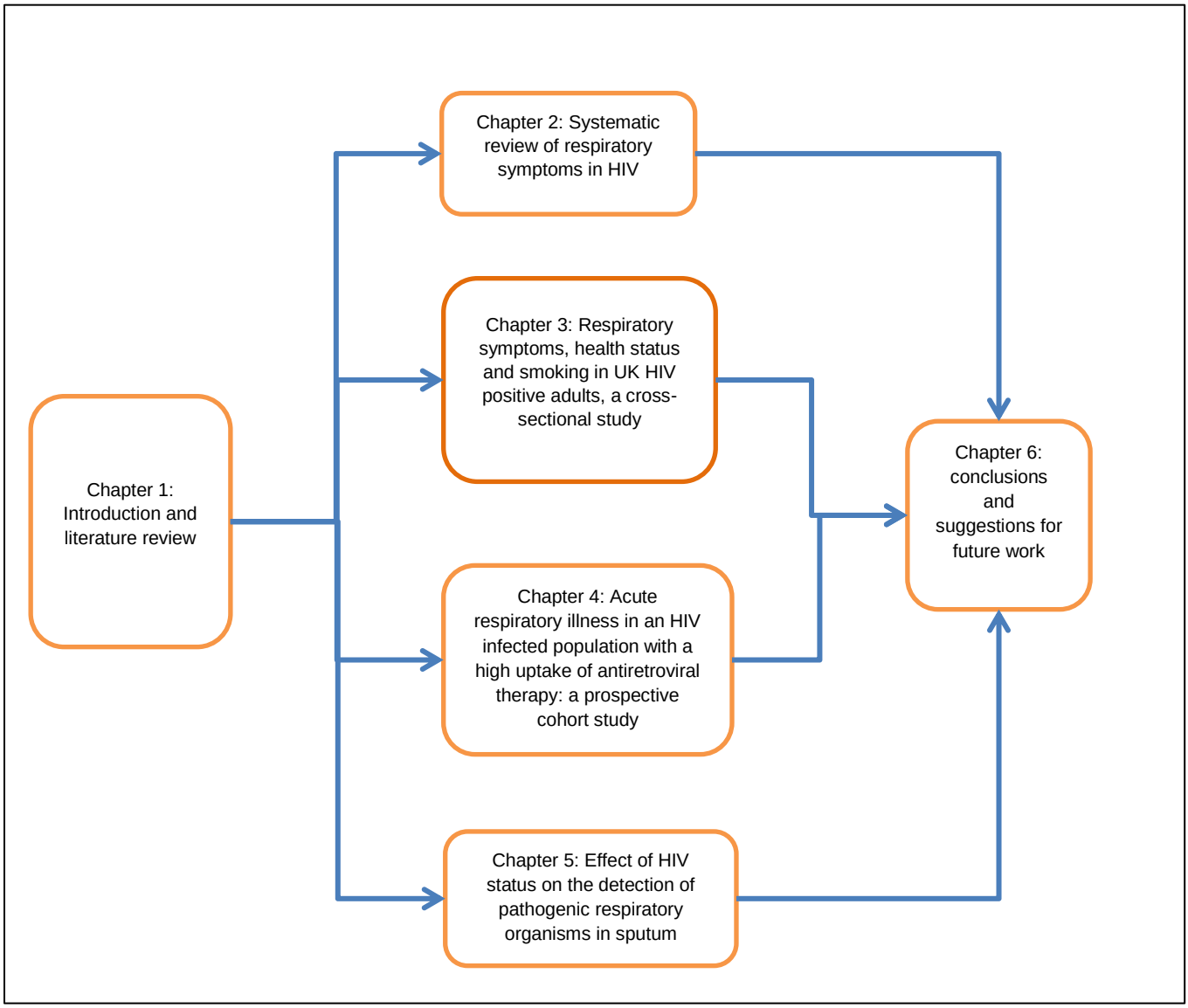
Successful immune reconstitution with antiretroviral therapy may not, however, mean that the risk of respiratory illness is the same as the HIV negative population (4). Many studies have suggested that HIV positive populations with access to ART may have higher rates of chronic non-AIDS conditions such as cardiovascular disease (5), osteoporosis (6), some types of cancer and neurological impairment (7, 8). The same may be true of chronic respiratory disease, with some authorities suggesting that conditions such as Chronic Obstructive Pulmonary Disease (COPD) occur more commonly in PLWH and may take an accelerated clinical course (9). Although ART reduces the incidence of respiratory infection, people living with HIV (PLWH) appear to remain at higher risk of infections such as bacterial pneumonia and tuberculosis than the general population (10, 11).

Significant uncertainties remain, however, regarding the relationship between HIV status and respiratory health in populations with good access to antiretroviral therapy, and there is therefore a need for research to guide patient care and public health policy.

This thesis explores these questions in the context of the UK HIV positive adult population, which has a high uptake of ART and good retention in care. Published evidence regarding the prevalence of respiratory symptoms in PLWH and how this has changed with the provision of ART are summarised in a systematic review. Results from a study of respiratory health status in the current UK HIV positive population are presented and the core of this doctoral thesis includes a study evaluating the frequency of acute respiratory illness in a contemporary HIV positive population, compared to HIV negative individuals with similar risk factors for respiratory disease. This included detailed molecular identification of bacterial airway pathogens at baseline. Finally, mechanisms to improve the respiratory health of PLWH will be evaluated and suggestions made for the future care of this population.

The hypothesis evaluated here is that despite the use of antiretroviral therapy, people living with HIV continue to experience a greater burden of respiratory illness than the general population. This results both from HIV-related effects and higher exposure to risk factors such as tobacco smoking. Identification of specific risk-factors for respiratory illness offers the opportunity to design interventions to improve the health-related quality of life of this population.

FIGURE 1 OVERVIEW OF THESIS



Chapter 1: Background and literature review

1.1 Respiratory illness in People Living with HIV prior to the development of antiretroviral therapy

Respiratory illness is a key manifestation of HIV infection; indeed the Acquired Immunodeficiency Syndrome (AIDS) was first recognised among individuals presenting with *Pneumocystis jirovecii* pneumonia (PCP) in the 1980s (12). Before effective antiretroviral therapy (ART) became available, respiratory infection and some forms of non-communicable respiratory disease were among the most important complications of HIV infection (3). This remains true today for individuals and populations unable to access ART. Although this thesis primarily concerns respiratory health of individuals using ART, a brief summary of respiratory illness in populations prior to the development of these treatments is provided here as this provides important context for later changes.

1.2 Respiratory infection in HIV positive populations without antiretroviral therapy

Infections are the predominant respiratory complication of HIV infection without ART. Most typically, as HIV infection progresses, CD4 T-cell numbers fall, significant immunodeficiency develops, and individuals become susceptible to a range of opportunistic respiratory infections. In the lung these include pneumonia caused by the yeast-like organism *Pneumocystis jirovecii* and (less commonly) other fungal pathogens such as *Cryptococcus neoformans* and (in endemic areas) *Histoplasma capsulatum* and *Penicillium marneffei* and viral pathogens such as Cytomegalovirus (CMV). As well as these opportunistic pathogens (which rarely cause infection in those with an intact immune system), organisms which can result in infections in immunocompetent individuals do so at a much higher frequency in those with HIV. Bacterial pneumonia, in particular caused by *Streptococcus pneumoniae*, occurs much more commonly, with invasive pneumococcal disease being up 20-100 times more common in PLWH (13). Tuberculosis also occurs at a far greater frequency and represents the most common cause of mortality among PLWH in many high-TB incidence settings such as Sub-Saharan Africa (14). Although opportunistic infections such as PCP are largely restricted to those with CD4 counts under 200 cells/μL, the incidence of bacterial pneumonia and TB increase soon after HIV seroconversion, indicating that significant immunological changes occur early in HIV infection (15).

These respiratory infectious consequences of HIV were documented and explored in numerous case reports, series and reviews (16-18). More systematic studies were made of prospectively followed cohorts including the Pulmonary Complications of HIV infection study (PACS) (19), Multicentre AIDS cohort study (MACS) and Women's Interagency Cohort study (WIHS), (20) (21) all of which studied HIV positive populations in the US, and included HIV negative comparator participants.

The Pulmonary Complications of HIV Infection study, recruited 1281 HIV positive and 165 HIV negative participants between 1988 and 1990 in 6 US cities (San Francisco, Los Angeles, Detroit, Chicago, Newark and New York) and followed this group prospectively to detail the incidence of respiratory illness (19). At recruitment, 74% of participants had CD4 counts above 250 cells/μL. Only limited antiretroviral therapies (the nucleoside analogues zidovudine (AZT), didanosine (DDI) or dideoxycytidine (DDc)) were available at this time. During the 18 months of prospective follow-up described by Wallace *et al* in 1997, bacterial pneumonia occurred in 4.8% of HIV positive participants (compared to 0.6% of those without HIV infection) and PCP pneumonia occurred in 3.9% (22, 23). Less severe (but more common) acute respiratory illnesses such as bronchitis, sinusitis and upper respiratory tract infections also occurred more frequently in those with HIV, with upper respiratory tract infections documented in 33% of HIV positive and 25% of HIV negative individuals, and bronchitis in 16% and 9% respectively during the 18 months of follow-up. Influenza like illness (defined as "acute self-limited febrile illness with myalgias and in some cases sore throat, coryza or cough, without radiographic evidence of pneumonia") was not more frequent among PLWH, and the evidence regarding susceptibility to influenza in HIV will be discussed in more depth in a later section.

In data specifically evaluating bacterial pneumonia in the Pulmonary Complications of HIV Infection study, Hirschtick *et al* demonstrated a much higher incidence of bacterial pneumonia (5.5 per 100 person years) in HIV positive participants, compared to 0.9 per 100 person-years in HIV negative participants(24). This incidence was higher at lower CD4 counts. Injecting drug users had a greater incidence of pneumonia, as did tobacco smokers in the lowest CD4 stratum.

The cohort recruited for the Pulmonary Complications of HIV Infection study was prospectively followed until May 1997. An analysis of the first 5 years of follow-up between 1988 and 1994 was published by Wallace *et al*: during this time, the level of immunocompromise in the cohort worsened as HIV infection progressed, with 19% having a CD4 count < 200 cells/μL at recruitment and 34% by year 5; by 5 years of follow-up, only 721 of the original 1143 participants were still surviving and being followed up. This study documented the progressive increase in respiratory infections in this cohort as HIV infection progressed, with bacterial pneumonia occurring at a frequency of 3.9 per 100 person-years in year 1 and 7.3 per 100 person-years in year 5, and PCP increasing from 2.8 to 9.6 per 100 person-years. Upper respiratory tract infection did not show a similar change over time, remaining at a frequency of between 35 and 52 per 100 person-years during follow-up. Overall, the occurrence of these upper respiratory tract infections was higher in those with HIV, occurring at a frequency of 42 per 100 person-years in the HIV positive and 29 per 100 person-years in the HIV negative participants.

Similar findings were reported by the Multicentre AIDS Cohort study, a cohort followed at six sites in the US, originally recruited in 1984 and comprising 6972 men who have sex with men either with, or at risk of, HIV infection(20). Recruitment occurred in three time periods: 1984; between 1987 and 1991 and 2001

to 2003. As with the Pulmonary Complications of HIV infection study, prior to the availability of ART, respiratory infection was much more common among PLWH, with the incidence of bacterial pneumonia recorded as 8.28 per 1000 person years among HIV positive and 0.41 per 1000 person years among HIV negative participants (OR 21.9, p <0.001); acute sinusitis or bronchitis were also more common among PLWH, albeit with a smaller relative difference – the absolute incidence rate of bronchitis of 35.89 per 1000 person years compared to 25.02 in HIV positive and negative participants respectively (OR (95%CI) 1.70 (1.46-1.97) p< 0.001), and of sinusitis of 75.05 and 65.04 per 1000 person years (OR 1.39 (95% CI 1.24-1.55) p <0.001) (25).

1.3 Non-communicable respiratory disease in HIV before the advent of ART

Although respiratory infection dominated the respiratory complications of HIV prior to the availability of antiretroviral therapy, there is also evidence from the pre-ART era of increased non-infectious pathologies. Most clearly related to HIV is the development of pulmonary Kaposi's sarcoma, although interstitial lung diseases were also associated with HIV infection including Non-Specific Interstitial Pneumonitis and Lymphocytic Interstitial Pneumonitis, however, these were relatively rare (occurring for instance in only 21 participants in the Pulmonary Complications of HIV study during 5 years of follow-up of 1143 participants) (22).

By the late 1980s and early 1990s investigators were suggesting that Chronic Obstructive Pulmonary Disease (COPD) might follow an accelerated course in some HIV positive individuals. This observation was first reported in the literature in 1989 by Kuhlman *et al* in a review of radiological evidence for emphysema in PLWH (26). This was followed by a report by Diaz *et al* of 4 cases of HIV positive non-smokers who had developed emphysema (27). A more systematic assessment of a cohort of HIV positive and negative individuals was then undertaken by these investigators, who compared 114 HIV positive and 44 HIV negative participants, with median ages of 34 and 33 years respectively, who underwent CT scans (28). These demonstrated that 17 of the HIV positive compared to only one of the HIV negative participants had radiological evidence of emphysema.

It should be noted, however, that these subjects studied by Diaz *et al* had a very high proportion of current smokers (60% and 56% respectively) and the HIV positive group had a greater pack-year exposure (7 vs 5 years) and 14 of the 17 PLWH with emphysema had over 12 pack-year exposure. No information on smoking of recreational drugs such as cannabis was provided in this report; it is possible therefore that this apparent effect of HIV is related to confounding by these risk factors.

The Pulmonary Complications of HIV infection study also evaluated lung function in PLWH and found a higher proportion with lung function impairment as assessed by DLCO (29). In this study, individuals with a CD4 count below 200/mm3 had a lower DLCO, as did subjects with a history of HIV-associated symptoms such as candidiasis, weight loss or herpes zoster.

Longitudinal follow-up of 474 HIV positive individuals documented that lung function worsened in those who developed pulmonary Kaposi's Sarcoma or PCP, and that recovery of lung function following an episode of PCP was impaired in smokers.

Data from the Multi-centre AIDS cohort study (MACS) prior to the availability of ART also suggested that chronic non-communicable respiratory disease, and specifically COPD, was more common among

PLWH, as the incidence of (a new diagnosis of) COPD was 0.61 vs 0.28 cases per 1000 person years (OR 2.84 (1.06-7.59) p=0.04)(30).

1.4 Changing epidemiology with the advent of antiretroviral therapy and implications for respiratory health

The first antiretroviral medication, zidovudine (AZT), was introduced in 1987 and by the mid-1990s effective combinations of antiretroviral medications were available which were able to control HIV replication and allow the immune system to recover. (31) Since then, combinations of antiretroviral medications that are capable of indefinitely suppressing viral replication if taken consistently have been available, transforming the treatment of PLWH.(32)

Over 40 different antiretroviral drugs and combination pills, utilising 5 different mechanisms of action are currently available in the UK (33). Current antiretroviral therapy (ART) typically involves the combination of three of these drugs, acting via different mechanisms (most commonly two nucleoside reverse transcriptase inhibitors plus a third agent, either a non-nucleoside reverse transcriptase inhibitor, protease inhibitor or integrase inhibitor) and these combinations of anti-HIV drugs will be referred to as “ART” in this thesis. In most individuals, these drugs can suppress HIV viral replication to the extent that HIV viral RNA is no longer detectable in peripheral blood (the current threshold for detection being 40 copies/ml, below this being defined as “undetectable”). As medications have become more tolerable and the benefits of using antiretroviral therapy before severe immunodeficiency has developed have become clearer,(34) clinical guidelines have moved away from recommending treatment at a particular threshold of CD4 count and towards recommending treatment for all HIV positive individuals.(35) Cohorts of PLWH diagnosed today are therefore potentially very different from those diagnosed 20 or even 5 years ago, having less time with uncontrolled HIV and higher nadir CD4 counts. This may have important implications for their future health In addition to the benefits to individuals of treatment, wider use of ART may have the effect of reducing onward transmission and thus the number of new infections (36).

The effect of widespread and effective antiretroviral therapy has been that the life expectancy of people living with HIV has greatly improved. Indeed, for people maintained in care and adherent to ART, it may not differ from that of the general population (37, 38). Despite this, HIV and AIDS still cause significant numbers of early deaths, including in the UK, particularly in those who don’t know their HIV status, present late to care, are within the first year after HIV diagnosis, or are not adherent to antiretroviral medication(39).

As the number of new HIV infections in the UK has been roughly stable in recent years, the greater life expectancy of PLWH means that the UK (like other similar settings) has a growing HIV positive population: from 55,923 in 2007 to 91,987 in 2017 (2). Furthermore, they are aging: the median age of people accessing care in the UK has increased over the past decade, from 39 in 2006 to 45 in 2015, and 38% are over the age of 50, (40) this is a trend that will continue and have a major effect on HIV care (41). This demographic transition inevitably means that the medical conditions affecting this population are changing (regardless of any specific risks associated with HIV). Management of age-related

comorbidities will therefore become an increasingly important part of HIV care. For instance, Smit *et al* estimated that by 2030 84% of HIV positive people in the Netherlands will have at least one non-communicable disease (compared to 29% in 2010) and 28% will have three or more comorbidities (41). These projections are likely to be similar in other populations such as the UK with similar levels of antiretroviral use.

Although aging alone will inevitably mean that non-AIDS co-morbidities become more frequent among PLWH, other factors result in some conditions occurring more often than in the general population. Some of these effects are not directly related to HIV – for instance PLWH are more likely to smoke than the general population and therefore have a higher incidence of smoking related comorbidities. Many PLWH come from migrant communities with different frequencies of some medical conditions, for instance the incidence of tuberculosis among PLWH in the UK who have migrated from high TB incidence countries remains greater than that of the UK born population despite the reduction in TB incidence cause by ART (42).

Further changes in the incidence of non-AIDS comorbidities may occur because of antiretroviral therapy. Examples of this include the dyslipidaemia caused by some protease medications (43), increased frequency of myocardial ischaemia associated with use of the nucleoside reverse transcriptase (NRTI) medication abacavir (44) and reductions in bone density and renal function associated with the NRTI tenofovir disoproxil fumarate (45, 46). To date, however, no such associations have been found between respiratory illness and the use of antiretroviral medications.

In addition to the higher frequency of non-AIDS non-communicable comorbidities related to demographic differences between HIV positive and negative populations, differences in exposure to important risk factors and the well described (although normally small) adverse effects of ART, there have been suggestions that direct effects of the HIV virus, or immunological sequelae of infection, may promote the development of some comorbidities. Ischaemic heart disease has been extensively studied and suggestions made that, for instance, the heightened state of “immune activation” associated with HIV infection causes changes in monocytes and macrophage phenotype which result in increased atherosclerosis (47). Whether such effects are apparent in respiratory disease will be discussed in a later section.

A note of caution should be raised when considering data regarding any increased risk of conditions associated with HIV. Observational studies can only describe associations and a causative relationship is not necessarily present. Although attempts can be made to adjust for the confounding effects of (for instance) tobacco smoking, this can only be successfully achieved when these confounding effects are known and can be accurately measured. This is often not the case. Residual confounding due to known and unknown factors is always therefore a possible explanation for the associations reported between HIV and non-AIDS comorbidities. The effect of such confounding factors is illustrated by a study from Denmark where the frequency of myocardial infarction in the (HIV negative) mothers of PLWH was

compared to population controls and found to be higher in this group (IRR 1.31 95% CI 1.08-1.80), therefore indicating that this increased risk of myocardial infarction is likely to be due to shared family lifestyle risks, rather than direct effects of HIV infection (48).

1.5 Respiratory infection and HIV in the antiretroviral era – is there still a problem?

Effective antiretroviral therapy has transformed the epidemiology of respiratory infection among PLWH. Continuous follow-up of the Multicentre AIDS cohort since 1984 allows this to be directly observed: here the incidence of PCP among PLWH fell from 30.59/1000 person-years to 2.51/1000 person-years between these two time-periods. Whilst a reduction in the frequency of bacterial pneumonia was also observed, this was much less pronounced, falling from 8.28 per 1000 person years in the period 1984-1994 to 6.29 in 1996-2008 (30). The incidence remained significantly higher than that among HIV negative participants (which was 2.31/1000 person-years in the same period). Similarly, in the Veterans Aging Cohort Study, bacterial pneumonia remained more common among PLWH, occurring at an incidence of 28 per 1000 person-years among PLWH (95% CI 27.2-28.8) compared to 5.8 (5.6-6) among HIV negative comparators. However, this included data until 2007 and the median CD4 count of these subjects was relatively low at 264 cells/µL (95% CI 108-451 cells/µL) with 65% of individuals using ART, and only 14% having an HIV viral load below 400 copies/ml.

The frequency of respiratory infection among PLWH is likely to have improved further, given that treatment guidelines now recommend treatment before more individuals before immunocompromise develops. However, despite ART, PLWH appear to remain at greater risk of respiratory infection than the general population. In a population-based cohort study in Denmark using data between 1995 and 2007, HIV positive individuals were significantly more likely to be hospitalised for pneumonia than the general population, including a subgroup with CD4 counts above 500 cells/µL in 2005-7, who were still around six times more likely to be admitted with pneumonia than the general population (IRR 5.9, 95% CI 4.2-7.6) (11). It should be noted, however, that a significant proportion of the HIV positive population documented in this study were not using antiretroviral therapy and median CD4 count at study entry was 313 cells/µL, so these findings may not be fully applicable to current populations where there is a greater use of ART.

Recently published data from the UK also suggest an ongoing higher frequency of acute infections among PLWH: a study evaluating causes of death in PLWH conducted by Croxford *et al* using data from Public Health England and death registrations, found that mortality rates have remained significantly higher among PLWH compared to the general population (76). Importantly, this study included all individuals within in a national database of HIV infections (which was then linked to national death registration data) and so avoided the biases associated with data arising from cohorts attending for HIV treatment, as including individuals who were not linked to care, or presented late, has the effect of significantly increasing the mortality rate associated with HIV. Although the risk of death was particularly high during the first year after HIV diagnosis (where the Standardised Mortality Rate (SMR) was 24.3 times that of the general population), a higher mortality rate persisted after 1 year of diagnosis (SMR for all-cause mortality 2.8 (95% CI 2.7-2.9)). Of note, non-AIDS infections continued to stand out as a cause of death, with an SMR for non-AIDS infections of 6.8 (5.9-7.8) times that of the general population

(representing an absolute number of 169 excess deaths above expected among PLWH in England and Wales each year). Bacterial pneumonia was the single largest cause of these deaths from non-AIDS infections (122 of 358 deaths, S. Croxford personal communication). As the source for this analysis included data between 1997 and 2012, it will be important to assess whether this excess mortality has been improved by greater use of antiretroviral therapy since this date: following this time period clinical guidelines have been revised to recommend antiretroviral therapy for all HIV positive individuals (rather than using a threshold for CD4 count below which ART was recommended, previously 350-500 cells/µL). As the Strategic Timing of Anti-Retroviral Treatment (START) study suggested that ART leads to a reduction in non-AIDS illness even in those with CD4 counts above 500 cells/µL, (77) further improvements in mortality might be hoped for with greater use of ART.

Despite antiretroviral therapy, there is still a need therefore to better understand and control the risk of serious infections among PLWH.

1.6 HIV and susceptibility to Influenza

Whether PLWH have a greater incidence or become more unwell when they acquire influenza infection is an important question. Influenza is a common infection, associated with significant morbidity in the general population and is vaccine-preventable and treatable with anti-viral medications (49). Clinical guidelines support the use of influenza immunisation for PLWH (50). Coverage in the US is significantly below target levels at 30-50% (51), although may be better in the UK (52). Unfortunately, there are relatively few, and sometimes conflicting studies regarding the effect of HIV on influenza incidence and severity on which to base clinical management.

Prior to the availability of antiretroviral therapy data on influenza in PLWH are scarce. As discussed already, no difference in the frequency of influenza-like illness was observed between HIV positive and negative participants in the Pulmonary Complications of HIV infection study between 1988 and 1997 (22). In a population with access to antiretroviral therapy in Quebec, Canada, Klein *et al* identified influenza virus in 20 of 50 PLWH presenting with acute respiratory illness, and suggested that influenza was an important cause of acute respiratory illness among PLWH (53). This group also noted that some of those presenting with illness had received the influenza immunisation. An evaluation of the duration of influenza illness and viral shedding among PLWH was reported by Patel *et al* (54), assessing a population in 2010-11 using antiretroviral treatment. These investigators concluded that viral shedding was similar to that reported in HIV negative people (10 days when assessed by PCR), although this conclusion was limited by the absence of direct comparison with an HIV negative group and small sample size of only 20 individuals with influenza.

A systematic review of studies assessing risk factors for serious outcomes from influenza conducted by Coleman *et al* in 2016 identified differences in outcomes in high compared to low and middle income countries (55). In high income settings the pooled relative risk for hospitalisation with influenza did not differ by HIV status (pRR 0.94 (95% CI 0.37-2.10)), however the authors identified only three studies assessing this outcome, and these yielded highly heterogeneous results (I^2 statistic 87%). In an analysis of the risk of death or Intensive Therapy Unit (ITU) admission, six studies from high income settings were identified and again the pooled relative risk suggested that PLWH with influenza were no more likely to require ITU care or die. In contrast, in Low and Middle-Income Countries (LMICs) the authors identified only one study (from South Africa) which assessed risk of hospitalisation, which was found to be higher in those with HIV. Data on antiretroviral therapy use were only available for 59% of HIV positive subjects in this study, of whom 50% were using ART and details of CD4 counts only available for 29% of HIV positive subjects, therefore limiting interpretation of this result. Three studies from LMICs assessed the risk of death or ITU care and all found a higher risk in those with HIV (pooled RR 2.17, 95% CI 1.29-2.91).

A further prospective study from Malawi (published after the systematic review by Coleman *et al*) which compared PLWH with a HIV negative comparator group was conducted by Ho *et al* (56). The HIV positive

participants in this study had a median age of 37 years; 65% were using ART and median CD4 counts were 390 (IQR 244-547). These investigators found that the frequency of Influenza-like illness (identified in participants presenting to hospital) was not significantly different between HIV positive and negative participants (IRR 1.21 (0.99-1.48) but PLWH were more likely to have influenza virus identified when they had an acute illness (10% of 229 illnesses vs 4% of 119 illnesses). In an associated case-control study exploring risk factors for severe hospitalised respiratory tract infection, the investigators identified being HIV positive as a significant risk for severe influenza (OR 4.98 2.09-11.88).

1.7 Techniques used in assessment of respiratory health and lung function

Respiratory health can be studied with several different approaches, including physiological evaluations of lung function parameters, patient reported evaluations of subjective respiratory symptoms, and functional impairments and imaging studies of lung structure. I will briefly summarise the more commonly used techniques, their advantages and disadvantages and discuss the methods used in this thesis.

Measuring lung function

Physiological evaluations of lung function range from simple measurements that can be undertaken using hand-held devices, to studies requiring sophisticated laboratory equipment. A full description of these techniques is beyond the scope of this thesis, and this section will provide an overview of those relevant here.

One of the simplest and best characterised assessments of respiratory function is spirometry, which measures volumes of inhaled and exhaled air and flow rates. The key parameters measured are the FEV_1 (the volume of air exhaled in one second of forced exhalation); the FVC (the total volume of air exhaled in a single forced exhalation) and the peak flow rate. Further information can be obtained studying patterns of abnormality in the flow / volume loop and measurements of flow such as the MEF_{50} - the maximal expiratory flow at 50 % of the forced vital capacity, which is felt to provide some measure of small airways obstruction.

Spirometry is a fundamental tool for the evaluation of lung function (for instance COPD is defined by spirometric impairment) and has the advantage that it can be performed using simple hand-held equipment. In addition, techniques are well standardised with accepted methods of quality control. Normal values have been measured in large and rigorous population-based studies, allowing the definition of normal or predicted values (with predictions based on measurement of age, height, gender and ethnicity) although such datasets are largely based on European, North American and Asian populations, with few data from other populations, in particular from Sub-Saharan Africa. Spirometry is a key tool of respiratory epidemiology and as such I have used this extensively in work contained in this thesis, using hand-held spirometers and defining normal values based on the Global Lung Initiative equations, which are derived from large and multi-ethnic population-based data sources.

Although spirometry has many advantages, as a measure of respiratory physiology it has some important limitations – many disease processes can lead to the same spirometric pattern, whilst significant lung function impairment can occur with completely normal spirometry. It is not surprising, therefore, that spirometry can correlate poorly with reported respiratory symptoms.

In both routine clinical practice and respiratory research, evaluation of lung volumes (including both respirated and non-respirable air) provides further information that can be supplemented by measurement of the gas-transfer capabilities of the lung. The standard measure uses the ability of the lung to take-up carbon monoxide and is referred to as the Diffusing capacity of the Lungs for Carbon Monoxide (DLCO) (which can be adjusted to the estimated alveolar volume to give the KCO). This therefore provides an estimate of the integrity of the gas-exchange part of the lung (which can be affected by many processes including loss of alveolar surface, reduced diffusion across the alveolar membrane, pulmonary vascular pathologies, shunts and impairment of oxygen uptake by haemoglobin). Measurement of DLCO is a key aspect of respiratory physiology and has been used in several studies in the field of HIV-related lung disease, as discussed elsewhere in this Introduction. However, assessing DLCO requires specialised equipment in a lung function laboratory and imposes a considerable burden of time, expense and on the participant themselves. It is therefore challenging to use in large-scale studies. Although assessment of physiological parameters beyond spirometry, such as DLCO, would have provided interesting additional data for this thesis, the constraints on time and resources available did not make this possible.

In addition to spirometry and measurements of lung volumes and gas transfer further techniques of physiological measurement can be used to evaluate respiratory health. These include measurement of small airway function by multiple-breath washout of gasses that are not taken up in the alveoli,(57) or by impedance oscillometry, a technique that uses sound waves to measure airway obstruction(58). Cardiopulmonary exercise testing, in which physiological evaluation during exercise is evaluated to determine the cause of exercise limitation, can also provide useful insights.(59)

Physiological measurements of spirometry and lung function enable us to evaluate “lung health”. Another approach identifies abnormalities through anatomical imaging. Although standard chest X-Ray can be used (and has the advantage of being relatively quick, cheap and of low radiation exposure to participants), they are difficult to standardise and provide limited information about lung abnormalities. Therefore, techniques utilising CT scanning are increasingly used. Such studies are resource-intense, both to obtain and interpret the scans, and also represent a much larger potential radiation exposure to participants. However, they have been used to define patterns of respiratory abnormality in HIV related lung disease – which is discussed elsewhere in this chapter.

1.8 Non-communicable lung disease in HIV positive populations with access to ART

Although improving treatments for HIV infection have resulted in reductions in the frequency of respiratory infection among PLWH, recent years have seen growing concerns that there may be a higher burden of chronic non-communicable respiratory disease in this population. As with other comorbidities, chronic respiratory disease is becoming more common among PLWH (4). This section will summarise current evidence regarding the epidemiology of chronic respiratory illness in HIV infected populations with access to ART.

As described previously, in the Multicentre AIDS Cohort Study (MACS) prior to the availability of ART, the risk of non-infectious pathology was higher among PLWH, with HIV positive participants nearly three times as likely to develop COPD (in the MACS study, adjusted hazard ratio 2.9, (95% CI, 1.02–8.4); p=0.046) (30). However, after ART became available this relationship weakened, with no significant association between HIV status and a diagnosis of COPD in the period 1996-2008, albeit with wide confidence intervals that do not exclude a similar magnitude of difference as that seen in the pre-ART era (OR 1.16 (0.28-4.68), p=0.84).

The MACS study only evaluated men, though similar findings were reported in the Women's Interagency Cohort Study (WIHS) of HIV positive and negative women (30). This cohort of 3,766 women with or at risk of HIV was followed at six centres in the USA from 1994. The 879 HIV negative participants had high rates of smoking and recreational drug use, with 54% being current smokers at baseline and 78% reporting current or previous recreational drug use. Not all of the HIV positive participants were on effective ART: the HIV positive participants had a median CD4 count of 382 (IQR 211-581) cells; 27% had HIV blood RNA levels >400 at baseline, and HIV positive participants had viral loads >400 at 53% of visits. This cohort therefore represents a population with significant levels of immunosuppression and findings from these studies may not be generalizable to populations with better uptake of ART and proportion with virological suppression. As with the MACS data, in this cohort respiratory infection remained more common among PLWH with an odds ratio for bacterial pneumonia of 13.5 (95% CI 4.3-42.6, p<0.001) and sinusitis of 2.27 (1.89-2.73, p<0.001). The incidence of non-communicable respiratory diagnoses did not differ between HIV positive and negative participants with an OR of 1.36 (0.49-5.64) for lung cancer or 0.9 (0.74-1.11) for asthma.

The Veterans Aging Cohort Study utilised routinely collected administrative data from 33,420 HIV-positive subjects and 66,840 demographically matched HIV-negative subjects within the Veterans Affairs (VA) healthcare system up to July 2007 (60). In an analysis based on ICD-9 codes, COPD was present in 4.6% of PLWH and 4% of HIV negative participants at baseline; incidence rate ratios (IRR) for the development of *new* respiratory pathology demonstrated higher incidences of COPD, lung cancer,

pulmonary hypertension, pulmonary fibrosis and TB with, for instance, an adjusted IRR of 1.17 (1.11-1.24, p <0.05) for the development of COPD in those under 50 and 1.08 (1.01-1.15, p<0.05) in those over 50.

As records of smoking status were thought likely to be incomplete and inaccurate in the routinely collected VA data, a nested sample of 3,707 HIV positive and 9,980 HIV negative individuals who had provided details regarding smoking status as part of the Large Health Survey of Veteran Enrolees in 1999 was included, allowing calculation of adjusted incidence rate ratios including smoking status (classified as never smokers or ever smokers if they reported greater than 100 lifetime cigarettes). In this analysis, incidence of COPD remained significantly higher in HIV positive individuals below the age of 50 (adjusted IRR 1.25 (1.08-1.43)) but not those over the age of 50 (1.11 (0.96-1.29)). Furthermore, those with a blood HIV viral load above 400 copies/ml had a higher prevalence of COPD and asthma.

One problem with using registry data in this way is that respiratory pathology is often undiagnosed and more direct evidence requires studies which directly measure lung function to identify disease. The AIDS Linked to Intravenous Experience (ALIVE) study has followed current or former Injection Drug Users in Baltimore since 1988 (61). Around 30% of this cohort were HIV positive, thus allowing direct comparisons between HIV positive and negative participants in a way that avoids the biases associated with studies that have different recruitment strategies for HIV positive and negative participants. A sub-study of the ALIVE cohort evaluated obstructive lung disease and the presence of respiratory symptoms(62). Participants in these studies had a mean age of 49 years, 65% were male and 91% of African American ethnicity. As expected, due to the nature of the study population, frequencies of smoking and recreational drug use were extremely high. All participants were current or former injection drug users, 85% were current tobacco smokers (with only 6% recorded as never smokers) and 38% reported smoking of heroin or cocaine.

Overall in the ALIVE cohort the prevalence of airflow obstruction (FEV1/FVC <0.7) did not differ by HIV status, being present in 15% of both HIV positive and negative groups. Although the prevalence of reported cough or wheeze also did not differ by HIV status, PLWH in this cohort were more likely to report breathlessness (as defined by an MRC dyspnoea scale score of 2 or more). The effect of HIV status on respiratory symptoms will be discussed in more depth in Chapter 2. In a smaller sub-study evaluating only individuals with airflow obstruction, the investigators found that PLWH who were using ART were less likely to have their airflow obstruction previously diagnosed and suggested that this might be related to an emphasis on direct HIV care rather than diagnosis and management of comorbidities by HIV care providers.

These investigators went on to evaluate whether HIV-related characteristics such as CD4 count or HIV load were associated with respiratory health. In these analyses they found that PLWH with an HIV load above 200,000 copies/ml were more likely to have airflow obstruction than HIV negative participants (OR

3.30 1.26-8.69). CD4 counts were not associated with the presence of obstructive lung disease in these cross-sectional analyses.

In a prospective study of this cohort over a median follow-up time of 2.75 years, the same investigators studied the effect of HIV status and HIV-related parameters on lung function change (63). Overall, decline in FEV1 or FVC was not different in HIV positive and negative participants, however PLWH with an HIV viral load above 75,000 copes/ml had a greater decline in FEV1 and FVC than HIV negative individuals (76ml/year decline in FEV1 and 86 ml/year decline in FVC); lung function decline was also greater among PLWH with a CD4 count less than 100 cells/µL than in HIV negative participants.

One hypothesis to account for the higher prevalence of airflow obstruction and accelerated loss of lung function observed among PLWH with higher levels of viraemia in the ALIVE cohort is that HIV, or the consequences of immunocompromise, directly cause lung damage. Other explanations for these findings are possible. In particular, residual confounding by risk factors such as tobacco smoking and recreational drug use may be present, as these may well be more common among the group of PLWH with higher viraemia than in the HIV negative group. Although the investigators adjusted for (self-reported) smoking, this is difficult to quantify accurately and to comprehensively adjust for in regression models. In the ALIVE study, smoking was only included as a binary variable (tobacco exposure of over 40 pack-years or not) in the adjusted analysis of the association between HIV status, viral load and obstructive lung disease. Such difficulties are inevitable in any observational study and are a limitation of this methodology which must be considered when interpreting such results. When the hypothesis that HIV viraemia is associated with accelerated lung function decline was tested in the context of a randomised trial in the START study (in which PLWH with CD4 counts above 500 cells/µL were randomly assigned to early or deferred antiretroviral treatment) no difference in spirometric change was found (64). Whether this definitively answers this question can be debated however, as the START study was stopped before its planned duration due to meeting its overall primary endpoint and inevitably many in the deferred ART arm started ART due to clinical indications. In an analysis adjusting for smoking, the overall difference in FEV1 change between early and deferred ART was –5·2 mL/year, but the 95% confidence interval spanned a 25ml reduction to a 15ml increase per year. As future randomised trials of antiretroviral therapy would no longer be ethical, it may not be possible to conclusively answer the question of whether HIV viraemia accelerates lung function decline.

The Examinations of HIV Associated Lung Emphysema (EXHALE) study (a sub-study of the VACS cohort discussed above) evaluated respiratory illness in more detail and included measurement of spirometry rather than relying on routinely collected coding data: baseline assessments of 180 HIV positive and 153 HIV negative individuals found a significantly higher prevalence of chronic respiratory symptoms in those with HIV (e.g. 25% vs 15% reporting chronic cough and 25% vs 16% having breathlessness) but there was no significant difference in the proportion of HIV positive and negative individuals with airflow obstruction (19% and 20% respectively) (65). Both HIV positive and negative participants in this study

had impaired diffusing capacity of the lung for carbon monoxide (DLCO) measurements, but again there was no difference according to HIV status. Chronic respiratory symptoms did correlate with functional impairment however, with a reduction in 6-minute walk distance of 42m in HIV positive individuals with chronic cough compared to HIV positive individuals without (the minimum clinically important difference in the 6MWT is felt to be between 14 and 30m).

A further collaboration between several centres in the US with an interest in HIV related lung disease collected data on respiratory health in HIV positive individuals between 2008 and 2013. Spirometric impairment was common in this study, with 37% of 908 participants having abnormal spirometry (27% with airflow obstruction and 10% with restriction) (66). Age, tobacco smoking, a history of having PCP and current respiratory symptoms were all associated with an increased likelihood of having abnormal spirometry. Again, chronic respiratory symptoms were reported in around a third of this population. Interestingly, the majority (56%) of those with chronic respiratory symptoms had normal spirometry. In contrast to findings from earlier studies such as the ALIVE study, neither increased HIV load or lower blood CD4 counts were associated with airflow obstruction in this study.

1.9 Evidence from populations other than the USA

All the data discussed so far has derived from clinical studies of HIV positive populations in the United States. It has been a significant bias in the published literature in this field that HIV positive populations elsewhere have are well studied. However, in recent years some studies evaluating HIV positive populations in Europe and elsewhere have been published which help to address this deficiency, although most are single-centre studies, and many have methodological limitations. Studies evaluating PLWH in low and middle-income settings are discussed in the next section.

Madeddu *et al* reported a higher prevalence of respiratory symptoms (47% vs 15%, p= 0.002) and COPD (23% vs 8%, p=0.008) in a study of 111 HIV positive and 65 HIV negative individuals in Sassari, Italy (67). Other studies have also documented a high frequency of respiratory illness with, for instance, airflow obstruction (FEV1/FVC <0.7) reported in 17% by Samperiz *et al* from Spain (68), 10% by Nakamura et al from Japan (69) and 11% by Guaraldi *et al* from Italy (70). A multicentre evaluation of HIV positive individuals over the age of 40 with at least a 20 pack-year smoking in France history demonstrated airflow obstruction in 26% (71).

In Modena, Italy, HIV positive patients attending an outpatient "HIV metabolic clinic" underwent investigations including thoracic or cardiac CT scans as part of clinical management and data from this clinical cohort has been analysed and described by Guaraldi *et al (70)*. The indications for referral into this clinic, and details about the wider HIV population from which this group was drawn were not described by the authors, so it is difficult to know whether the group investigated were representative of the wider HIV population, but nonetheless this represents a useful source of data regarding the prevalence of cardiorespiratory impairment in this population, particularly as data are available regarding a relatively large group of individuals (1460 in total). All these patients were using antiretroviral therapy (for at least 18 months) and the median age was 48 years; 94% of the participants had an undetectable HIV viral load and the mean CD4 count was 612 cells/µL; 40% were current smokers and 28% had a history of previous injecting drug use.

In this group, CT scans were abnormal in 49% of subjects, with 13% having evidence of bronchiolitis, 19% emphysema and 16% both. The investigators performed most of these scans primarily as cardiac CTs and therefore had less complete imaging of the lungs. The prevalence of emphysema was 41% in those with full scans and 34% in those with cardiac scans only, suggesting that in this group some cases of emphysema may have been missed. As expected, being a current smoker, age and a history of injecting drug use were independently related to the presence of emphysema, as was a prior history of PCP, although only in univariable analyses.

This large dataset provides an insight into the high prevalence of radiographic lung abnormalities in a contemporary HIV positive population. In contrast to some other groups evaluated, the subjects were universally using ART, with good levels of viral suppression and CD4 counts. However, as there was no

HIV negative comparator group in these analyses it is difficult to draw clear conclusions regarding the extent to which these changes were driven by HIV infection rather than established risk factors such as smoking and age. A further study by the same authors in the same population described airflow obstruction in 11% of 903 individuals who underwent spirometry (this test was added as part of assessments in 2011, and hence was not available for all participants) (72). Again, tobacco smoking was the strongest predictor of respiratory impairment, with the number of pack-years of tobacco exposure strongly related to the presence of either heart or lung disease. (72)

To provide some comparison with an HIV negative group, data from this HIV cohort in Modena was compared to general population data from a population-based Italian national registry (the CINECA ARNO database), which is an observational study aggregating data from primary care records, prescriptions and hospital records. (73) In analyses stratified by age, this demonstrated a significantly higher prevalence of a range of non-communicable comorbidities (cardiovascular disease, hypertension, renal impairment, bone fracture and diabetes mellitus) (74), but respiratory illness was not included in these comparisons. For instance, among those aged 51-60 years, renal failure was documented in 9% of HIV positive and 0.29% of HIV negative individuals and cardiovascular disease in 6% and 3% respectively. Unfortunately, the interpretation of these data is complicated by the different methods of evaluation and data capture in the HIV positive and negative groups, with active (and very detailed) investigation of the HIV positive group compared to only passive collection of routine clinical data in the HIV negative comparator group, plus the possibility of biases in the selection of the HIV positive group if individuals who were more likely to have co-morbidities were preferentially referred to the clinic for investigation. Therefore, these data represent an information source at significant risk of bias, but nonetheless do demonstrate the high prevalence of non-communicable comorbidity that may be present in HIV positive patients in resource rich settings.

The prevalence of COPD in an HIV positive population in France was evaluated as part of a trial of chest CT screening for lung cancer (75). This study only examined individuals over the age of 40, with at least a 20 pack-year smoking history and a nadir CD4 count below 350 cells/μL who were current smokers or gave up smoking less than 3 years prior to recruitment. This cannot therefore provide information on the HIV positive population in this setting as a whole, but nonetheless does yield some useful insights. The HIV positive participants were then compared to a general population control group derived from a population-based epidemiological study (the CONSTANCES cohort), with HIV positive individuals matched by age and gender to a HIV negative comparator, and again only individuals aged above 40 with at least a 20 pack-year smoking history were included. A proxy measure of COPD prevalence using pre-bronchodilator spirometry was used (as post-bronchodilator spirometry was not undertaken in the CONSTANCES cohort), obstructive lung disease was therefore defined as a pre-bronchodilator FEV1/FVC ratio less than 0.7 plus FEV1 less than 80% predicted.

This analysis found a greater prevalence of airways disease among the HIV positive group, with 19% of HIV positive compared to 9% of HIV negative individuals having evidence of airflow obstruction. In a multivariable linear regression model using FEV1/FVC ratio as the dependent variable, HIV status, age, gender, Body Mass Index, tobacco smoking, and hepatitis C status were all independently associated with a lower FEV1/FVC ratio. As with the data presented by Guaraldi *et al*, methodological imitations complicate interpretation of these results: in particular, the different recruitment methods used for the two study groups and the possibility of biases in recruitment of HIV positive participants. Therefore, the differences found between HIV positive and negative individuals could be caused by residual confounding or biases in the study populations. The data could demonstrate a cross-sectional association, which may not necessarily imply that a causative relationship exists between HIV status and airways disease. Nonetheless this study provides further evidence of a high prevalence of obstructive lung disease among some HIV positive populations.

A further large and well-conducted European study comparing an HIV positive population with a general population control group has been undertaken by Ronit *et al* in Denmark (76). This study evaluated a cohort of 1098 PLWH who were compared to a general population sample derived from the Copenhagen General Population Study (CGPS). In this study matching did not attempt to obtain an HIV negative group with equivalent risk factors and the prevalence of tobacco smoking was significantly higher among PLWH (with 29% compared to 13% being current smokers) but instead regression models were used to assess the importance of different participant characteristics. In this population there was no difference in the prevalence of airflow obstruction defined as an FEV1/FVC less than the (age, gender and ethnicity standardised) lower limit of normal: by this definition airflow obstruction was present in 10.6% of PLWH and HIV negative controls. However, when airways disease was defined by the Global Initiative for Obstructive Lung Disease (GOLD) criteria of FEV1/FVC < 0.7 and FEV1< 80% predicted, a significantly greater proportion of the HIV positive group were found to have impairment: 10% vs 5.8%, ($p<0.0001$). This appeared to be primarily driven by concurrent reduction in FEV1 *and* FVC, and was associated with a lower nadir CD4 count, suggesting that prior immunosuppression may have been an important driver of lung function derangement in this population.

1.10 Data from Low and Middle-Income Countries

In contrast to the HIV positive populations in resource rich settings, where large proportions of PLWH are established on antiretroviral therapy, low and middle-income countries (LMICs) have to date had much less access to ART, with important implications for the respiratory health of these populations. Chronic respiratory impairment has rarely been assessed in these settings (although as will be discussed in Chapter 2 there is some evidence regarding the frequency of respiratory symptoms). Gupte *et al* investigated risk factors for respiratory impairment among people living with HIV in South Africa (77). In a study of 730 predominantly (85%) female individuals, with a median age of 36 years, in an urban setting (Soweto) in South Africa, only 25% were using antiretroviral therapy; 30% of this population had ever smoked, yet only 8% were current smokers, (in marked contrast to populations in the US and Europe). Only 5% of the population had airflow obstruction, which was significantly associated with older age, current smoking and a higher blood CRP level.

Two other recent studies from Sub-Saharan Africa provide further data regarding respiratory illness in HIV positive populations in resource poor settings. An analysis of 356 PLWH over the age of 30 in Nigeria, found that airflow obstruction (post-bronchodilator FEV1/FVC <0.7) was present in 15%, despite only 17% of this cohort ever having smoked.(78) Perfura-Yone *et al* conducted a case-control study comparing HIV infected individuals in Yaoundé, Cameroon with age and sex matched HIV negative controls and found no significant difference in the proportion with airflow obstruction (2.2% HIV positive and 0.7% HIV negative individuals with FEV1/FVC <0.7) (79), however this was a relatively young population (mean age 42 years) with limited tobacco exposure (87% and 82% of HIV positive and negative participants respectively were never smokers), in which a past history of TB was the strongest predictor of airflow obstruction. These studies illustrate how diverse populations, with differing exposures to important risk factors have different burdens of respiratory illness. How this changes as antiretroviral therapy becomes more easily available, and resource poor settings have growing populations of HIV positive individuals using antiretroviral therapy will be an important question in future decades.

1.11 Chronic lung disease in individuals with vertically acquired HIV infection in low and middle-income countries

A specific group which appears to have a particular phenotype of HIV-related chronic lung disease are individuals with vertical acquisition of HIV infection who survive to adolescence without treatment. Although this group are not the subject of this thesis, they do offer insights into the potential impact of HIV on the lung and possibly mechanisms of disease. Whilst in most settings they may represent a relatively small population, in sub-Saharan Africa it is estimated that over 3 million HIV positive children and adolescents were alive in 2015 (80). Without antiretroviral treatment, about a third of vertically HIV infected infants typically survive to adolescence. A group of these "slow-progressors" was evaluated by Ferrand *et al* in Harare, Zimbabwe, who found a very high burden of chronic respiratory illness, with chronic cough reported by 66% and exercise limitation by 21%. (81) Thirteen percent were hypoxic at rest and 29% on exercise. Fifty-six of these individuals went on to have CT scans, and here the predominant findings were suggestive of small airways obstruction (characterised by mosaic attenuation on CT) and bronchiectasis.

Similar analyses of vertically infected children and adolescents have been undertaken in Nairobi, Kenya, and Blantyre, Malawi, which have recapitulated these findings – Mwalukomo *et al* found evidence of both small airways disease and bronchiectasis in 160 children with a mean age of 11 in Malawi who had a similarly high prevalence of respiratory symptoms and Attia *et al* also identified a high prevalence of hypoxia and tachypnoea in 258 adolescents in Nairobi (82, 83).

This group of vertically infected individuals in some ways represents an extreme end of the spectrum of HIV exposure, in which lung development and maturation occur in the presence of often uncontrolled HIV viraemia. Such individuals are likely to have experienced recurrent respiratory infections throughout childhood with important consequences for their lung health. This group illustrate the potential impact of HIV infection on the lung and their future management will be an important clinical issue in sub-Saharan African countries.

1.12 Effect of HIV on measures of lung function other than spirometry

Most studies evaluating respiratory health in HIV positive populations have used spirometry to assess lung function. Whilst this has the advantage of being relatively quick, well-standardised and able to be completed in a clinic room, this approach may miss more subtle, yet potentially important, lung function changes. To date, relatively few studies have used techniques such as evaluation of the diffusing capacity for carbon monoxide (DLCO) of the lung in a systematic way to assess respiratory impairment in HIV positive populations.

In a study of 167 HIV positive individuals recruited from the University of Pittsburgh between 2007-9, Gingo *et al* demonstrated that 64% of these participants had a DLCO of less than 80% of the predicted value (whereas only 21% had airflow obstruction) (29). Impairment was associated with greater number of pack-years smoked and a CD4 count of less than 200 cells/µL. As this study only evaluated HIV positive individuals, the relative importance of HIV status compared to established risk factors such as tobacco smoking could not be studied. Further work from the same group suggested that in HIV positive never smokers DLCO impairment was still common, with mean DLCO of 73% predicted (74).

Only two studies to date have systematically compared DLCO measurements in HIV positive and negative individuals. Crothers *et al* examined 300 HIV positive and 289 HIV negative men recruited as part of a cross- cohort study from the MACS and Veterans Aging Cohort Study cohorts (30). PLWH in this study were more likely to be current smokers (47% vs 35%) and had a greater total cigarette pack years (median 26 vs 20 pack-years), as well as being more likely to be current cannabis users and more likely to have had previous bacterial or *Pneumocystis jirovecii* pneumonia. However, in a multivariable linear regression model adjusting for ethnicity, smoking exposure and study site, HIV status remained associated with lower percentage predicted DLCO. This association was stronger in those with a CD4 count below 200 cells/µL.

The study of Crothers *et al* was restricted to men, and Fitzpatrick *et al* conducted a similar evaluation enrolling 63 HIV positive and 36 HIV negative women from the Women's Interagency HIV study (28). Here, exposure to tobacco smoking was again greater in the HIV positive group (10 pack-years compared to 4.2). Again, DLCO was found to be significantly lower in the HIV positive group (mean percentage predicted of 65% vs 72%, p=0.01) and HIV status was associated with lower DLCO in a multivariable linear regression model. In a regression model including only HIV positive participants, prior history of bacterial or PCP, current use of cocaine and cannabis use (ever) were also associated with lower DLCO.

Impairment in DLCO in PLWH was reported by several groups prior to the availability of ART, who noted impairment in gas transfer associated with worsening immunosuppression, even when opportunistic

infections such as PCP had been confidently excluded. (84-89) Diaz et al explored the physiology of this impairment in more detail, estimating the relative contribution of obstruction to diffusion at the alveolar membrane (due for instance, to the accumulation of an inflammatory exudate in the alveolar space) and loss of pulmonary vascular beds – their conclusion was that the predominant cause of this impairment was a loss of the pulmonary vascular beds, and concluded that this could indicate that PLWH are experiencing the early onset of emphysema (which is associated with loss of alveolar volume including the vascular beds). (90) There has been relatively little exploration of the pathophysiology of diffusion capacity impairment in PLWH using ART. Gingo *et al* investigated this in 158 PLWH (33 of whom were never-smokers) including comprehensive assessments of lung function, and CT imaging for 117 of the cohort. They concluded that different pathological mechanisms might be operating in smokers and non-smokers, with impairment among smokers characterised by airflow obstruction and radiological evidence of emphysema, whereas in never smokers there were stronger associations with a reduction in FVC and neutrophilic airway inflammation(91).

Ronit *et al* evaluated small airway dysfunction using a technique of Multiple Breath Washout for nitrogen to calculate the Lung Clearance Index (LCI) (75). This technique may allow assessment of small airways disease before changes in conventional lung function tests (such as FEV_1) become apparent. In order to avoid the confounding effects of tobacco and cannabis smoking, Ronit *et al* evaluated only people who had never smoked, recruited from the Copenhagen Co-morbidity in HIV infection study, compared to HIV negative subjects recruited from hospital staff and outpatient clinics. This group found that HIV status was associated with lower LCI, which persisted after adjustment for age (which was also found to be associated with lower LCI in univariable analysis). However, although HIV positive participants reported more respiratory symptoms, with slightly higher scores using the St George's Respiratory Questionnaire (3.5 vs 1.4), LCI was not significantly associated with SGRQ scores.

1.13 Summarising the epideimiology of lung function impairment and HIV in the antiretroviral era

As discussed in this section, many studies have evaluated lung function in PLWH and attempted to determine whether or not HIV infection results in an increased likelihood of respiratory impairment (Table 1). This field as a whole is limited by the fact that the majority of studies are cross-sectional in design, use only spirometry as a measure of lung function, are often not able to adjust for important confounding effects and in many cases do not include an HIV negative comparator group. Therefore, the ability of such data to confidently answer the question of whether HIV infection leads to lung function impairment is limited.

Studies with more detailed phyisiological measurements (such as of lung diffusion capacity), with prospective follow-up and which could more fully assess the effect of confounding factors would provide a significantly improved level of evidence. Researchers can only hope to address the question of whether or not PLWH are more likely to have lung function impairment if they make direct comparisons with HIV negative comparators. If we look specifically at studies which have done so and reported prevalence of airflow obstruction in HIV positive and negative groups in populations with access to antiretroviral therapy (each discussed individually in this chapter), overall there appears to be no significant association between HIV status and airflow obstruction. The study by Madeddu *et al* was the only one which reported a significantly greater prevalence of airflow obstruction, and this appears to be an outlier when compared to the other studies.

TABLE 1 STUDIES EVALUATING FREQUENCY OF AIRFLOW OBSTRUCTION IN HIV POSITIVE POPULATIONS

First Author	Country	N	Prevalence of airflow obstruction (FEV1/FVC <0.7)	HIV negative comparator group?
Drummond **(66)**	USA	908	27%	No
Fitzpatrick **(92)**	USA	124	27%	No
Makinson**(71)**	France	338	26%	No
Madeddu**(67)**	Italy	111	23%	**Yes**
Gingo**(93)**	USA	167	21%	No
Crothers**(94)**	USA	300	18%	**Yes**
Samperiz**(68)**	Spain	275	17%	No
Drummond**(95)**	USA	316	16%	**Yes**
Akanbi **(96)**	Nigeria	356	15%	No
Hirani**(97)**	USA	98	14%	No
Guaraldi **(72)**	Italy	903	11%	No
Nakamura**(69)**	Japan	49	10%	No
Kristoffersen**(98)**	Denmark	88	9%	No
Risso **(99)**	France	624	9%	No
George **(100)**	USA	234	7%	No
Kunisaki **(64)**	Multi-site	1026	5%	No
Gupte **(77)**	South Africa	730	5%	No
Cui**(101)**	Canada	119	3%	No
Onyedum **(78)**	Nigeria	100	3%	No
Pefura Yone **(79)**	Cameroon	461	2.20%	**Yes**

Progress in our understanding of the relationship between HIV status and respiratory health, and where any specific associations between HIV and lung impairment exist, requires studies which do more than cross-sectional assessments of spirometry; more detailed physiological measurements of diffusion capacity or small airways disease and studies including prospective follow-up would add to the available data. Furthermore, all studies comparing spirometry in HIV positive and negative people have been conducted in Europe or the United States. Most PLWH live in low and middle-income countries, and the epidemiology of lung disease in these settings may well be different. We know that the prevalence of lung function impairment varies hugely in the general population in different settings, such that in the Burden of Obstructive Lung Disease (BOLD) study of the epidemiology of COPD, the prevalence of airflow obstruction ranged from 11% (in Guangzhou, China) to 24% (in Cape Town, South Africa). Different exposures to known risk factors such as tobacco smoking only partially explained these differences in prevalence (102). There is a need therefore to investigate the impact of HIV status on respiratory health in low and middle-income countries, as the proportion of PLWH globally who can access to ART grows.

1.14 Potential mechanisms for HIV-related lung disease

Investigators have suggested multiple mechanisms which may drive the development of chronic lung disease in PLWH (103). One of these is that lung damage may occur before an individual starts ART which could have long-term and persistent effects: although ART treatment guidelines now recommend starting therapy before significant immunocompromise develops, most PLWH have had a period of untreated infection which may last many years. Over this time, respiratory infections may result in lung damage that subsequently leads to chronic respiratory illness. Having an episode of PCP appears to be specifically associated with more chronic airflow obstruction than an episode of bacterial pneumonia, as demonstrated by Morris *et al* (104), and recovery of lung function following PCP may be particularly poor in smokers. It may therefore be that the effects of HIV prior to treatment have a persisting and negative impact on respiratory health.

Airway inflammation (which might be clinically manifested as chronic cough) may play an important role in the development of chronic lung disease, both in those with and without HIV (105) (106). The finding that neutrophilic airway inflammation (defined by the percentage of neutrophils in induced sputum samples) is associated with reduced DLCO in HIV infected non-smokers (91) is consistent with this hypothesis.

1.15 Immune dysregulation and HIV

Although peripheral blood CD4 counts are used as the primary clinical measure of immunocompromise in HIV infection, more complex derangements of immune function occur. Chronic HIV infection may be associated with a state of heightened “immune activation” as defined by circulating inflammatory markers (such as CRP and soluble CD14) and innate and adaptive immune cell phenotypes (defined by cell surface markers) (107). Disruption of the gut mucosal immune defence and consequent translocation of bacteria from the gut into the bloodstream has been suggested as a possible driver of this immune activation, a hypothesis supported by the association between serum levels of soluble CD14 and lipopolysaccharide. It has been suggested that this immune activation acts as a driver of the development of chronic comorbidities in PLWH: for instance, the severity of coronary atherosclerosis has been found to correlate with markers of immune activation (108). Similarly, Attia *et al* demonstrated an association between soluble CD14 in the blood and radiographic emphysema, suggesting that a state of generalised immune activation, or possibly premature immune senescence, may contribute to the development of respiratory illness in PLWH (109). These studies were, however, cross-sectional in nature and thus cannot establish temporality: the association found does not necessarily imply a causative relationship. A prospective study by Kirkegaard-Klitbo *et al* in Denmark evaluated the association between soluble CD163 (a marker of monocyte and macrophage activation) and the development of non-AIDS comorbidities in subsequent years, with the cohort followed-up from 2004-5 until 2014.(110) In this analysis, a higher CD163 at baseline was associated with an increased risk of developing chronic kidney

lung or liver disease (but not cardiovascular disease, cancer or diabetes mellitus), with a three-fold higher incidence of chronic lung disease in the highest sCD163 quartile compared to the lowest (Hazard ratio 3.2 95% CI 1.34-7.46). Although the authors suggested that their findings supported the hypothesis that monocyte/macrophage activation is involved in the pathogenesis of non-AIDS comorbidity, as with any such observational data, the association found may not indicate that the inflammatory marker studied is part of the causative pathway of these chronic diseases.

1.16 Immunological mechanisms of lung impairment in HIV

HIV causes systemic acquired immunodeficiency primarily by depleting CD4+ T helper lymphocytes (111). However, multiple specific mechanisms in the lung may contribute to pulmonary pathology (112). This is relevant both to the increased susceptibility to particular infections (such as *Streptococcus pneumoniae* and *Mycobacterium tuberculosis*) the incidence of which is greatly increased in chronic HIV Infection before significant depletion of peripheral blood CD4 cell numbers occurs (15, 113) and the development of non-communicable respiratory disease (9).

The HIV virus has the ability to directly infect lung immune cells: both alveolar macrophages and lung T cells express CD4, CCR5 and CXCR4 receptors and are therefore permissive to HIV infection (114). Infection of these cells is well documented, although only a small minority (typically 1 in 100 lymphocytes and 1 in 1000 alveolar macrophages) are typically directly infected by the virus (115). Disruptions of normal immune response, rather than by direct infection of lung effector cells, may therefore be responsible for many of the deleterious effects of HIV infection on lung immunity.

During early infection, the HIV virus primarily utilises CCR5 as a viral co-receptor to facilitate cellular entry ("CCR5 tropism") and preferentially infects the population of memory CD4+ T cells expressing this co-receptor (in particular the effector memory subset). The period of apparent stability in total CD4 numbers observed during chronic (untreated) HIV infection, which often lasts several years, represents a balance between HIV-mediated loss of these cells and replacement from the (relatively spared) central memory and naïve T cell subsets (116). As the effector memory subset represents the major cellular population in extra-lymphoid effector tissues such as the gut, this leads to a severe depletion of lymphocytes in these tissues: in the gut mucosa, primary HIV infection is followed by a rapid and profound depletion of mucosal immunity, with a loss of the gut associated lymphoid cell populations (117). This has a significant impact on gut mucosal defence and the integrity of this mucosal barrier to pathogens.

Immunological consequences of HIV infection seem to be compartmentalised, with differing results in the gut, lung, peripheral blood and other sites. In the lung, depletion of mucosal lymphoid numbers does not appear to occur to the same extent as in the gut early in HIV infection, as lung CD4+ cell populations are relatively preserved in chronic HIV infection (118). However, a well-established finding in untreated HIV infection is an increase in CD8+ T cell numbers in the lung (119, 120). This "lymphocytic alveolitis"

appears to be a cytotoxic response directly targeting HIV, as the degree of CD8+ infiltrate correlates with the concentration of virus in the lung, and is associated with a poorer prognosis (120). This CD8+ T cell infiltrate in the lung appears to be reduced by antiretroviral therapy (121).

In addition to the depletion of lymphocyte populations, impairment of innate immunity in the lung also contributes to HIV pathogenesis (122) (123). Alveolar macrophages are the predominant immunological cell and are the main effector phagocyte in the lung. Although some data are conflicting, most studies suggest that phagocytosis by alveolar macrophages is not impaired by HIV infection – for instance, Gordon *et al* found no difference in phagocytosis of *Streptococcus pneumoniae* by alveolar macrophages from HIV positive and negative individuals (124). This may not tell the whole story however, as more subtle derangements of phagocytic function may be present: Jambo *et al* suggested that although overall phagocytic activity (based on the internalisation of reporter beads) by alveolar macrophages is unaffected by HIV status, there was a reduction in proteolytic activity, interpreted as an impairment in phagosomal function within these cells(125). This impairment of proteolysis was not restricted to macrophages directly infected with HIV but was a generalised property of cells derived from the lungs of HIV positive individuals.

Collini *et al* undertook further work elucidating the mechanisms underlying the higher susceptibility to pneumococcal infection *(126)*. In analyses comparing HIV positive participants using ART with an undetectable HIV viral load with healthy volunteers, this group found that alveolar macrophages had impaired ability to kill phagocytosed pneumococci. This defect appeared to be related to the HIV envelope glycoprotein gp120, via post-translational modification of Mcl-1, a regulator of macrophage apoptosis. Although participants had no detectable HIV RNA in peripheral blood, in some participants there was evidence of ongoing viral replication in the lung, either through detection of viral RNA, p24 in alveolar macrophages or gp120 in bronchoalveolar lavage fluid.

At the level of global proteomic analysis of immune function, Nguyen *et al* demonstrated downregulation of immune pathways in HIV positive individuals in an analysis of bronchoalveolar lavage fluid (127). This disruption of the immune environment may persist despite antiretroviral treatment as Jambo *et al* demonstrated ongoing changes in lung cytokine networks even after a median of 5.5 years on antiretroviral therapy (128).

1.17 Respiratory infection and chronic lung disease

Respiratory infection may be one mechanism by which chronic lung disease can develop. Many respiratory infections may lead to long-term lung damage – for instance acute bacterial pneumonia can cause bronchiectasis with subsequent persistent symptoms and recurrent infection. Tuberculosis frequently leads to chronic lung disease which, at a global scale, is likely to be an important source of ill-health.(129) Also opportunistic lung infections including PCP in people living with HIV not taking ART or on ineffective treatment is likely to significantly contribute to chronic lung disease. Taken together, these severe respiratory illnesses, particularly in resource limited settings where the burden of respiratory infection is very high, may promote chronic lung disease in people living with HIV.(104) What is less well-documented is whether more common respiratory infections, such as bronchitis could also contribute to worsening respiratory health in HIV populations.

1.18 Mechanisms of non-communicable lung disease in HIV

This discussion of the mechanisms of lung disease in HIV has focussed on immune function and susceptibility to infection. Possible mechanisms contributing to non-communicable pulmonary disease in HIV are less well characterised (130). Several have been proposed by which HIV infection may promote lung damage and in particular the development of emphysema or airflow obstruction. Yearsley *et al* noted that HIV positive alveolar macrophages were more commonly found in areas of pulmonary emphysema in a histological analysis of lung samples removed at operation from people living with HIV, and suggested that these cells could be contributing to the destruction of lung tissue, perhaps by the release of inflammatory cytokines or proteinases(131). It is possible that specific pathogenic pathways contribute to the development of COPD in HIV positive individuals: Popescu *et al* compared HIV positive individuals with and without COPD and identified a particular CD8 T-cell alveolitis phenotype in those with COPD, plus depletion and dysfunction of CD4 T-cells compared to HIV positive individuals without COPD (132).

Changes to the pulmonary vascular system may also contribute to lung pathology. Several groups have noted a higher incidence of pulmonary arterial hypertension in PLWH, and HIV related vascular injury may contribute to this (133, 134). Although endothelial cells of the pulmonary vascular system are not susceptible to HIV infection, they may undergo apoptosis when exposed to HIV proteins and damage to endothelial cells could contribute to lung pathogenesis (135, 136).

1.19 Effects of HIV on the airway epithelium

Impairment of respiratory function may also result from HIV-related changes in the behaviour of airway epithelial cells, particularly in the small airways which are thought to be the initial site of pathology in the development of COPD. Although these small airway epithelial cells are not susceptible to productive HIV infection, Chung *et al* have demonstrated *in vitro* that HIV can bind to airway basal cells, and that this induces activation of intracellular signalling pathways that result in an increased production of matrix metalloproteinases (which can degrade lung tissue) and a change in cellular behaviour by which these cells acquire a “cell-invasion” phenotype (137). Whether these pathogenic mechanisms are of importance *in vivo,* and the extent to which this is a significant mechanism in individuals using antiretroviral therapy, however, has not been established.

Brune *et al* conducted a further analysis of the effect of HIV on the airway epithelium (138). These investigators cultured human airway epithelial cells *in vitro* and assessed the effect of HIV exposure on cellular permeability and intracellular signalling. They suggested that exposure to CXCR4-tropic HIV viruses (but not CCR5 tropic viruses) induced changes in these epithelial cell layers. Although epithelial cells do not have CD4 receptors, CXCR4 tropic viruses interacted with the CXCR4 receptor and were internalised by these epithelial cells but could not then replicate. Exposure to these viruses was associated with increased permeability of the cellular layers, decreased expression of E-cadherin and activation of intracellular inflammatory signalling pathways (ERK signalling and ICAM-1 expression).

In contrast to the findings of Brune *et al*, Chinnapayan and colleagues also evaluated lung epithelial cells and found that these cells express not only CD4 receptors, but also CCR5 and CXCR4 co-receptors and *were* susceptible to infection by both CXCR4 and CCR5 tropic viruses (139). Exposure to the HIV protein Tat appeared to interfere with normal epithelial and ciliary function and there was a reduction in cystic fibrosis transmembrane conductance regulator (CFTR) production and ciliary function. These authors suggest that impairment of airway mucociliary clearance could be a mechanism of pathogenesis in PLWH leading to increased frequency of infection and chronic airway inflammation.

The studies evaluating airway epithelial function demonstrate possible mechanisms by which HIV could promote the development of lung disease that are distinct from the pathways mediated by immune compromise or inflammatory responses to HIV. However, whether such effects occur *in vivo*, or have any clinical significance in PLWH using ART has not yet been established.

1.20 How complete is immune reconstitution with ART in the lung?

The extent to which antiretroviral therapy allows the immune system to return to a state equivalent to that of individuals without HIV is an important question in current HIV research (140). It appears that despite ART, PLWH continue to be more susceptible to some infections: the frequency of invasive Pneumococcal disease may continue to be 30 times higher than in the HIV negative (141) (142). Part of the reason for this may be ongoing immune changes despite virological suppression and recovery of peripheral blood CD4 numbers. Even with suppression of viral replication to the extent that HIV is not detectable in peripheral blood, analysis of encoded proviral DNA within alveolar macrophages suggests that the lung may be one “sanctuary site” where HIV persistence and replication continues, which may have ongoing effects on macrophage function (143).

Whether HIV positive individuals using ART have ongoing defects in T cell function is the subject of some debate. In an analysis of HIV positive and negative individuals including an HIV positive group with over 4 years ART exposure, although total CD4+ numbers did not differ, there were some deficiencies in particular T cell subsets and antigen-specific T cell responses (to Influenza and *Mycobacterium tuberculosis*) in particular in polyfunctional (i.e. multiple cytokine producing) CD4 T cell responses(144). Recovery of CD4+ T cell numbers with antiretroviral therapy may not imply that the overall breadth of immune responses recovers. Analysis of the diversity of T cell receptors present in HIV positive individuals using RNA-sequencing techniques suggests that there is a significant perturbation of receptor diversity with a reduced overall diversity and over-expression of some sequences (145). Relatively short-term antiretroviral use does not appear to reverse these changes, although whether long-term antiretroviral use does is not known.

1.21 Summary

Multiple studies have evaluated the impact of lung disease in HIV positive populations in recent years. These have yielded very variable estimates of the prevalence of airflow obstruction in different populations, ranging from under 3% to over 25% of PLWH, reflecting important differences in the global epidemiology of HIV and exposures to risk factors such as tobacco smoking. A major limitation of the evidence available is that relatively few studies have included an HIV negative comparator group, and consequently cannot adequately adjust for important confounding factors such as tobacco smoking and age of the cohorts. There continues to be relatively little high-quality data addressing the question of whether HIV independently increased the likelihood of developing chronic lung disease.

The improvement in life-expectancy and quality of life attributable to ART use is undisputed. However, important unanswered questions exist regarding the extent to which PLWH remain at greater risk of infection and non-communicable comorbidities and underlying mechanisms of disease. Whether PLWH who achieve viral suppression with ART are still at higher risk of infections such as bacterial pneumonia is not known (and guidelines therefore continue to recommend Pneumococcal and influenza immunisation for all HIV positive individuals). Acute respiratory illnesses are a common cause of morbidity in any population, but relatively little is known about these illnesses in PLWH now that antiretroviral therapy is widely and successfully used. Despite the large amount of data that have become available in the last 5 years, the importance of chronic lung disease in HIV is debated, and the relative importance of HIV as an independent driver of lung disease verses the contribution of well-established risk factors such as tobacco smoking remains the subject of ongoing research.

1.22 Aims of this thesis

This thesis aims to contribute to our understanding of respiratory health in PLWH by providing new data regarding respiratory illness in a current HIV positive population with a high uptake of antiretroviral therapy. Data arising from these studies will be of relevance to other HIV positive populations with similar levels of access to antiretroviral therapy, and to the increasing proportion of people living with HIV globally who are able to access ART.

To complete the research contained in this thesis I have used several methodologies including systematic review and quantitative meta-analysis of existing data. I have conducted systematic measurement of participant reported symptoms, relevant exposures and risk factors and lung function to determine the prevalence of respiratory ill-health in the population under examination. In addition, I have used molecular microbiology techniques for pathogen detection in respiratory samples to examine airway bacterial populations. A key novel aspect of this work is the use of detailed prospective follow-up of a well characterised cohort of HIV positive and negative participants to examine the frequency of acute respiratory illness in this population.

The work presented in this thesis extends and differs from the existing published literature in several important ways. Much of the data presented explores respiratory symptoms and health status (rather than lung function impairments alone) in contrast to most other published data, and therefore provides an insight into the effect on quality of life of impaired respiratory health of people living with HIV. Relatively few existing studies have prospectively compared people living with HIV with HIV negative comparator groups, and data presented here adds to this literature. In particular, this thesis includes data prospectively evaluating the frequency of acute respiratory illness in a cohort of HIV positive and negative individuals using diary cards to measure the onset of new symptoms in a comprehensive way, a type of study that has not previously been undertaken in an HIV positive population.

Chapter 2: Respiratory symptoms in HIV positive individuals and the effect of antiretroviral therapy, a systematic review and meta-analysis

This chapter describes a systematic review undertaken regarding respiratory symptoms in HIV positive (compared to HIV negative) individuals and how this has changed with the provision of antiretroviral therapy.

2.1 Aims of the study

As summarized in Chapter 1, respiratory illness is a common consequence of HIV infection. Respiratory complications of HIV infection include both acute (normally infective) illnesses such as bacterial pneumonia, PCP, tuberculosis etc., but also chronic respiratory conditions including Chronic Obstructive Pulmonary Disease (COPD) and pulmonary hypertension. In addition, other non-AIDS chronic comorbidities (in particular cardiovascular disease) manifest with “respiratory” symptoms such as breathlessness.

This systematic review had the following aims:

(a) To summarise existing data from studies comparing HIV positive and negative individuals which quantify the extent to which chronic respiratory symptoms are associated with HIV infection

(b) To evaluate the change in chronic respiratory symptoms among PLWH since the provision of antiretroviral therapy

(c) To highlight areas where the evidence base concerning chronic respiratory symptoms in people living with HIV is lacking

As discussed in the Introduction, although objective impairment of lung function has been the subject of considerable recent research, less attention has been paid to the symptoms that individuals report. For this reason, we focused this systematic review on respiratory symptoms (rather than changes in objectively measured lung function). This is important, as lung function impairment can correlate poorly with symptoms and functional status (146). We therefore aimed to provide a comprehensive analysis of existing data regarding respiratory symptoms in people living with HIV with the aim of understanding how this has changed with antiretroviral therapy, and where deficiencies in the current evidence base exist.

2.2 Methods

2.2.1 Search strategy

The aim of this systematic review was to evaluate and summarise the currently available published evidence regarding the relative frequency of respiratory symptoms in HIV positive and negative populations.

We therefore chose search terms that would capture any respiratory symptom (excluding those arising from pathology of the ear, or obstructive sleep apnoea).

We did not attempt to set rigid definitions (for instance regarding the duration of symptoms required to distinguish chronic from acute cough) and instead used those provided by the investigators of the primary studies.

The protocol of this systematic review was registered with the PROSPERO database (Ref: PROSPERO 2015:CRD42015013762), details are available online:

http://www.crd.york.ac.uk/PROSPERO/display_record.asp?ID=CRD42015013762

A systematic literature search was undertaken in the MEDLINE, EMBASE, Cochrane and CINAHL databases between 1946 and August 2015. Only publications in peer-reviewed journals were included, but we undertook manual search of abstracts from the Conference of Retroviruses and Opportunistic infections (CROI), International AIDS Society (IAS) and American Thoracic Society conferences since 1982 (where these were available) to find abstracts that may have been published as full papers. There was no language restriction on searches. Searches were limited to studies in humans.

The search terms used for each database are listed in Figure 3.

FIGURE 2 SEARCH TERMS USED

EMBASE (Ovid)

HIV.mp OR Human Immunodeficiency Virus OR Acquired Immunodeficiency Syndrome

AND (Wheezing or coughing or dyspn?ea or h?emoptysis).mp

MEDLINE (Ovid)

Exp "signs and symptoms respiratory"/ OR (signs and symptoms, respiratory).mp

OR (cough$ or breathless$ or dyspn?ea or h?emoptysis or (short$ adj2 breath)).mp

AND Exp HIV OR Exp HIV Infections OR (HIV or human adj immunodeficiency adj virus) or acquired adj immunodeficiency adj syndrome).mp

CINAHL (EBSCO)

(MH "Human Immunodeficiency Virus+" OR MH ("Acquired Immunodeficiency Syndrome") OR MH "HIV Infections+") OR (MH "HIV Infected Patients+")) OR ((HIV) OR (human immunodeficiency virus) OR (AIDS) OR (Acquired Immunodeficiency Syndrome)) AND ((MH "Signs and symptoms, Respiratory (Non-CINAHL)+") OR "Cough" OR "Dyspn?ea" OR "h?emoptysis" OR "short*adj2 breath")

2.2.2 Review team

The study was designed and led by myself with assistance from my primary and secondary supervisors. Advice regarding study design and conduct, and assistance with searches and data extraction were provided by Dr Anjana Roy (Public Health England).

In addition, statistical advice was provided by Dr Ross Harris (Public Health England). I conducted all the final statistical analyses presented here.

2.2.3 Inclusion and exclusion criteria for systematic review

We included studies if they were published in a peer-reviewed journal, reported data from HIV positive and negative individuals, measured respiratory symptoms in each group and provided quantitative data. No age restriction was applied and there was no language restriction, (although searches were conducted in English).

Exclusion criteria were as follows:

1. Studies which did not include HIV negative individuals
2. Studies reporting symptoms of obstructive sleep apnoea (such as hyper-somnolence)
3. Studies reporting symptoms arising from the ear (such as ear pain, discharge or hearing change)
4. Studies reporting data with the intention of describing the symptoms associated with a specific condition (such as tuberculosis, bacterial or *Pneumocystis jirovecii* pneumonia) rather than the occurrence of symptoms within the general or HIV positive population.
5. Studies which only assessed hospital in-patients, as these would not be representative of the population as a whole.

We did not exclude population-based studies if, by chance, they contained people with prevalent respiratory conditions as part of the population studied.

2.2.4 Study selection and data extraction

All abstracts were independently reviewed by two members of the review team (JB and AR). A short-list was then compiled of possibly relevant articles for review in full-text. These full text articles were accessed and jointly reviewed by two reviewers based on the inclusion/ exclusion criteria. Where necessary, discrepancies were resolved by discussion with the senior member of the team (ML). After selection of relevant studies had been completed, data were extracted on the following study characteristics:

1. Study design
2. Date of study
3. Age and gender of participants
4. Prevalence of tobacco smoking
5. Risk of acquisition of HIV
6. Geographical location(s) of study
7. Number of HIV positive and HIV negative participants
8. Frequency of respiratory symptoms
9. Description of respiratory symptoms / tool used to evaluate
10. Availability of antiretroviral treatment
11. Treatment provided for the respiratory illness
12. Level of immunocompromise in the HIV positive group (e.g. median blood CD4 count).

In order to assess the risk of bias in each study, we used a modified Newcastle Ottawa Scale based on that reported by Herzog *et al* (147). This is shown in Table 2. The Newcastle Ottawa scale provides a quantitative assessment of risk of bias in a study relevant to observational studies (in contrast to the Cochrane risk of bias tools which are more appropriate to randomized trials of two or more interventions). The Newcastle Ottawa scale awards up to 8 points to each study: three for adequate selection of participants, three for comparability of HIV positive and negative participants included in the study and two for the assessment of symptoms and reporting of statistical tests (148). In this scale, stars are awarded for higher scores for each criterion, giving a total score out of a possible 8 stars. High quality studies were defined as those that scored 6 to 8 stars, moderate quality 5 or 6 stars and low quality those with 0-3 stars.

TABLE 2 MODIFIED NEWCASTLE-OTTAWA SCALE USED

Selection	Representativeness of sample	Truly representative of the target population (random sampling) *	*
		Somewhat representative of the target population (non-random sampling) *	*
		Selection of population No description of sampling strategy	
	Non-respondents	Comparability between respondents and non-respondents is established and the response rate satisfactory	*
		Poor response rate, or comparability between respondents and non-respondents is not established	
		No description of response rate or characteristics of non-respondents	
	Ascertainment of HIV status in HIV negative participants	HIV test undertaken or contemporaneous clinic record of HIV test available	*
		Self -report of HIV status or not tested in HIV uninfected group or details of HIV testing for HIV negative participants not described	
		Information not provided	
Comparability	Subjects in HIV positive and negative groups are comparable based on recruitment strategy	Same recruitment strategy used for both groups *	*
		Recruitment strategy different for HIV negative group or recruitment strategy not mentioned	
	Smoking status:	Details of smoking history provided for all participants, including evaluation of pack-year history*	*
		Limited information on smoking status (e.g. number of current smokers only provided)	
		Data on smoking not provided	
	Level of immunocompromise in HIV positive participants	Mean or median CD4 count reported with range or standard deviation*	*
		CD4 count reported as range or range / standard deviation not given*	*
		No data on CD4 counts	
Outcome	Ascertainment of respiratory symptoms	Validated tool*	*
		Non-validated tool	
		Other	
	Statistical test	Association between HIV status and respiratory symptoms reported and appropriate statistical tests provided, including confidence intervals and probability (p value)*	*
		Association between HIV status and respiratory symptoms not evaluated or details of statistical test used not described.	

2.2.5 Statistical analysis

The difference in prevalence of respiratory symptoms in HIV positive and negative individuals was compared using odds ratios for the occurrence of each symptom (e.g. cough, breathlessness) in each group. Although the absolute frequency of these symptoms varied significantly according to how they are defined and measured by the investigators of each study, the definitions would be the same for all participants in each study the relative frequency of symptoms in HIV positive and negative participants could therefore be compared by means of odds ratios, allowing estimation of the effect of HIV infection on the likelihood of having these symptoms.

Studies of adults were stratified by (a) location of study in resource-limited or resource-rich settings (which also equated with high and low TB prevalence and high and low HIV prevalence settings) and (b) the availability of ART in the population studied. This stratification was performed because the causes of respiratory illness were likely to differ between these settings, and this would allow the effect of antiretroviral therapy provision to be evaluated.

Studies evaluating children were not included in the quantitative analyses because HIV positive children represent a unique population and these studies were highly heterogenous in methodology and outcome.

Study data were summarised in Microsoft Excel. Meta-analyses were conducted using RevMan 5.3 (Cochrane collaboration, 2014).

Pooled odds ratios for the presence of specific respiratory symptoms were calculated within each stratum wherever more than 1 study provided data. The DerSimonian and Laird random effects model was used to account for between-study heterogeneity (149). Statistical heterogeneity was assessed using the Cochran χ^2 test and the I^2 statistic, calculated using RevMan software and used to summarise the degree of heterogeneity.

The possibility that small studies have more extreme results (due to reporting bias) was assessed by the visual inspection of funnel plots.

2. 3 Results

2.3.1 Identification of relevant studies

A total of 5,788 publications were identified by the database search after removal of duplicate publications. Of these, 5,611 were excluded after review of the article title and abstract. 192 potentially relevant articles were reviewed in full-text of which 154 were excluded; reasons for exclusion are given in Figure 2.2. Twenty-four publications were included in the final list for review (Full list of studies is provided in Table 2.2).

Figure 3 PRISMA flow chart of studies included in systematic review

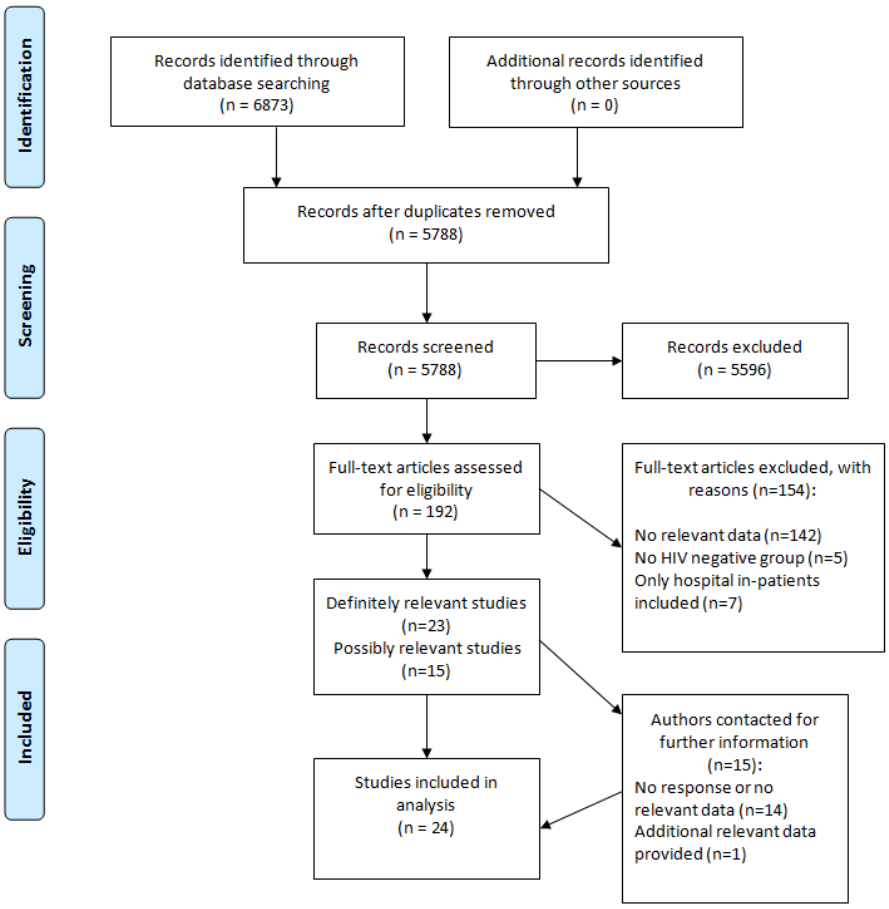


TABLE 3: STUDIES INCLUDED IN THE REVIEW

	1st Author & reference	Year of publication	Number of study participants	% HIV positive	Risk for HIV	Study location
1	**Lepage (150)**	1991	431	50%	General population	Rwanda
2	**el Sadr (151) (Multicentre AIDS Cohort Study)**	1992	223	56%	IDU‡	USA
3	**Hoover (152) Multicentre AIDS Cohort Study Visit 3**	1993	2854	33%	MSM◊	USA
	Hoover - visit 7	1993	2627	29%	MSM	USA
4	**Norrgren (153)**	1998	2215	9%	General population	Guinea-Bissau
5	**Spira (154)**	1999	401	54%	Vertical transmission	Rwanda
6	**Olayinka (155)**	1999	272	45%	Vertical transmission	Zimbabwe
7	**Nilses (156)**	2000	1213	21%	General population	Zimbabwe
8	**Taha (157)**	2000			Vertical transmission	Malawi
9	**Diaz (158)**	2003	379	85%	MSM	USA
10	**Galli (159)**	2003	280	75%	Vertical transmission	Italy
11	**Lewis (160)**	2009	1955	29%	General population	South Africa
12	**Read (161)**	2009	1317	84%	Vertical transmission	Malawi, Tanzania, Zambia
13	**Kheaw-On (162)**	2010	210	50%	General population	Thailand
14	**Corbett (163)**	2010	8979	21%	General population	Zimbabwe
15	**Drummond (164) ALIVE cohort**	2010	974	30%	IDU	USA
16	**Onyedum (78)**	2010	200	50%	General population	Nigeria
17	**Gounder (165)**	2011	3937	37%	General population	South Africa
18	**Crothers (94) Lung HIV Study**	2013	589	51%	Mixed	USA
19	**Fitzpatrick (92) WHIS**	2013	97	64%	Mixed	USA
20	**Madeddu (67)**	2013	176	63%	MSM	Italy
21	**Antwal (166)**	2014	1546	35%	Not detailed	India
22	**Campo (167) EXHALE sub-study, VACS cohort**	2014	340	53%	Mixed	USA
23	**Gingo (30) (MACS)**	2014	1896	71%	MSM	USA
24	**Gingo (WIHS)**	2014	1976	71%	Mixed	USA
25	**Telisinghe (168) Prison cohort**	2014	846	25%	General population	South Africa

2.3.2 Risk of bias in the studies included

Using the modified Newcastle Ottawa Scale to assess risk of bias, 6 studies were rated as low quality/ high risk of bias, 14 as moderate and 4 as high quality / low risk of bias (Table 4).

The main reasons for lower study quality scores were potential biases in the selection of study participants, who were rarely randomly selected from the populations being studied and only a minority of studies provided details of non-respondents. In contrast, ascertainment of HIV status was good, and most studies used the same recruitment strategies for HIV positive and negative participants. Smoking history among study participants was incompletely reported with only 8 studies reporting full details (including a measure of exposure such as pack-year history). In assessing the outcomes, most studies did not utilize a validated patient reported outcome tool. Overall, therefore, the published evidence regarding the association between HIV status and respiratory symptoms had at least a moderate risk of bias, and this represents a significant limitation of the evidence available to answer this question.

TABLE 4 NEWCASTLE-OTTAWA SCALE SCORES

Study	Selection			Comparability			Outcome		Quality / risk of bias		
	Representativeness (a-c)	Non-respondents (a-c)	Ascertainment HIV status (a-b)	Comparable (a-b)	Smoking (a-c)	Immuno-compromise (a-c)	Ascertainment symptoms (a-c)	Statistical test (a-b)	Star rating	Overall rating	Quality
Lepage	C	C	A*	A*	C	B*	B	A*	4*	50%	**Moderate**
el Sadr	B*	C	A*	A*	C	A*	B	A*	5*	62%	**Moderate**
Hoover	B*	C	A*	A*	C	B*	B	A*	5*	50%	**Moderate**
Norrgren	C	A*	A*	A*	C	A*	B	A*	5*	62%	**Moderate**
Spira	C	B	A*	A*	C	B*	B	A*	4*	50%	**Moderate**
Olayinka	C	C	A*	A*	C	C	B	A*	3*	37%	**Low**
Nilses	A*	A*	A*	A*	C	C	B	A*	5*	62%	**Moderate**
Taha	B*	B	A*	A*	N/A	A*	B	A*	5*	50%	**Moderate**
Diaz	C	C	C	B	A*	A*	A*	A*	4*	50%	**Moderate**
Galli	C	A*	A*	A*	C	C	B	B	3*	37%	**Low**
Kheaw-On	C	C	C	A*	A*	B*	B	B	3*	37%	**Low**
Lewis	C	A*	A*	A*	C	C	B	A*	4*	50%	**Moderate**
Read	B*	A*	A*	A*	N/A	B*	B	B	5*	50%	**Moderate**
Corbett	A*	B	A*	A*	B	C	B	B	3*	37%	**Low**
Drummond	B*	C	A*	A*	A*	A*	A*	A*	7*	87%	**High**
Onyedum	C	C	C	A*	N/A	B*	B	A*	3*	37%	**Low**
Gounder	C	B	A*	A*	C	A*	B	A*	4*	50%	**Moderate**
Crothers	B*	C	C	A*	A*	A*	A*	A*	6*	75%	**High**
Fitzpatrick	B*	C	A*	A*	A*	A*	A*	A*	7*	87%	**High**
Madeddu	B*	C	C	B	A*	A*	A*	A*	4*	50%	**Moderate**
Antwal	B*	C	A*	A*	C	A*	B	B	4*	50%	**Moderate**
Campo	B*	C	C	A*	A*	B	A*	A*	5*	62%	**Moderate**
Gingo	B*	C	A*	A*	A*	A*	A*	A*	7*	87%	**High**
Telisinghe	C	A*	A*	A*	B	C	B	B	3*	37%	**Low**

2.3.3 Characteristics of included studies and data available

All studies included were observational studies, although two papers investigated cohorts recruited as part of randomized trials.

The earliest study recruited patients from 1985 (152); the most recent provided data collected in 2012 (167). Eight articles reported research conducted in the USA (detailing 9 separate studies), with 12 studies conducted in Africa, 2 in Asia and 2 Europe (both from Italy). Nineteen studies included adult patients and 5 evaluated infants only.

The presence of cough and breathlessness were the most common symptoms evaluated: data on cough were presented by 17 of 19 studies in adults. Definitions of cough varied, with the most common being the presence of cough for >2 weeks. Frequency of breathlessness was reported by 11 studies. Usually this was only defined as the presence of “dyspnoea”, “breathlessness” or “shortness of breath”, though four studies (all evaluating adults with access to ART) used the MRC dyspnoea score as a standardised measure.

2.3.4 Studies assessing respiratory symptoms in HIV infected adults without access to ART

Twelve studies provided data regarding respiratory symptoms in HIV positive adults without access to ART (Tables 5 and 6). All of these demonstrated a greater proportion of HIV positive participants with respiratory symptoms. Five studies reported prevalence of breathlessness (2 from the USA, others from Nigeria, Zimbabwe and India).

Nine studies reported the prevalence of respiratory symptoms in HIV positive and negative individuals without access to ART in resource-limited settings, 7 of these were in Sub-Saharan African populations (Table 5). The earliest of these used data from Rwanda collected in 1988, the most recent was from Antwal et al in 2014 (169). Only 2 studies evaluated populations outside of the USA and Africa (Kheaw-on *et al* in Cambodia and Antwal *et al* in India) (162).

Three studies provided data regarding respiratory symptoms in HIV positive populations in the first decade of the HIV epidemic in the USA. The Multicentre AIDS Cohort study collected data from a cohort of men who have sex with men at 4 sites from 1985 onwards; el Sadr *et al* studied a cohort of injection drug users in New York and Diaz *et al* evaluated a cohort of predominantly white men in Ohio (151, 158). Data on respiratory symptoms in these populations are presented in Table 6.

TABLE 5 RESPIRATORY SYMPTOMS IN RESOURCE-LIMITED SETTINGS WITHOUT ACCESS TO ART

1st Author	Year of publication	Study location	Total N	% HIV +	HIV + on ART (%)	Mean / median blood CD4*	% HIV+ Smokers	% HIV- smokers	Definition of cough used	% HIV+ with cough	% HIV- with cough	Definition of breathlessness	% HIV+ with breathlessness	% HIV- with breathlessness
Lepage (150)	1991	Rwanda	431	50%	0	24% CD4:CD8 <0.5	NR	NR	"Persistent cough > 1 month"	14%	7%			
Norrgren (153)	1998	Guinea-Bissau	2215	9%	0	715 (men) 430 (women)	NR	NR	"Cough > 1 month"	3%	1%			
Nilses (156)	2000	Zimbabwe	1213	21%	0	NS	NR	NR	"Cough"	4%	2%	"Dyspnoea"	1%	1%
Kheaw-On (162)	2009	Thailand	210	50%	NR	261	36%	15%	"History of chronic cough"	57%	31%			
Lewis (160)	2009	South Africa	1955	29%	NR	NR	NR	NR	"New or worsening cough, any duration	4%	2%			
									> 2 weeks	2%	1%			
									>3 weeks"	2%	1%			
Corbett (163)	2010	Zimbabwe	8979	21%	NR	NR	NR	NR	"Cough for 2 weeks or more"	8%	2%			
Onyedum (170)	2010	Nigeria	200	50%	0	71% CD4 <200	0	0	"Cough"	48%	8%	"Breathlessness"	32%	0%
Gounder (165)	2011	South Africa	3937	37%	NR	333	NR	NR	"Cough for 2 weeks or longer"	7%	4%			
Antwal (169)	2014	India	1546	35%	0	241	NR	NR	"Cough with sputum"	10%	2%	"Breathlessness"	4%	1%

TABLE 6: RESPIRATORY SYMPTOMS IN RESOURCE-RICH SETTINGS WITHOUT ACCESS TO ART

1st Author	Year	Study location	Total number of participants	% HIV +	Risk for HIV	HIV+ on ART (%)	Mean / median blood CD4*	% HIV+ Smokers	% HIV- smokers	Definition of cough used	% HIV+ with cough	% HIV- with cough	Definition of breathlessness	% HIV+ with breathlessness	% HIV- with breathlessness
el Sadr (151)	1992	USA	223	56%	IDU	0	200-500	NR	NR	"Persistent cough"	17%	7%			
Hoover visit 3 (152)	1993	USA	2854	33%	MSM	0	619	NR	NR	"New or unusual cough lasting > 2 weeks"	5%	2%	"persistent shortness of breath"	1%	2%
Hoover - visit 7	1993	USA	2627	29%	MSM	0	552	NR	NR		5%	2%		3%	2%
Diaz (158)	2003	USA	379	85%	MSM	0	370	54%	50%	"Do you usually have a cough?"	40%	25%	"are you troubled by shortness of breath when hurrying on the level or walking up a slight hill?"	42%	8%
										"Do you usually cough like this on most days For ≥ 3 consecutive months of the year?"	24%	12%	"Do you have to walk slower than most people of your own age on the level ground because of breathlessness ?"	20%	0%

2.3.5 Studies that examined symptoms in HIV positive children

Five studies reported the prevalence of respiratory symptoms in HIV positive and negative children (Table 7). All these studies evaluated cohorts of vertically infected infants followed up from birth, and no studies assessed older children. The earliest was conducted in Rwanda from 1988 with subsequent work in Malawi and Zimbabwe. None of these study populations had access to ART.

One study evaluated symptoms in HIV positive infants born in Chieti, Italy; this suggested a *lower* frequency of “wheezy respiratory illness” in HIV infected infants despite more maternal smoking, premature delivery, low birth rate and formula feeding. However, the design of the study (based on routine clinic records) did not allow appraisal of the possibility of differences in health-seeking behaviour or reporting of acute respiratory illness which could bias the comparison of respiratory symptoms between the different groups.

TABLE 7: STUDIES EVALUATING RESPIRATORY SYMPTOMS IN HIV POSITIVE AND NEGATIVE CHILDREN

1st Author	Year of publication	Study location	Total Number of participants	% HIV+	% HIV+s on ART	Duration of follow-up (from birth)	Respiratory data collected	Notes / comments
Spira	1999	Rwanda	401	54%	0%	Median 27 months (HIV positive group), 51 months (HIV negative group)	Chronic cough (>14 days)	Measured cumulative incidence of developing a cough lasting >14 days.
Olayinka	1999	Zimbabwe	272	45%	0%	24 months	Persistent cough	
Taha	2000	Malawi	689	19%	0%	Median 18 months	Cough since last review	2 HIV negative groups - exposed uninfected infants and unexposed. Rate of cough the same in each.
Galli	2003	Italy	280	75%	25%	24 months	History of wheezy illness collected at clinic appointments	Presents proportion of infants with wheezy illness in 1st 2 years of life
Read	2009	Malawi, Tanzania, Zambia	1317	84%	0%	12 months	Retraction of intercostal muscles, wheezes, cough, active nasal flaring or rales at follow up visits	HPTN 024 study cohort, studied 2001-4

2.3.6 Studies that examined respiratory symptoms in HIV positive adults with access to antiretroviral therapy

Six studies (using data from 7 cohorts) reported on adults with access to ART. All were from resource-rich settings other than one from South Africa. Data on respiratory symptoms in these populations is presented in tables 8 and 9.

All contained a high proportion of current smokers in both the HIV positive and negative groups and, where reported, often had high rates of former or ongoing recreational drug use.

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TABLE 8: STUDIES FROM RESOURCE-RICH SETTINGS WITH ACCESS TO ART

Author	Year	Location	Total number of participants	% HIV+	HIV+ on ART (%)	% undetectable HIV plasma load	Mean / median blood CD4	% HIV+ smokers	% HIV- smokers	Definition of cough	% HIV+ with cough	% HIV- with cough	Definition of breathlessness	% HIV+ with breathlessness	% HIV - with breathlessness
Drummond (62) ALIVE cohort	2010	USA	974	30%	54%	16.5%	320	83%	86%	"Cough present"	3%	29%	MRC Dyspnoea score ≥ 2	35%	28%
										"Morning cough ≥ 4 days per week"	19%	19%			
										"Morning cough ≥3 months"	15%	15%			
Crothers (94) Lung HIV Study	2013	USA	589	51%	89%	84%	493	47%	35%	"Unusual cough"	28%	20%	MRC Dyspnoea score ≥ 2	15%	11%
Fitzpatrick (92) WIHS	2013	USA	99	64%	81%	NS	426	46%	50%				Dyspnea	35%	31%
Madeddu (67)	2013	Italy	176	63%	78%	71%	541	57%	58%	"Cough"	32%	14%	MRC Dyspnoea score ≥ 2	30.6%	15.4%
Campo (167) EXHALE sub-study, VACS cohort	2014	USA	340	53%	89%	NS	431	64%	58%	"Chronic cough"	24%	15%	MRC Dyspnoea score ≥ 2	25%	16%
Gingo (25) **MACS**	2014	USA	1896	48%	77%	70%	572	31%	23%	"Cough"	42%	38%	Dyspnoea	14%	9%
Gingo – WIHS	2014	USA	1976	71%	76%	56%	502	39%	52%	"Cough"	54%	58%	Dyspnea	36%	37%

2.3.7 Studies that reported respiratory symptoms in adults in resource-rich settings

The first systematic comparison of respiratory symptoms in an HIV positive population with access to ART with HIV negative comparators is provided by the AIDS Linked to Intravenous Experience (ALIVE) cohort of former and current injection drug users in Baltimore, USA.(62) This found no difference in frequency of current cough, although a history of "wheezing" was reported by 50% of HIV positive and 37% of HIV negative participants.

Several cohort studies have addressed respiratory symptoms in HIV infected individuals with access to ART in the USA: the Women's Interagency HIV Study (WIHS); Multi-Centre AIDS Cohort Study (MACS) and the EXHALE sub-study of the Veterans Aging Cohort Study. Tobacco use was high in these cohorts, and smoking was more frequent in the HIV positive than HIV negative groups (reported in 39-64% and 23-58% respectively). The prevalence of respiratory symptoms was high in these populations: for instance Fitzpatrick *et al* described breathlessness in 35% of HIV positive and 31% HIV negative participants in the WIHS study and Campo et al reported cough in 25% of HIV positive and 16% in HIV negative participants in the EXHALE VACS sub-study (92, 167).

There is little published information comparing respiratory symptoms in HIV negative and HIV positive individuals with access to ART outside of North America. The only study providing data from Europe documented the presence of any respiratory symptom in 47% HIV positive participants and 23% of HIV negative participants, with 32 vs 14% reporting cough (p=0.006) and 31 vs 15% breathlessness (p=0.02) (67).

The prevalence of tobacco smoking was only consistently reported in studies undertaken in resource-rich settings with access to ART. In all these studies a large proportion of both HIV positive and negative participants were current smokers.

2.3.8 Data from resource-limited settings with access to ART

In the only data published from resource limited settings with access to ART at the time of searching, Telisinghe *et al* evaluated a prison population in South Africa (with a 25% HIV prevalence) in which cough was present in 25% of HIV positive and 19% of HIV negative individuals (168). 36% of the HIV positive participants reported the use of ART.

TABLE 9: STUDIES EVALUATING RESOURCE-POOR SETTINGS WITH ACCESS TO ART

Author	Year	Study location	Total number of participants	% HIV+	HIV+ on ART (%)	% undetectable HIV plasma load	Mean / median blood CD4	% HIV+ smokers	% HIV- smokers	Definition of cough	% HIV + with cough	% HIV - with cough
Telisinghe (168) Prison cohort	2014	South Africa	846	25%	41%	NS	NS	59%	59%	"Cough > 2 weeks"	13%	8%

2.3.9 Quantitative data synthesis

In total, from the 19 studies evaluating HIV positive adults, data were available on 12,075 HIV positive individuals compared with 24,450 individuals without HIV infection. The symptoms for which sufficient data were available to allow quantitative synthesis were cough and breathlessness. Figures 5 and 6 demonstrate the odds ratios for the presence of cough and breathlessness in those with and without access to ART, and in resource rich and resource limited settings.

Meta-analysis suggested that the availability of ART has been associated with a reduction in the difference in prevalence of respiratory symptoms between HIV positive and negative individuals, yet these symptoms remained more common in people living with HIV. There was little evidence regarding respiratory symptoms in HIV positive populations in resource limited settings. The pooled odds ratio (OR) for the presence of cough in resource limited populations without access to ART was 3.05 (95% CI 2.24-4.16, I^2= 68%); in resource-rich populations without access to ART this was 2.18 (1.55-3.04 I^2 = 0%); and in those from resource-rich settings with access to ART the odds ratio for the presence of cough was 1.26 (0.97-1.63). There was, however, a substantial degree of heterogeneity in this analysis (I^2= 74%). Only one study reported frequency of cough in a high prevalence setting with access to ART.

FIGURE 4 META-ANALYSIS OF ODDS RATIOS FOR PRESENCE OF COUGH, STRATIFIED BY AVAILABILITY OF ART AND LOCATION

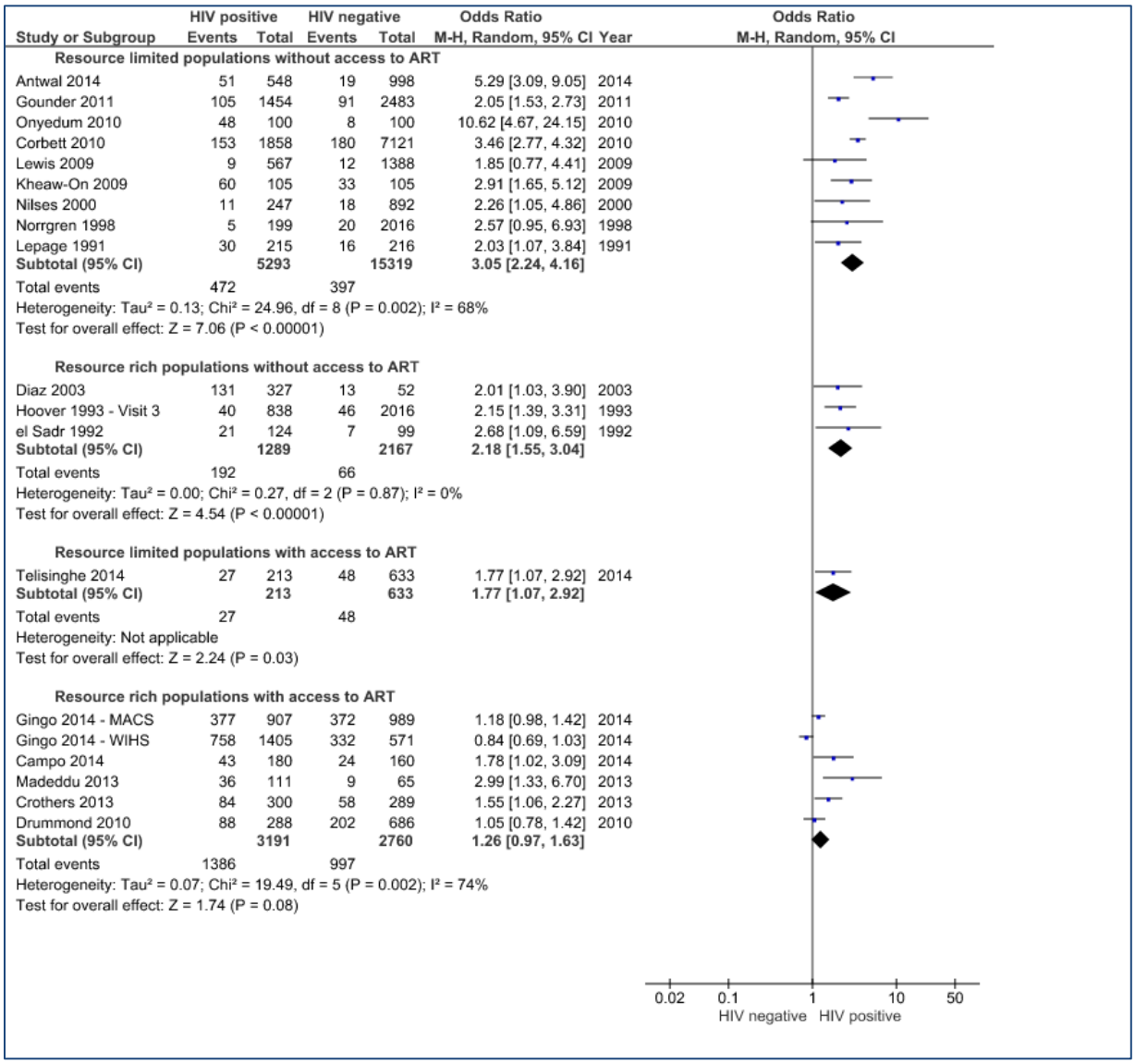
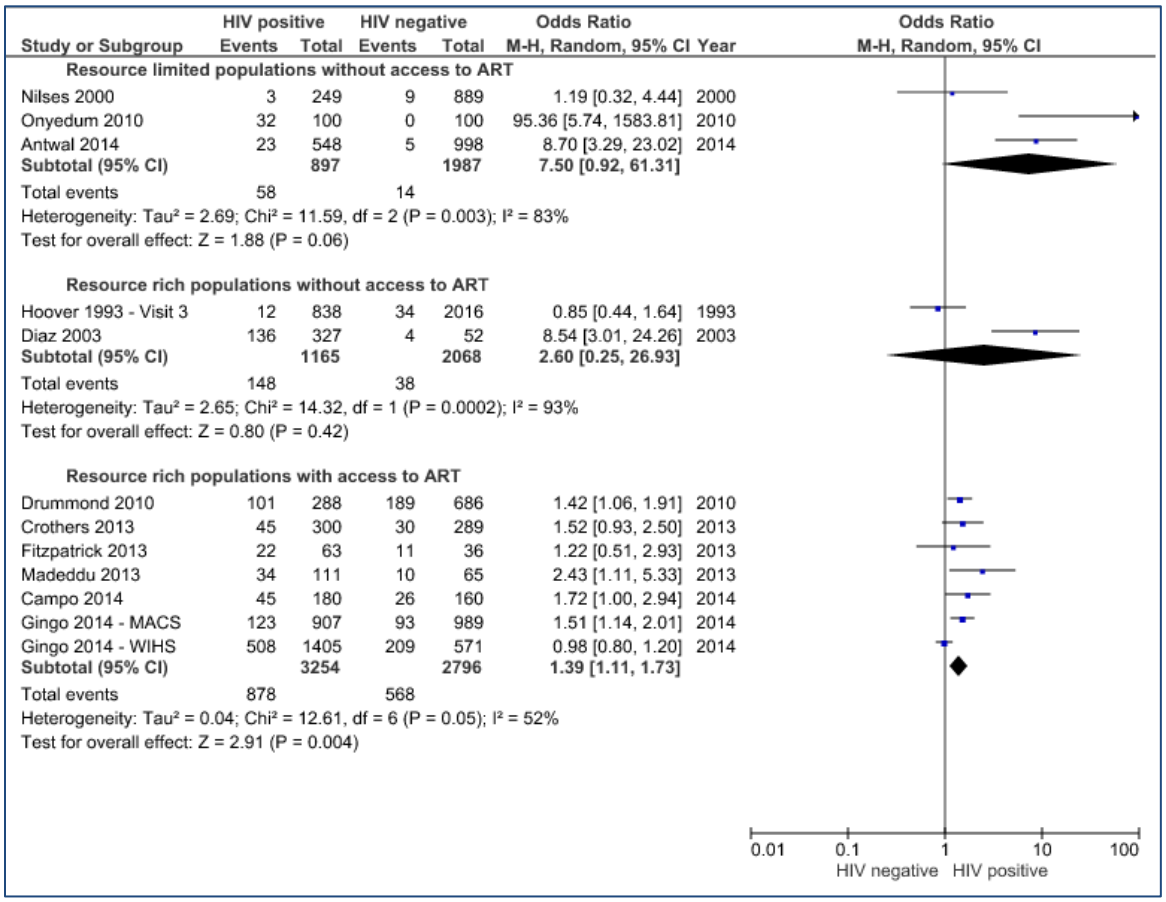


FIGURE 5 META-ANALYSIS OF STUDIES EVALUATING PREVALENCE OF BREATHLESSNESS, STRATIFIED BY LOCATION AND ACCESS TO ANTIRETROVIRAL THERAPY



Breathlessness was also assessed by quantitative meta-analysis, although fewer studies reported this. In resource limited settings without access to ART the OR for the presence of breathlessness was 7.5 (0.92-61.31, I^2 = 83%). Only 2 studies were available from resource-rich settings without access to ART, and meta-analysis of these data provides an OR of 2.60 (0.25-26.93, I^2 = 93%). In resource-rich settings with access to ART there was evidence for a higher frequency of breathlessness in HIV positive individuals with an OR of 1.39 (1.11-1.73, I^2 52%, p=0.004).

Funnel plots were inspected to evaluate the possibility of reporting or publication bias where there were enough studies within each stratum of ART availability and location to make this useful. For the most part these did not suggest the presence of significant reporting bias (e.g. Figure 7) although this was possible for the apparent association between breathlessness in HIV positive populations with access to ART (Figure 8). As there were fewer than 10 studies within each stratum, we did not use quantitative tests of reporting bias, in accordance with the Cochrane Handbook guidance.

FIGURE 6 FUNNEL PLOT, ODDS RATIO FOR COUGH, RESOURCE-LIMITED POPULATIONS WITHOUT ACCESS TO ART

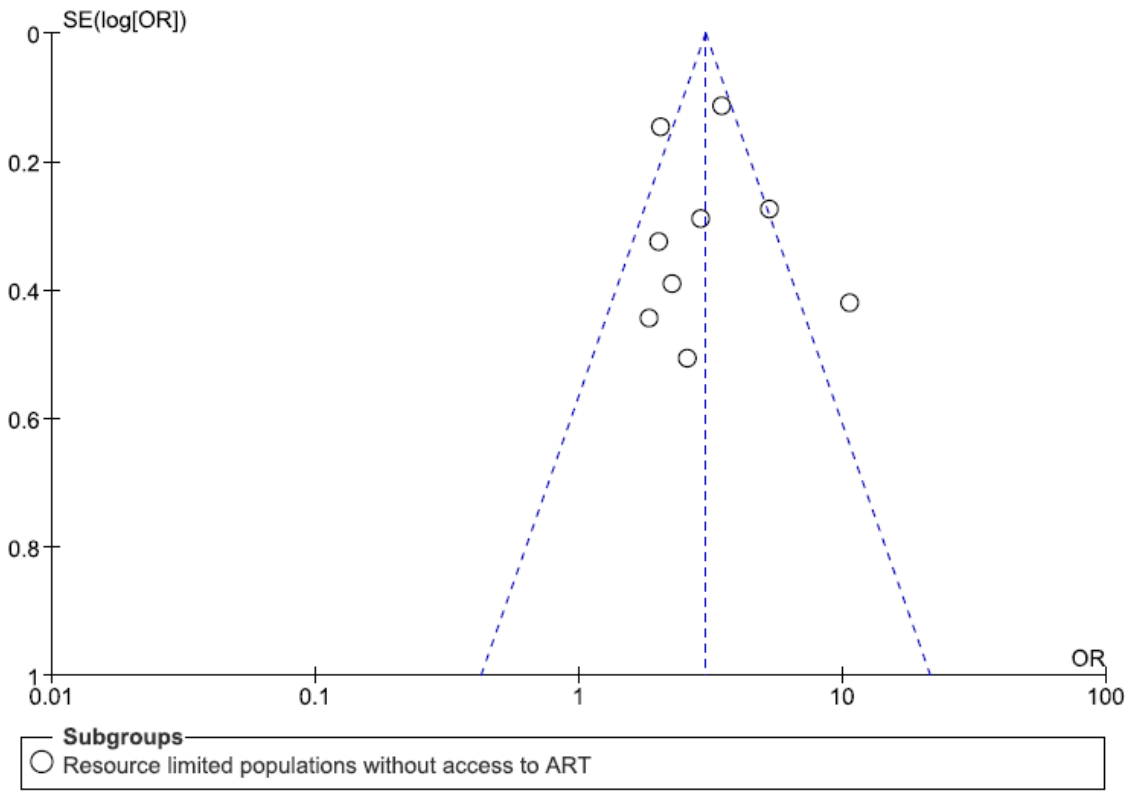
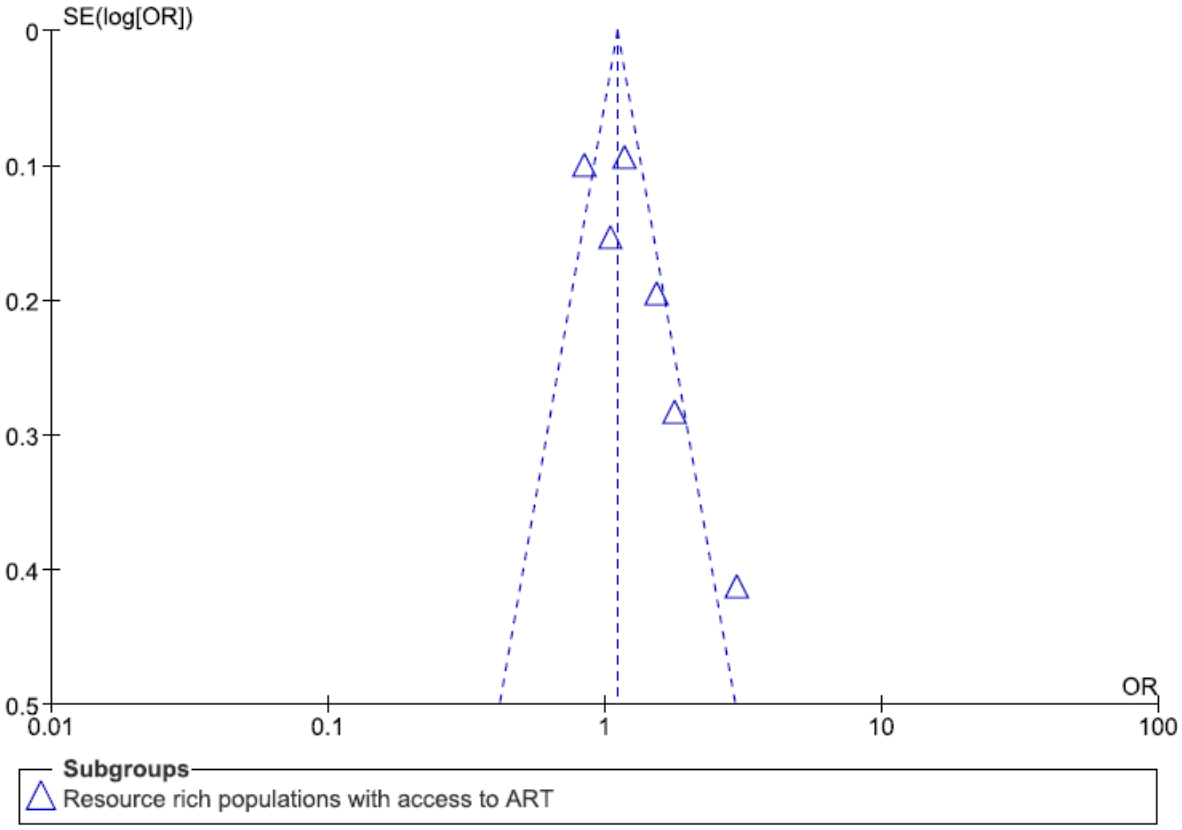


Figure 7 Funnel Plot: odds ratio for breathlessness in populations with access to ART

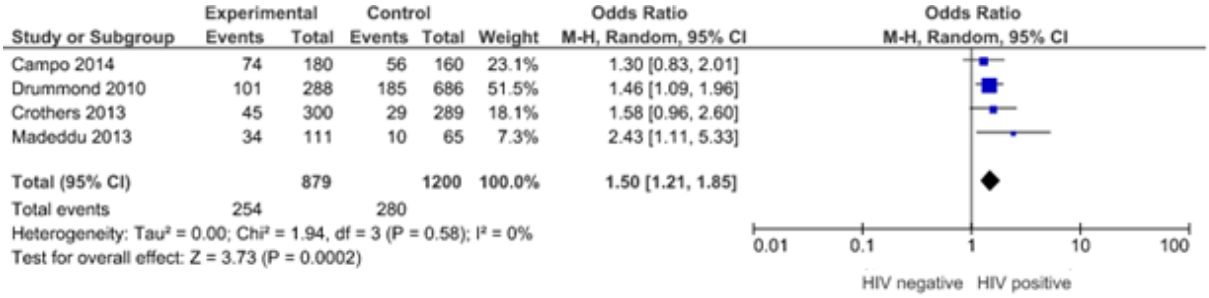


2.3.10 Sensitivity analyses

Two studies (Onyedum 2010 and Antwal 2014) included in the analysis of respiratory symptoms in populations without access to ART were judged to be at high risk of bias due to the inclusion of significant numbers of participants presenting for care with acute respiratory illnesses due to their methodology (which involved recruitment within acute care services). Exclusion of these studies reduced somewhat the effect size for the prevalence of cough to an OR of 2.84 (2.45-3.30) and reduced the statistical heterogeneity in this analysis (I^2 = 55%).

Four studies (all in populations with access to ART) used the MRC dyspnoea scale to measure breathlessness (see Table 2.7). A meta-analysis including only studies that used the MRC dyspnea scale (with breathlessness defined as a score of ≥2) did not significantly change the effect size (compared to the analysis of all studies reporting the prevalence of breathlessness in populations with access to ART) but reduced heterogeneity significantly (OR for having MRC dyspnoea ≥2 = 1.50, 95% CI 1.21-1.85, I^2= 0%, Figure 2.7).

FIGURE 8 META-ANALYSIS OF STUDIES IN RESOURCE-RICH SETTINGS USING MRC DYSPNEA SCORE TO EVALUATE BREATHLESSNESS IN HIV POSITIVE AND NEGATIVE POPULATIONS



2.4 Discussion

This systematic review and meta-analysis suggests that despite the availability of ART, HIV positive populations continue to experience more respiratory symptoms than comparable HIV negative groups, at least in resource-rich settings for which there are sufficient data to draw conclusions. It also highlights several research needs: in particular the lack of rigorous data concerning respiratory symptoms in HIV positive populations in low and middle-income settings where the majority of the world's HIV positive individuals live.

The successful provision of ART means that HIV positive populations are experiencing considerable improvements in life-expectancy and quality of life. This has been reflected in a reduction in the prevalence of respiratory symptoms in HIV positive populations. However, aging HIV infected populations will have an increasing frequency of non-AIDS co-morbidities and respiratory illness is likely to become increasingly important over time. The data summarised in this chapter demonstrate that the prevalence of respiratory symptoms among PLWH has reduced since ART has become available, although this also indicates that the evidence available is unevenly distributed, with little data from the resource-limited settings where most PLWH live. An improved understanding of the health needs of aging HIV positive populations is required, as even in resource-rich settings the impact of respiratory illness on individuals (measured by instruments designed to evaluate health related quality of life) or at a societal health-economic level has been infrequently determined in HIV positive populations.

In resource-rich settings, HIV positive populations often have greater cigarette and recreational drug use than the general population. Interventions to reduce this are therefore important, given that there is accumulating evidence of increased rates of both cardiovascular and respiratory illness. In some high HIV prevalence settings tobacco smoking is rising fast, with important implications for the future health of people living with HIV. The true effect of HIV status on respiratory symptoms may be exaggerated by the higher proportion of HIV positive subjects who were current smokers in several of the included studies, as few provided data adjusted for smoking status.

2.4.1 Strengths and limitations of this analysis

Strengths of this analysis include a comprehensive search strategy and robust methods for study selection. Limitations include the lack of standardized assessments of respiratory symptoms in the primary studies, heterogeneity of assessments used and incomplete reporting of important confounding factors (such as smoking and recreational drug use) which may influence the effect sizes found. Incomplete reporting of exposures such as tobacco smoking and drug use mean that the extent to which the association identified between HIV status and respiratory symptoms is a direct result of HIV is uncertain. A wide range of terms might be used to describe respiratory symptoms and use of more search terms, (for instance including upper respiratory tract symptoms such as sneezing, sore throat, blocked nose or pain in nose or face) might have yielded relevant studies regarding these symptoms. We decided not to include ear symptoms in the search terms, yet the middle ear is arguably part of the upper respiratory tract.

One difficulty when evaluating respiratory symptoms is the challenge of standardising assessments: The wide range in the frequency of respiratory symptoms found arises in part from the diversity of methods used to evaluate them: the definitions used for symptoms such as cough or breathlessness were often not stated, and only a minority of studies used standardised tools such as the St George's Respiratory Questionnaire (171), American Thoracic Society Division of Lung Disease questionnaire, or the MRC Dyspnoea scale (172) (see Tables 5 to 9) for details of data collected in each study). Furthermore, investigators usually presented results the proportion or number with respiratory symptoms, and no study reported estimates adjusted for potential confounders.

It is important to note that although populations may have access to ART, most studies included a significant proportion of participants who were either not using ART, or who do not have an undetectable HIV load. This meta-analysis did not include patient-level data and so could not evaluate the effect of HIV infection at an individual level in those with a favourable virological and immunological response to ART. Furthermore, most of the data regarding populations with access to ART derives from the USA and there is limited information from other settings. Exposures of importance that could lead to respiratory disease may differ in other locations, and the findings of the studies included in this review may not be generalizable - for instance to HIV positive African populations using ART.

2.5 Conclusions

This analysis suggests that despite the availability of antiretroviral therapy, HIV positive individuals remain more likely to experience respiratory symptoms than those without HIV. Although the impacts of these symptoms cannot be measured in the data available here, it is likely that these have significant consequences – this includes an impairment on the quality of life of the individuals affected, including on their mental health, and impacts on wider society such as loss of capacity for work and caring responsibilities. Furthermore, it may be that this greater prevalence of respiratory ill-health results in greater healthcare resource utilisation such as consultations with healthcare providers and episodes of hospitalisation.

Interventions are required to address this health need, which might include reducing the frequency of tobacco smoking among PLWH, greater uptake of immunisation against respiratory infections and an earlier use of antiretroviral therapy. However, as highlighted in this review, there is little available evidence regarding the determinants of respiratory illness in HIV positive individuals in resource limited settings, where the majority of the PLWH live.

Chapter 3: Respiratory symptoms, health status and smoking in UK HIV positive adults, a cross-sectional study

3.1 Background and rationale for study

As detailed in chapter 2, the currently available data suggest that respiratory symptoms are common among people living with HIV and continue to occur at a higher frequency than in HIV negative individuals despite access to antiretroviral therapy.

Few studies have examined respiratory health in PLWH in a way that enables adjustment for important confounding effects such as tobacco smoking. There is also little information from the UK. At the time of conducting this analysis, the only published data available regarding the prevalence of respiratory illness in the UK HIV positive population was a study by Dickson *et al* in abstract form that reported 15% of 133 HIV positive participants attending for HIV care in Brighton had evidence of airflow obstruction by spirometry (173). My hypothesis was that, based on existing data from other HIV positive populations, despite the use of antiretroviral therapy people living with HIV would continue to have a greater prevalence of respiratory symptoms, a greater proportion with obstructive lung disease and that tobacco smoking would be more prevalent among people living with HIV than HIV negative comparators. We sought to collect data that would allow us to explore the relationship between HIV and respiratory health, and to estimate the extent to which this relationship could be explained by confounding by tobacco smoking.

We therefore sought to evaluate the prevalence of respiratory illness in individuals attending ambulatory HIV care at the Royal Free London NHS Trust. In addition to the frequency of respiratory symptoms and spirometric impairment, we also explored tobacco smoking and use of recreational drug use, health-related quality of life and health beliefs regarding smoking and lung disease.

3.2 Methods

3.2.1 Study population

We conducted a cross-sectional observational study in the HIV ambulatory care service and Sexual Health Clinics (at Royal Free and Barnet Sites) of the Royal Free London NHS Trust, London, UK from February to July 2015. The recruitment of an appropriate HIV negative comparator group is an important consideration in the design of studies evaluating chronic ART-treated HIV infection. In many settings, including the UK, HIV positive populations have greater exposures to relevant risk factors (in particular tobacco smoking and recreational drug use) than the general population (174). Therefore, selection of a comparator population with similar risk factors is required if a study aims to evaluate the effect of HIV status. This, however, can be difficult to achieve, and a balance between recruitment of an ideal study population and the feasibility of completing recruitment is necessary.

The Royal Free London NHS Trust manages a relatively large patient cohort of around 3,500 PLWH. This clinic maintains a longitudinal database of patients, allowing this patient group to be compared to national data. Uptake of ART and levels of virological suppression is good, with around 89% of the clinic population having an undetectable HIV viral load. Demographic details of the clinic population are broadly similar to those of PLWH nationally, although a slightly greater proportion are male (74% compared to 69% nationally) (175).

We chose Sexual Health clinics as the site for recruitment of HIV negative participants as this population was anticipated to have similar lifestyle characteristics, including smoking behaviours, to the HIV clinic population. Clinics at Barnet Hospital (the Clare Simpson clinic) and the Royal Free Hospital (Marlborough clinic) were selected as sites for recruitment.

Consecutive clinic patients were invited to take part in the study when they attended routine care appointments. Subjects completed written informed consent. As service users in the Sexual Health clinics were significantly younger than those attending HIV care, recruitment in these clinics was restricted to those over the age of 35 to achieve a sample which approximated the age of the HIV positive participants. There were no exclusion criteria for the HIV positive group. If individuals declined to participate in the study this choice was noted; we did not record any further details about individuals who declined to participate.

Participants were given a written information sheet detailing the study and provided written consent to participate; if they preferred, participants could take part in the study anonymously, or decline consent to access hospital medical records.

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3.2.2 Contributions to this work

Recruitment of participants for this study was conducted by me with the assistance of Dr Jennifer McGowan. Additional data were collected for a collaborative project concerning psychological resilience among PLWH, the findings of which have been published separately and do not form part of this thesis (176). In addition to input from my primary supervisor for this project, further advice and support was provided by Dr Alison Rodger and Dr Fiona Lampe and I am grateful for their invaluable input. Advice regarding statistical analyses was provided by Dr Colette Smith; I completed all analyses presented here.

3.2.3 Research Ethics Committee approval

Ethical approval for this study was granted by the London – Camden and Islington Research Ethics Committee (14/LO/1646) on the 17th October 2014.

3.2.4 Procedures

Participants completed a questionnaire including items on risk factors for respiratory illness, smoking and recreational drug use and health related quality of life and mental health (depression and anxiety - the questionnaire used is provided as appendices 1 and 2). Where possible we utilised standard instruments to assess patient-reported outcomes. In addition to providing validated measures, this approach allows comparison between data arising from this study and those conducted in other populations. Respiratory health status was measured using the St George's Respiratory Questionnaire (SGRQ), and the MRC dyspnoea (breathlessness) scale, a 1-5 scale. Anxiety and depression were measured using the PHQ-9 score. The questionnaires / scales used in this study are described in more detail in Table 10.

The questionnaire was developed by reviewing the literature regarding instruments that could be used to evaluate respiratory health and to measure important confounding effects such as tobacco smoking and recreational drug use. Ideally, we wished to find instruments that measure respiratory health as a whole, rather than being specific for a particular condition such as asthma or COPD. Many validated disease-specific symptom and health status measures are available, such as the Asthma Control Questionnaire (ACQ) or COPD Assessment Tool (CAT),(177, 178) but these are not appropriate for use in individuals without these conditions. We therefore chose the SGRQ as the best available existing validated tool to evaluate respiratory health. It should be appreciated, however, that this was originally developed in the setting of COPD and is largely focussed on symptoms of obstructive airways disease. It is therefore by no means perfect for this objective and represented a compromise between measuring the symptoms and health impacts that we wanted to capture and the use of a validated and widely used instrument. The questionnaire once developed was piloted in a small number of individuals from the HIV positive population under study, to assess the feasibility of completing the questionnaire and time required to do so. There was no formal validation process for the questions specifically created for the questionnaire as these were not intended to be used as part of a quantified score or instrument, but rather to capture data on confounding variables such as smoking.

In addition to the respiratory-specific questionnaires we also included a generic health related quality of life instrument. We chose the Euro-Qol 5D-5L score – this is a simple and well characterised scoring system that evaluates general quality of life using 5 domains. There is extensive literature on its performance, and it is widely used in research and assessments, in particular within health-economic evaluations (where it is the basis for calculation of Quality-Adjusted Life Years, QALYs).

As differences in mental health could affect the relationship between HIV status and respiratory health, we also measured symptoms of depression, using the PHQ-9 score(179)

TABLE 10 QUESTIONNAIRES / SCALES USED IN THIS STUDY

Instrument / assessment	Description / details
St Georges Respiratory Questionnaire (SGRQ)(171)	The SGRQ is a patient reported outcome measure that aims to quantify respiratory health status using a 50 item self-completed questionnaire. This evaluates not only symptoms but the impact on activities and quality of life. Responses are translated onto a 0-100 scale in which higher scores indicate worse respiratory health status. Individual domains assess Symptoms, Activities and Impacts as well as a Total score. Although initially developed for use in asthma and Chronic Obstructive Pulmonary Disease (COPD) it has been widely used in other respiratory conditions and can be considered to evaluate general respiratory health status, rather than being disease-specific. The SGRQ has been shown to produce valid results both when used to discriminate between patients and to evaluate changes in condition within patients at different time points. The minimum clinically important difference (MCID) in the SGRQ (assessed by a variety of methods) has consistently been reported to be 4 points (180). The SGRQ is therefore sensitive to changes in patient condition (and hence is frequently used as an outcome measure in clinical trials).
MRC dyspnoea scale(172)	The MRC dyspnoea (breathlessness) scale is a simple and widely used measure of breathlessness which uses a simple 5-point scale and has been used in respiratory research and epidemiology since the 1950s. It reports breathlessness related to activity or perceived respiratory disability rather than being a measure of the symptom of breathlessness its self (as is quantified by, for instance the Borg scale). Although having the advantage of simplicity and face-validity, it is relatively insensitive to change, and therefore less useful in evaluating effects of treatment or longitudinal change over time.
PHQ-9(181)	The PHQ-9 is a self-completed, 9 item questionnaire, with each symptom question scored from 0 “not at all” to 3 “nearly every day”, giving a total score out of 27. It has been shown to be valid for the assessment of depression, with a score of 10 or more having a sensitivity and specificity of 88% when compared to a structured mental health professional interview (179, 181)
EuroQoL 5D-5L(182)	The EuroQol 5D (EQ5D) seeks to measure general (rather than disease or organ specific) health status. It consists of a descriptive system of five dimensions: mobility, self-care, usual activities, pain / discomfort and anxiety/ depression. In the “5L” form each of these is described in 5 possible levels, from “no problems” to “extreme problems”. These answers can then be combined to give a 5 digit “health state”, and there are therefore a total of 1325 possible health states described by different combinations of responses to the EQ5D-5L questions. In addition, there is a visual analogue scale in which individuals are asked to rate their health status on a 0-100 scale from ‘The worst health you can imagine’ to ‘The best health you can imagine’. The characteristics of the EQ5D have been extensively evaluated, in particular for use in health-economic analyses, where its ability to allow comparisons between health-states in different conditions is useful. As well as providing the description of each of the five dimensions, “index values” have been calculated for each of the possible health states. As preference for different health states may differ between populations, these are population-specific, and derived from population-based studies.

3.2.5 Spirometry

We performed spirometry according to standard procedures using a Carevision Micro I spirometer (Carevision, San Diego, USA). We gave subjects verbal instruction in the technique, including what the test entails and how they may feel during and after the test. Participants performed spirometry a minimum of 3 times, with appropriate single use one-way filter. Subjects underwent spirometry without bronchodilation and had their height and weight measured to allow calculation of normal values and body mass index.

FEV1, FVC and peak flow, plus quality of results were recorded at each attempt. All attempts were recorded and the best used as study measure. If an unexpected abnormal result were obtained this was highlighted to the participant and they were advised to discuss this with their General Practitioner.

We used the Global Lung Function Initiative equations to provide normal values for spirometry. (183)

3.2.6 Other data collected with the questionnaire.

In addition to the standardised questionnaires (such as the EQ5D) used in the questionnaire, further information was collected regarding:

1. General demographic information (gender, age, ethnicity, sexual orientation, educational attainment, employment)
2. Comorbid conditions and medications
3. Smoking and recreational drug use, including (for smokers) beliefs about quitting smoking
4. Health beliefs and perceptions regarding respiratory disease and smoking.

The questionnaire used for this study is provided in the Appendix.

3.2.7 Results of blood tests

No blood samples were obtained from participants for this study. However, as HIV-related factors (such as current or nadir CD4 count) could potentially influence outcomes of the study, we sought specific consent from participants to access medical records. 168 of 197 (85%) HIV positive participants consented to this.

As blood samples were not taken for study purposes and Sexual Health records are stored in pseudo-anonymised format using clinic numbers (to which we did not seek consent to access), we relied on self-reported HIV status for HIV negative participants in this study, and this was not independently confirmed.

3.2.8 Statistical analysis

I compared participant characteristics and results using Chi Squared and Fisher's exact tests for categorical values, with t tests, and Mann Whitney tests for continuous variables as appropriate. Multivariable regression models were used to evaluate the relationship between participant characteristics and results. For the analysis of St Georges Respiratory Questionnaire results, these values were log-transformed to approximate a normal distribution and linear regression models used, with the resulting beta-coefficients being back transformed for presentation as adjusted fold-changes in SGRQ score. For analysis of MRC dyspnoea scale scores, these results were dichotomised into values of 1, or 2 or more and logistic regression models used to evaluate the odds of having a score of 2 or more.

3.2.9 Calculation of required sample size

The number of subjects required for this study was originally calculated to evaluate the difference in smoking rates between those with and without HIV infection as the primary outcome. Data on expected smoking rates have been obtained from national surveys of smoking to estimate smoking rates in the sexual health clinic and existing data on smoking in the HIV clinic at the Royal Free Hospital collected as part of routine clinical care. Prior to the study we expected that the prevalence of current smoking in the sexual health clinic would be around 25%, and that around 40% of the HIV clinic population would be current smokers. To have an 80% power to detect this difference (i.e. 25% vs. 40%) with a type 1 error of 5% we would need 165 individuals in the HIV infected and uninfected groups, giving a target for recruitment of 330 participants.

Although we originally intended to have equal numbers of cases and comparators, recruitment from the Sexual Health clinic proved to be slower than anticipated, and during completion of the study we decided to aim for a 2:1 case-to-control ratio as a pragmatic means of achieving the required recruitment target. The greater number of HIV positive individuals also allowed exploration of subgroups and characteristics specific to the HIV positive group (such as CD4 count and duration of HIV infection).

3.2.10 Data Management

Data from the participant-completed questionnaires were entered into spreadsheets by myself and Dr Jennifer McGowan, in the case of the SGRQ utilising the scoring spreadsheet provided by the developers of the questionnaire (http://www.healthstatus.sgul.ac.uk/sgrq). Results were entered into this database and then data-entry double-checked by a second researcher. A final master dataset was stored, identified by participant number, in secure hospital computer systems. Pseudo-anonymised data were analysed using the SPSS software system for statistical analysis.

3.3 Study Results

3.3.1 Participant recruitment

We invited 402 individuals to participate in the study, of whom 290 (72%) agreed: 197 HIV positive and 93 HIV negative. The response rate was 75% amongst HIV positive and 73% amongst HIV negative individuals (though recruitment of HIV negative individuals was lower as fewer eligible individuals attended these clinics during the recruitment times). We did not record details regarding participants who declined to take part.

3.3.2 Details of study participants

The median CD4 count of HIV positive participants was 627 cells/µL (IQR 456-838); 171 (94%) of PLWH reported using ART with a median duration of treatment of 7 years. 89% of all participants and 93% of those using ART had an undetectable HIV load (<40 copies/ml) at their last clinic visit. The median nadir CD4 count of study participants was 250 cells/µL (122-365). Eighty-nine percent of all PLWH and 93% of those using ART had an undetectable plasma HIV load. No significant differences were found in gender, educational attainment or being non-UK born between HIV positive and negative participants, but PLWH were more often of White ethnicity (72% vs 60%, p=0.001). HIV negative participants had a lower median age (43 vs 50 years, p=0.05) and were more likely to be heterosexual (71% vs 32%, p<0.01) than those recruited from the HIV ambulatory care clinic. Details of participant characteristics are provided in Table 11 and 12.

The HIV positive and negative groups had a similar reported prevalence of a range of physical comorbidities (asthma, COPD, diabetes, heart disease or stroke). Symptoms of depression were more common in the HIV positive group (thirty-nine (20%) of the HIV positive and 13 (14%) of the HIV negative group had PHQ-9 scores of 10 or more) although this difference did not reach statistical significance (p=0.25). When analysed as a continuous variable, PHQ-9 scores were higher among HIV positive participants with median scores of 4 (IQR 1-8) vs 2 (0-6) points (p=0.002).

3.3.3 Smoking and recreational drug use

Sixty (30%) HIV positive and 31 (33%) HIV negative participants were current smokers; 54 (28%) and 22 (25%) respectively were ex-smokers (Table 12). In smokers, PLWH reported more intensive smoking, with a median of 15 (IQR 8-20) vs 10 (5-13) cigarettes per day for current smokers ($p<0.001$).

Past recreational drug use was more often reported in those with HIV infection, with 60% indicating drug use ever compared to 48% of HIV negative participants ($p=0.05$). No significant differences were found in the proportion of participants indicating any recreational drug use in the past 3 months.

TABLE 11 COMPARISON OF THE DEMOGRAPHIC DIFFERENCES BETWEEN HIV POSITIVE AND NEGATIVE PARTICIPANTS

		HIV positive n=197	HIV negative n=93	P value
Gender				
Male, n (%)		158 (80%)	64 (71%)	0.09*
Age, years, median (IQR)		50 (42-55)	43 (38-52)	0.025#
BMI, kg/m^2, mean (SD)		25.84 (5.04)	25.36 (4.23)	0.434×
Race /ethnicity				
	White n (%)	143 (72%)	54 (60%)	**0.003**□
	Black n (%)	37 (20%)	15 (17%)	
	Other n (%)	15 (8%)	24 (26%)	
Born in UK, n %		121 (62%)	49 (54%)	0.24*
Gender / Sexuality	MSM	131 (66%)	25 (27%)	<0.001□
	MSW	27 (14%)	37 (40%)	
	Female	37 (20%)	24 (28%)	
	Not stated	0	5 (5%)	
Highest educational attainment	None n (%)	24 (12%)	10 (12%)	
	GCSE or equivalent, n (%)	28 (14%)	7 (8%)	0.64□
	A level or equivalent, n (%)	31 (16%)	14 (16%)	
	University degree or higher, n (%)	106 (54%)	50 (59%)	
	Other, n (%)	6 (3%)	4 (5%)	
Employment	Full-time, n (%)	92 (47%)	59 (67%)	
	Part-time, n (%)	22 (11%)	11 (12%)	0.015□
	Unemployed, n (%)	25 (13%)	5 (6%)	
	Retired, n (%)	18 (9%)	4 (4%)	
	Student, n (%)	5 (3%)	1 (1%)	
	Not working due to ill health, n (%)	28 (14%)	4 (5%)	
	Other, n (%)	6 (3%)	5 (5%)	

*** Chi square test** **# Mann Whitney U test** □ Fishers exact test × Independent samples T-test

3.3.4 Spirometry, respiratory symptoms and health-related quality of life

Spirometry was within normal limits in most people: 18 (11%) HIV positive and 7 (9%) of HIV negative participants had evidence of airflow obstruction (FEV1/FVC <0.7), (p=0.55).

The MRC dyspnoea and St Georges Respiratory Questionnaire (SGRQ) scores suggested a higher prevalence of breathlessness and respiratory health status impairment in PLWH (Table 14). SGRQ scores were higher in the HIV positive group for all domains, with median SGRQ Total scores of 12 in the PLWH and 6 in HIV negative participants (p <0.01). Breathlessness was also more common in the HIV positive group, with 47% having an MRC dyspnoea score ≥2 (on a 1-5 scale) suggesting at least moderate breathlessness compared to 25% of the HIV negative participants (p=0.001); 13% of HIV positive vs 1% of HIV negative participants had MRC dyspnoea scores of 3 or more (p= 0.001).

There was no significant difference in general health-related quality of life scores between the HIV positive and negative groups with median EQ5D-5L (UK) index values of 0.88 and 0.85 (p=0.06) respectively and median VAS scores of 78 and 72 (p=0.46).

TABLE 12 COMPARISON OF COMORBIDITIES AND THEIR MANAGEMENT BETWEEN HIV POSITIVE AND NEGATIVE PARTICIPANTS

		HIV positive N=197	HIV negative N=93	P value
Diagnosis of comorbid conditions (self-report), ever.				
Asthma, n (%)		36 (18%)	21 (23%)	0.34□
COPD / emphysema, n (%)		9 (5%)	1 (1%)	0.18□
Cancer (any), n (%)		9 (5%)	3 (3%)	0.76□
Heart disease, n (%)		11 (6%)	4 (4%)	0.78□
Stroke, n (%)		3 (1%)	1 (1%)	1 □
Diabetes, n (%)		10 (5%)	6 (7%)	0.59□
Currently receiving treatment for depression, n (%)		40 (20%)	8 (9%)	0.02*
PHQ-9 score, median, IQR		4 (1-8)	2 (0-6)	0.002#
PHQ-9 score ≥10, n (%)		39 (20%)	13 (14%)	0.25□
Self-reported history of immunization against:				
Influenza in past 12 months, n (%)		138 (70%)	27 (30%)	<0.01□
Streptococcus pneumonia (ever), n (%)		50 (26%)	6 (7%)	<0.01□
Use of inhaled medications (any), n (%)		24 (12%)	15 (17%)	0.30*
Undertakes physical activity at least once per week		115 (59%)	63 (71%)	0.05*
History of acute respiratory illness in past year	sinusitis	23 (12%)	7 (8%)	0.41□
	bronchitis	6 (3%)	3 (3%)	1.0□
	chest infection	39 (20%)	11 (12%)	0.13□
	cold or flu serious enough to miss work or stop normal activities	54 (28%)	19 (21%)	0.31□
	pneumonia	3 (1.5%)	0	0.55□
	Any acute respiratory illness	93 (47%)	30 (32%)	0.02*

□ Fisher's exact test * Chi squared test # Mann Whitney U Test

TABLE 13 SMOKING AND RECREATIONAL DRUG USE

	HIV Positive N= 197	HIV negative N= 93	p value
Smoking			
Current smoker, n (%)	60 (30%)	31 (33%)	0.81□
Ex-smoker, n (%)	54(27%)	22 (24%)	
Never smoker, n (%)	80 (41%)	37 (40%)	
Not stated, n (%)	3 (1.5%)	3 (3%)	
Cigarettes smoked per day (current smokers only) median (IQR)	15(8-20)	10 (5-13)	<0.01#
Most cigarettes smoked per day in the past, median (IQR)	20 (15-30)	12.5 (7.5-20)	0.04#
Currently using electronic cigarettes	20 (18%)	8 (16%)	0.83*
History of recreational drugs use, ever.			
Any	118 (61%)	41 (48%)	0.07□
Cannabis, n (%)	98 (51%)	35 (41%)	0.15□
Cocaine (smoked), n (%)	23 (12%)	3 (3%)	0.03□
Cocaine (sniffed or rubbed in gums), n (%)	71 (37%)	21 (25%)	0.05□
Ecstasy / GHB / Ketamine / crystal meth, n (%)	72 (36%)	13 (14%)	<0.001□
Heroin smoked, n (%)	13 (7%)	1 (1%)	0.07□
Heroin injected, n (%)	7 (4%)	1 (1%)	0.44□
History of recreational drug use in last 3 months			
Any, n (%)	58 (30%)	18 (20.7%)	0.15□
Cannabis, n (%)	39 (20%)	14 (16.1%)	0.51□
Cocaine (smoked), n (%)	3 (1%)	0	0.55□
Cocaine (sniffed or rubbed in gums), n (%)	13 (7%)	8 (9%)	0.47□
Ecstasy / GHB / Ketamine / crystal meth, n (%)	22 (11%)	3 (3%)	0.025□
Heroin smoked, n (%)	3 (1%)	0	0.55□
Heroin injected, n (%)	0	0	-

□ Fisher's exact test * Chi squared test # Mann Whitney U Test

TABLE 14 RESPIRATORY SYMPTOMS AND HEALTH STATUS

	HIV positive (N=197)	HIV negative (N=93)	p-value
FEV_1, L, mean (SD),	3.49 (0.87)*	3.25 (0.76)*	0.56[Δ]
% predicted	93%	91%	
FVC, L mean (SD),	4.29 (1.05)*	3.95 (0.91)*	0.23[α]
% predicted	91%	89%	
FEV_1/FVC < 0.7, n (%)	18 (11%)*	7 (9%)*	0.50[□]
MRC dyspnoea scale			
1. Not troubled by breathlessness except on strenuous exercise, n (%)	99 (53%)	61 (75%)	0.02[□]
2. Short of breath when hurrying or walking up a slight hill, n (%)	62 (33%)	19 (24%)	
3. Walk slower than contemporaries on level ground or have to stop for breath when walking at your own pace, n (%)	8 (4%)	1 (1%)	
4. Stop for breath after walking about 100m or after a few minutes on level ground, n (%)	13 (7%)	0 (0%)	
5. Too breathless to leave the house, or breathless when dressing/undressing, n (%)	4 (2%)	0 (0%)	
St George's Respiratory Questionnaire			
Symptoms, median (IQR)	25 (7-48)	18 (0-29)	<0.01[#]
Activity, median (IQR)	17 (6-36)	12 (0-19)	<0.01[#]
Impacts, median (IQR)	5 (0-15)	0 (0-6)	< 0.01[#]
Total, median (IQR)	12 (6-25)	6 (2-14)	< 0.01[#]

*157 (80%) HIV positive and 74 (80%) HIV negative participants had acceptable spirometry results

[Δ] T-test; comparison of FEV % predicted. [α] T-test; comparison of FVC % predicted

3.3.5 Factors associated with respiratory health status impairment in univariable analyses

In addition to HIV status, we explored other possible contributors to impaired respiratory health among the whole study sample (Table 15). As expected, higher SGRQ scores were associated with impaired lung function, with a median Total SGRQ score of 28.5 (IQR 7.2-41.9) found in those with an FEV1 <80% predicted compared to 9.1 (4.4-17.4) in those with an FEV1 in the normal range ($p<0.01$). Symptoms of depression were associated with impaired self-reported respiratory health status: median SGRQ total scores were 7.7 in the 238 participants with PHQ-9 scores less than 10 compared to 26.5 in the 52 participants with scores of 10 or more.

Combining HIV positive and negative groups, no significant associations were found between gender, ethnicity, smoking status or recreational drug use and impaired respiratory health status in univariable analyses. An association between BMI and SGRQ Total score was seen (with higher scores in those with BMIs below 20 or above 25) that approached statistical significance ($p=0.07$).

TABLE 15 ASSOCIATIONS BETWEEN PARTICIPANT CHARACTERISTICS AND RESPIRATORY HEALTH STATUS IMPAIRMENT

		N*	St Georges Respiratory Questionnaire Total Score Median (IQR)	P value	MRC dyspnea score mean (SD)	p-value**
HIV status	HIV positive	197	11.64 (5.70-25.16)	**p = 0.001**	**1.72 (0.99)**	**p<0.01**
	HIV negative	93	6.01 (1.92-13.92)		1.25 (0.47)	
Gender	Male	222	9.25 (4.38-19.69)	p = 0.15	1.50 (0.82)	**p = 0.01**
	Female	65	12.77 (5.59-30.67)		1.88 (1.04)	
Sexual orientation	Heterosexual	125	9.24 (4.37-23.61)	p = 0.758	1.80 (0.90)	p=0.19
	MSM	139	10.79 (4.54-22.78)		1.57 (0.89)	
	Bisexual	18	10.90 (4.57-20.37		1.41 (0.62)	
Use of recreational drugs, ever	Yes	120	9.90 (5.05- 20.26)	0.24	1.56 (0.91)	p=0.47
	No	159	8.07 (3.48- 24.51)		1.60 (0.88)	
Use of recreational drugs, past 3 months	Yes	207	11.31 (4.48- 25.30)	0.121	1.64 (0.94)	p=0.61
	No	76	8.97 (4.43- 19.69)		1.56 (0.88)	
Age (years)	<40	78	7.50 (2.36-14.62)	**p = 0.024**	1.42 (0.74)	**p = 0.22**
	40-50	103	9.60 (5.24-23.73)		1.49 (0.71)	
	50-60	77	12.05 (5.76-33.34)		1.74 (1.01)	
	60+	32	11.53 (4.47-28.43)		1.83 (1.26)	
Body Mass Index (kg/m^2)	<20	17	15.36 (6.01-28.33)	p = 0.07	1.81 (0.83)	**p=0.02**
	20-25	110	8.21 (3.48-15.12)		1.41 (0.74)	
	25-30	75	10.50 (5.07-24.48)		1.46 (0.65)	
	30+	34	9.76 (4.65-39.57)		1.91 (1.15)	
Ethnicity	White	197	10.55 (4.78-20.37)	p = 0.36	1.57 (0.90)	p=0.19
	Black Caribbean or African	54	7.81 (4.71-23.73)		1.69 (0.80)	
	Asian	8	9.85 (4.42-29.66)		1.63 (1.06)	
	Mixed ethnicity	7	11.46 (3.87-19.56)		1.33 (0.52)	
	Other	20	5.48 (1.85-11.37)		1.21 (0.43)	
Self-reported anxiety or depression	no or mild anxiety or depression	224	7.86 (3.84-14.96)	**p <0.001**	**1.33 (0.76)**	**p<0.001**
	moderate or severe anxiety or depression	66	29.09 (9.50-45.70)		1.83 (0.93)	
PHQ score	PHQ <10	238	7.7 (3.8-15.1)	**<0.001**	1.44 (0.77)	<0.001
	PHQ ≥ 10	52	26.5 (15.0-39.6)		2.16 (1.11)	
Smoking status	never smoker	127	8.55 (3.57-24.44)	p = 0.28	1.62 (0.93)	p=0.51
	Current smoker	87	11.56 (6.30-22.78)		1.57 (0.83)	
	Ex-smoker	76	9.19 (4.41-17.12)		1.51 (0.90)	
FEV1 % predicted	<80%	40	28.49 (7.19-41.92)	**p = 0.008**	**2.03 (1.18)**	**0.01**
	80-100%	120	10.07 (4.65-16.66)		1.52 (0.73)	
	>100%	71	8.25 (4.16-16.65)		1.44 (0.84	
FEV1/FVC	<0.7	24	15.20 (9.25-32.78)	**p= 0.016**	**1.88 (1.12)**	**0.14**
	>0.7	207	9.17 (4.38-19.56)		1.55 (0.84	

*values may not sum to full study sample due to missing data

3.3.6 Associations between HIV-related parameters and respiratory health

In analyses restricted to HIV positive participants, neither current nor nadir CD4 count were significantly associated with higher SGRQ Total scores (although trends were seen for higher scores with lower current or nadir blood CD4 counts). No significant difference in median SGRQ Total score was found between those with and without a HIV load <40 copies/ml (Table 16). After adjustment for age in a log-scale linear regression model, there was a trend towards higher SGRQ Total scores in those who had been diagnosed with HIV infection for longer. A significant association was found between having a longer period between HIV diagnosis and starting ART and a higher SGRQ Total score.

TABLE 16: ASSOCIATIONS BETWEEN HIV RELATED FACTORS AND SGRQ TOTAL SCORE

		HIV positive participants N (%)	SGRQ Total (Median, IQR)	Unadjusted fold change in SGRQ* (95% CI)	Age-adjusted fold-change in SGRQ*	P value*
Current CD4 (cells/µL)	0-350	19 (11%)	20 (6-35)	1.53 (0.88-2.18)	1.57 (0.94-2.62)	0.11
	350-500	30 (18%)	16 (9-34)	1.39 (0.91-2.54)	1.40 (0.90-2.19)	
	500+	119 (71%)	11 (6-20)	Reference		
Viral load < 40 copies/ml at last clinic review	No	19 (11%)	15 (5-67)	1.31 (0.78-2.19)	1.52 (0.89-2.57)	0.12
	Yes	149 (89%)	13 (6-25)	Reference		
Nadir CD4 (cells/µL)	0-100	35 (21%)	17 (7-43)	1.80 (0.98-3.32)	1.65 (0.88-1.74)	0.11
	100-250	47 (28%)	16 (8-33)	1.52 (0.85-2.73)	1.40 (0.76-2.55)	
	250-500	67 (40%)	12 (6-17)	1.04 (0.60-1.81)	1.0 (0.57-3.09)	
	500+	17 (10%)	10.5 (4-14)	Reference		
Time since HIV diagnosis	20+ years	47 (27%)	16 (8-37)	1.72 (1.14-2.61)	1.56 (1.01-2.43)	0.07
	10-20 years	62 (36%)	11 (6-25)	1.24 (0.85-1.83)	1.19 (0.80-1.76)	
	0-10 years	62 (36%)	10 (4-20)	Reference		
Years between HIV diagnosis and ART	>10	33 (20%)	21 (11-48)	1.90 (1.23-2.91)	1.79 (1.17-2.75)	0.004
	5-10	43 (25%)	9 (5-26)	1.00 (0.68-1.45)	0.95 (0.64-1.39)	
	0-5	93 (55%)	12 (5-21)	Reference		
Years of ART exposure	>10	68 (49%)	17 (7-37)	1.73 (1.13-2.64)	1.58 (1.0-2.49)	0.15
	5-10	26 (19%)	13 (7-24)	1.54 (0.87-2.70)	1.41 (0.78-2.52)	
	1-5	46 (33%)	9 (4-21)	Reference		

* log-scale linear regression model

3.3.7 Multivariable analysis of factors associated with respiratory health status including all participants

To allow adjustment for potential confounding factors we used multivariable (log scale) linear regression models including all participants, with log SGRQ as the dependent variable. In addition to those factors chosen *a priori* (smoking status, age and gender), PHQ-9 scores and BMI were also included as they reached statistical significance at the 5% level in univariable analysis.

After adjustment for these other factors, HIV infection remained independently associated with an increased SGRQ Total score, with a 54% higher SGRQ total score compared to HIV negative individuals (adjusted fold-change 1.54 (1.14-2.09), p=0.005) (Table 17). Depression (PHQ-9 score ≥10) was also independently associated with a higher SGRQ Total score (adjusted fold change 1.90 (1.42-2.53), p<0.001).

Similar factors were independently associated with an MRC dyspnoea score ≥2 in a multivariable logistic regression model (Table 18). Here, the adjusted odds ratio for an MRC dyspnoea score of ≥2 was 2.84 (1.35-6.00), P=0.006 in HIV positive compared to HIV negative participants. Independent associations were found with female gender (adjusted OR 4.69 (1.85-11.45), p=0.001) and depression (aOR=6.30 (2.75-14.46), p <0.001).

TABLE 17 ASSOCIATIONS WITH ST GEORGES RESPIRATORY QUESTIONNAIRE TOTAL SCORE, MULTIVARIABLE LOG-STALE LINEAR REGRESSION

		Adjusted fold-change in SGRQ* (95%CI)	P value
HIV status	HIV positive	**1.58 (1.18-2.12)**	**0.002**
	HIV negative	Reference	
Age (per year)		1.01 (1.00-1.02)	0.33
Gender	Female	1.37 (0.96-1.09)	0.08
	Male	Reference	
Depression (PHQ-9 score)	≥10	**2.77 (1.98-3.88)**	**<0.001**
	<10	Reference	
Body Mass Index (kg/m^2)	<20	1.18 (0.68-1.74)	0.68
	20-25	Reference	
	25-30	1.18 (0.85-1.37)	
	30+	1.23 (0.8-1.42)	
Smoking	Current smoker	1.23 (0.89-1.24)	0.41
	Ex -smoker	1.01 (0.72-2.57)	
	Never smoker	Reference	

TABLE 18 MULTIVARIABLE LOGISTIC REGRESSION OF ASSOCIATIONS BETWEEN MRC SCORE ≥2 AND PARTICIPANT CHARACTERISTICS.

Covariate		Mean (SD) MRC dyspnea score (1-5 scale)	MRC sore ≥2 n (%)	p-value*	Unadjusted odds ratio of MRC score >1	adjusted Odds Ratio of MRC dyspnoea score >1, multivariable logistic regression**
HIV status	HIV positive	1.72 (0.99)	87 (47%)	**0.001**	**2.68 (1.5-4.8) p=0.001**	**2.84 (1.35-6.00) P=0.006**
	HIV negative	1.25 (0.47)	20 (25%)		1	1
Gender	**Female**	1.88 (1.04)	35 (58%)	**0.001**	**2.57 (1.42-4.62) p= 0.002**	**4.27 (1.79-10.14) 0.001**
	Male	1.50 (0.82)	72 (35%)		1	1
Depression (PHQ-9 score)	≥10	2.16 (1.11)	35 (70%)	**<0.001**	4.70 (2.41-9.16)	6.30 (2.75-14.46) P<0.001
	<10	1.44 (0.77)	72 (33%)		1	1
BMI	<20	1.81 (0.83)	10 (62%)	**0.02**	3.76 (1.26-11.27) p=0.018	2.56 (0.76 -8.68) P=0.13
	20-25	1.41 (0.74)	31 (31%)		1	
	25-30	1.46 (0.65)	32 (39%)		1.45 (0.76-2.76) p=0.255	1.05 (0.50-2.19) P= 0.99
	>30	1.91 (1.15)	17 (53.1%)		2.56 (1.13-5.77) p=0.023	1.39 (0.41- 3.79) P=0.518
Smoking status	Current smoker	1.51 (0.90)	25 (34%)	0.43	1.03 (0.58-1.86) p=0.897	1.18 (0.52-2.63) p=0.69
	Ex-smoker	1.57 (0.83)	34 (43%)		0.72 (0.38-1.29) p=0.254	0.72 (0.31-1.68) p=0.44
	Never smoker	1.62 (0.93)	48 (42%)		1	1

* Chi-squared test ** age included in model in addition to covariates listed

3.3.8 Comparing HIV positive participants with an undetectable HIV viral load with HIV negative participants

As other studies have suggested that untreated HIV is associated with chronic respiratory impairment, a higher prevalence of respiratory symptoms within the PLWH as a whole might be the result of increased symptoms only among HIV positive individuals not yet taking ART, or on ART without virological suppression. We therefore undertook a subgroup analysis comparing HIV negative participants with HIV positive participants whose HIV load was undetectable (<40 copies/ml) within 6 months of the study visit. Participants who declined consent to access clinical records were excluded from this analysis, leaving 157 PLWH with documented virological suppression, compared to the 93 HIV negative participants. Those with virological suppression had similar demographic details to the PLWH study population as a whole (Table 19), and a median CD4 count of 684 (473-839) cells/µL. The differences in respiratory health scores between HIV positive (HIV supressed) and HIV negative groups were similar to those present in the complete dataset: median SGRQ Total scores were 12 (IQR 6-25) compared to 6 (2-14) ($p<0.001$) and 70 (47%) of the HIV positive group had MRC dyspnoea scores of ≥2 compared to 20 (24.7%) of the HIV negative group $p= 0.001$. In a log-scale linear regression model (including the same predictive factors as the whole-group analysis), HIV status remained independently associated with a higher SGRQ Total score, with a similar effect size to that in the whole group (1.53 (1.13-2.06), $p= 0.007$).

TABLE 19 COMPARISON OF HIV POSITIVE PARTICIPANTS WITH AN UNDETECTABLE HIV LOAD AND HIV NEGATIVE PARTICIPANTS

		HIV positive, undetectable VL (n=157)	HIV negative (n= 93)	p value
Gender	Male	125 (80%)	64 (71%)	0.13*
	Female	32 (20%)	26 (29%)	
Age, years, Median (IQR)		50 (44-56)	43 (38-52)	0.001#
Smoking status	never smoker	69 (44%)	42 (45%)	0.50*
	Current smoker	41 (26%)	29 (31%)	
	Ex-smoker	47 (30%)	22 (24%)	
PHQ-9 score (mean, SD)		5.48 (5.65)	3.74 (4.69)	0.005#
PHQ-9 score ≥10	<10	126 (80%)	80 (86%)	0.25*
	≥10	31 (20%)	13 (14)	
Sexual orientation	Missing / declined to state	0	5 (5%)	<0.001**
	MSM	106 (67%)	25 (27%)	
	MSW	19 (12%)	37 (40%)	
	Female	32 (20%)	26 (28%)	
Body Mass Index ($kg/m^{2)}$	<20	10 (8%)	4 (5%)	0.24**
	25-30	43 (35%)	23 (28%)	
	30+	21 (17%)	10 (12%)	
	20-25	49 (40%)	44 (54%)	

* Chi Squared test # Mann Whitney U test ** Fisher's exact test

3.3.9 Sensitivity analyses

To further explore the possible effect of the difference in age distribution between the HIV positive and negative groups, we undertook a sensitivity analysis in which we examined only those aged 52 or less (the 75th centile of the HIV negative participants). Using this restricted analysis (of 127 HIV positive and 69 HIV negative participants) the age distributions were similar - with a median age of 42 in both groups. The previously-demonstrated differences remained, with median (IQR) SGRQ Total scores of 11.2 (6-20) in the PLWH and 6.2 (3-15) in the HIV negative group p=0.01). MRC dyspnoea scores were also higher, with 55 (46%) of the HIV positive and 16 (27%) of the HIV negative participants having a MRC Dyspnoea score ≥2 (p=0.01).

3.3.10 Attitudes and health beliefs towards respiratory illness

In addition to comparing respiratory health status and risk factors for respiratory illness in the HIV positive and negative groups evaluated, we sought to explore health beliefs and attitudes towards respiratory health in these populations (Table 20).

The overwhelming majority of both HIV positive and negative participants agreed that smoking increases the risk of lung cancer and heart disease; similarly, there were no significant differences in the responses to questions about influenza, and immunisation with influenza and pneumococcal vaccines.

Only the HIV positive participants were asked about HIV and lung disease. Overall, 53% were unsure as to whether HIV infection is associated with the early onset of smoking-related lung disease, with only 21% agreeing with this statement (and the same proportion disagreeing). Similar responses were found to a question asking if PLWH are at greater risk of developing lung cancer. Respondents were more likely to agree with the statement that PLWH are more likely to get pneumonia (78% agreed).

TABLE 20 PARTICIPANT THOUGHTS ABOUT LUNG HEALTH

		Agree or strongly agree n (%) HIV positive (n=197)	Agree or strongly agree n (%) HIV negative (n=93)	P value
Most smokers will develop lung disease		155 (79%)	62 (68%)	0.55
Smoking increases the risk of heart disease		174 (88%)	76 (82%)	0.23
Only people who are old or overweight or pregnant die of the 'flu:		20 (10%)	7 (7%)	0.50
Having the 'flu vaccine:	Means I can't catch the 'flu at all this year:	57 (29%)	18 (19%)	0.22
	Means I can still get the 'flu, but it might be less severe:	121 (61%)	51 (55%)	0.75
	Will make me feel terrible:	36 (18%)	13 (14%)	0.05
Having the pneumococcal vaccine (Pneumovax or Previnar):	Means I won't ever get pneumonia in the future	32 (16%)	13 (14%)	0.2
	Will give me some protection from pneumonia for a few years	92 (47%)	28 (30%)	0.33
	Will make me feel terrible	13 (12%)	8 (9%)	0.13
HIV is associated with early onset of smoking related lung disease (emphysema/ chronic bronchitis)		33 (18%)	--	--
The risk of developing lung cancer is increased in people living with HIV		53 (27%)	--	--
People with HIV are more likely to get pneumonia.		113 (57%)	--	--

3.4 Discussion

The results of this study suggest that HIV infection continues to be associated with impaired respiratory health despite viral suppression on ART. We compared HIV positive individuals with an HIV negative group with similar exposures to risk factors such as tobacco smoking. Although some differences were present in the age and ethnicity composition of these two groups, these did not account for the differences in respiratory health status seen after adjustment in multivariable analyses.

HIV positive participants commonly reported breathlessness, with 47% of PLWH reporting breathlessness of at least moderate severity, compared to 25% of the HIV negative participants. Using the SGRQ respiratory health questionnaire (which assesses not only respiratory symptoms but the impact on activity and quality of life) we found a 6-point difference in the median Total score between HIV positive and negative groups (a minimum clinically important difference in SGRQ being around 4 points), suggesting that there is a meaningful impairment of the respiratory health of PLWH. Of note, this difference was present despite the prevalence of airflow obstruction in our HIV positive subjects (11%) being lower than that reported in other HIV positive populations (for instance 23% in Italy and 27% in the USA)(66, 67). There was also no difference in the prevalence of airflow obstruction in our study between the HIV positive and negative groups.

To our knowledge, no other study has used patient-reported outcomes to compare respiratory health status in HIV positive and negative populations. Two previous reports used the SGRQ to evaluate respiratory health in HIV positive adults: Hirani *et al* evaluated 98 consecutive HIV positive individuals (84% male) attending HIV care in Philadelphia (97), USA, and found a mean SGRQ Total score of 7, in contrast, Leung *et al* reported a mean SGRQ Total score of 32 in 199 HIV positive men attending care in Vancouver, Canada (146). Our data therefore provide the first estimate of the difference in respiratory health (as experienced by individuals) between HIV positive adults with optimised access to ART and HIV negative adults.

What might be contributing to these findings? Impairment of lung function not measured by spirometry may be present: as discussed earlier, reductions in transfer factor (DLCO) and measures of small airways impairment have been found to be more common among PLWH than equivalent HIV negative comparators. This might be the result of a higher frequency of respiratory infection prior to effective ART and lead to long-term lung damage: this could also result from the direct effect of HIV in the lung. Other possibilities include heart disease or other non-respiratory comorbidities which can lead to respiratory symptoms. Depression was strongly associated with impaired respiratory health in our population and this may contribute to the burden of physical symptoms (although it should be noted that the difference in respiratory health status persisted after adjustment for the presence of depression in our population).

As discussed in the Introduction to this thesis, several hypotheses have been advanced to try and account for the apparent association between HIV and impairment in respiratory health. These have included direct HIV mediated damage to the lung epithelium (138) or pulmonary vasculature (135),

disruption or dysregulation of immune responses caused by HIV or the host immune response to it (109, 132, 184), or residual damage due to prior immunosuppression (perhaps the result of opportunistic infections) which persists after antiretroviral therapy has been started (76). Mechanistic studies, in particular those with prospective follow-up, may allow the relative importance of these possible mechanisms to be determined.

3.4.1 Strengths and limitations of this data

The strengths of our study include the presence of an HIV negative control group with similar exposures to tobacco smoking and recreational drugs and the use of well-validated measures of respiratory health status. Our high response rate (72%) suggests that participants were representative of the wider clinic population, who in turn are similar to the UK HIV population as a whole.

Limitations include the relatively small sample size, recruitment at a single study site and the cross-sectional nature of the data collected – meaning that temporality cannot be established. As people were not randomly selected to participate, recruitment bias is possible. Spirometry was our only objective measure of lung function, however as discussed in the Introduction, impairment of gas transfer may be more common than airflow obstruction in PLWH. There were also differences in age and ethnicity between groups which could have influenced our results; though, our findings persist after adjustment in multivariable analyses. Our sensitivity analysis is reassuring in that the difference in age distribution between HIV positive and negative participants appeared to have little impact on the results.

The choice of HIV negative comparator group requires discussion – as we wished to derive findings reflective of the HIV positive population as a whole, we did not restrict recruitment of people living with HIV to one risk-group (e.g. MSM) , However recruitment of the HIV negative comparator group occurred primarily within Sexual Health clinics, and consequently may not adequately capture the diversity within our HIV positive participants, such as migrants from high HIV incidence settings and injecting drug users. A wider recruitment strategy could have mitigated this issue – and provided more confidence in our findings.

Finally, we relied on self-reported HIV status for the HIV negative participants, so we cannot exclude the possibility that HIV positive or undiagnosed individuals were included in this group. However, we believe that this is unlikely to be a major source of bias, as although we do not have data directly measuring the prevalence of undiagnosed HIV infection in this sexual health clinic population, data based on anonymised testing of residual samples in the Unlinked Anonymous HIV Prevalence Monitoring Programme (UAPMP) reported a low HIV prevalence in sexual health clinic attendees of 2%. However (as this surveillance programme used anonymised samples) the proportion with undiagnosed HIV infection is unknown. Furthermore, more recent data from Public Health England (PHE) have demonstrated significant declines in the number of new HIV diagnoses from Sexual Health clinics, with positive HIV results in around 1-2% of HIV tests undertaken in individuals not tested in the previous 2 years.(185) Therefore it is unlikely that significant numbers of undiagnosed

HIV positive individuals were present in our comparator group. Also, if this did occur, it would have acted to weaken the association between HIV and respiratory health status impairment.

3.5 Conclusions

Interventions that can preserve the respiratory health of people with chronic HIV infection are needed. The earlier use of ART may yield reductions in non-AIDS comorbidities including respiratory illness – although (notwithstanding a median duration of follow-up of only 2.8 years) this was not associated with differences in lung function decline in the recent START trial (64)., The impaired respiratory health of PLWH despite effective virological suppression found in our study suggests that more than ART alone is required to maintain population health. Reducing the effect of known risk factors such as tobacco is key to this (many HIV positive populations have high rates of smoking) and the provision of appropriate smoking cessation services should be a priority. Though further evidence is required to determine why HIV infection is associated with worse respiratory health even in people who have never smoked.

Chapter 4: Acute respiratory illness in an HIV positive population with a high uptake of antiretroviral therapy: a prospective cohort study

4.1 Background and rationale for the study

As discussed previously, there is evidence that ART reduces the incidence of respiratory infections such as bacterial pneumonia and tuberculosis (11, 42), however, most studies evaluating respiratory illness in the modern HIV population have been cross-sectional in nature and therefore cannot determine whether acute respiratory illnesses continue to be more frequent among PLWH. These illnesses are common in the general population and associated with significant morbidity and healthcare utilisation (186). Therefore, if these remain at a higher incidence in HIV positive individuals (or if they are more severe or longer lasting) this would have a considerable impact on health-related quality of life and might contribute to the development of chronic lung disease in people living with HIV. The identification of specific risk factors for acute respiratory illness in this population could also enable interventions to reduce the incidence or severity of these illnesses (such as smoking cessation, immunisations and treatment for respiratory conditions) to be more effectively targeted.

This study aimed to prospectively measure the frequency of acute respiratory illness among HIV positive individuals with a high uptake of antiretroviral therapy compared to HIV negative comparator participants.

4.2 What is known about the effect of HIV status on the frequency of acute respiratory illness

HIV positive individuals have been known to experience a high frequency of acute respiratory illness since the start of the HIV epidemic. The epidemiology of respiratory illness prior to the use of ART was detailed by the Pulmonary Complications of HIV Infection Study, which followed 1,353 HIV positive and 183 HIV negative individuals in the USA between 1988 and 1994 (22). The most prevalent respiratory diseases in this study were upper respiratory tract infections (which occurred at a rate of 42/100 person years in those with HIV infection compared to 29 per 100 person-years in those without) and bronchitis (which was twice as common in those with HIV infection). Although less prevalent, bacterial pneumonia was six times more common in PLWH.

The use of effective antiretroviral therapy has changed the epidemiology of respiratory disease. Several large observational cohorts and population-based registry studies have provided evidence regarding the rates of respiratory illness since the widespread introduction of ART. These suggest that the frequency of acute respiratory illness may remain higher in people living with HIV. The Multicentre AIDS Study since 1996 (when ART became available) suggest that the odds ratio was 1.51 for bronchitis (95% CI 1.24-1.84); 1.46 (1.28-1.68) for sinusitis and 4.14 (2.43-7.08) for bacterial pneumonia compared to the HIV negative study participants (30). The Women's Interagency HIV study, which recruited women in the US since 1994, reported an OR of 2.17 for sinusitis ($p<0.001$), 1.46 for acute bronchitis ($p= 0.22$) and 9.55 ($p <0.001$) for pneumonia since the development of ART (30).

The Veterans Aging Cohort Study in the USA has used registry data to evaluate rates of disease and reports that pneumonia is around 5 times more common in those with HIV infection compared to those without (60). The investigators found that higher CD4 count and lower plasma HIV load were protective against bacterial pneumonia in this study.

These studies all evaluated HIV positive cohorts in the US, where access to care and uptake of antiretroviral therapy may be lower than in European settings. A Danish cohort study evaluated all HIV positive individuals receiving care in Denmark since 1995 and compared them to population controls selected from the Danish Civil Registration System (11). Data regarding hospitalisation for pneumonia were extracted from Danish National Hospital Registry data, with discharge diagnoses of pneumonia recorded. The results demonstrated a significant reduction in hospitalisation for pneumonia among people with HIV between 1995 and 1999, associated with increased uptake of antiretroviral therapy. After that, the incidence of hospitalisation for pneumonia remained around 18-20 per 1000 person-years among the HIV positive population. This compared with an annual incidence of 1.5-3 hospitalisations for pneumonia per 1000 person-years among the HIV negative comparator group.

The finding that a higher frequency of bacterial pneumonia persists among people living with HIV is supported by the work of Coxford *et al*, which used Public Health England data (187). This evaluated causes of death among HIV positive people in England between 1997 and 2012. Although a

particularly high mortality rate occurred soon after HIV diagnosis, in this data set, mortality remained higher even if those in the first year after HIV diagnosis were excluded. In particular, non-AIDS infections were significantly more common as a cause of death, with Standardised Mortality Ratios of 5.8 in HIV positive men and 10.7 in women.

Although population-level epidemiological data are appropriate to evaluate comparatively rare, but severe, events such as death from bacterial pneumonia, there is little information available about the frequency of acute respiratory illness as a whole. There is therefore a need to better understand the epidemiology of acute respiratory illness among people living with HIV using prospective follow-up of a well characterised cohort.

4.3 Study aims

This study aimed to test the hypothesis that people living with HIV experience a greater incidence of acute respiratory illness than people without HIV despite the use of antiretroviral therapy. In addition, we sought to evaluate the severity and duration of these illnesses, the associated healthcare use, and impact on health-related quality of life. Furthermore, we evaluated the relationship between individual characteristics (such as tobacco smoking or prior immunosuppression) and the frequency of acute respiratory illness.

Specific pre-defined study objectives (as documented in the study protocol and reviewed by the Research Ethics Committee) were as follows:

4.3.1 Primary Study Outcome

The annual incidence of acute respiratory illness in HIV positive compared to HIV negative adults.

4.3.2 Secondary study outcomes

1. Duration of symptoms during respiratory tract illness in HIV positive and negative participants
2. Health-related quality of life measured by the St Georges Respiratory Questionnaire and EuroQoL-5D
3. Healthcare resource utilisation arising from acute respiratory illness
4. The prevalence of positive microbial isolation at baseline
5. The prevalence of positive microbial isolation during acute respiratory illness
6. The baseline prevalence of obstructive lung disease

4.3.3 Study registration

We registered this study with the ISRCTN registry (http://www.isrctn.com/ISRCTN38386321)

4.4 Methods

4.4.1 Recruitment strategy

(a) Recruitment of HIV positive participants:

HIV positive participants were recruited from individuals attending for HIV ambulatory care at the Royal Free Hospital HIV care service. During the first 6 months of recruitment, potential participants were randomly selected from clinic lists to be invited to take part in the study, with three individuals being selected from each clinic list. However, this strategy did not meet the required recruitment targets (as some selected individuals might not attend for their clinic visit) and recruitment from more than one list at a time was difficult. Therefore, after interim review the recruitment strategy was altered to include unselected invitation from clinic lists.

Individuals were initially asked if they might be interested in participating in the study by their HIV care consultant. Those who suggested that they might be interested in doing so were then given verbal details of the study as well as a written participant information leaflet. Potential participants were given at least 24 hours to consider whether they wished to take part.

Limited demographic details of individuals who were invited to participate but declined to do so were recorded to allow quantification of the response rate and any biases in recruitment.

(b) Recruitment of HIV negative participants:

The ideal HIV negative participants in this study would have socio-economic and social backgrounds similar to the HIV positive participants and have similar exposure to risk factors such as tobacco smoking. However, recruitment of such a control group is not easy. Experience gained during recruitment for the study described in Chapter 2 suggested that although Sexual Health clinics serve a population with similar characteristics to the HIV positive population, the numbers of potentially suitable people attending these clinics who would be interested in participating in this study would be relatively low. We therefore did not select this as the primary source for recruitment. Nonetheless, study information with details of how to contact the study team were displayed in the Sexual Health clinics and staff there were given information about the study and asked to provide written information to any potentially interested individuals.

The main source of recruitment of HIV negative participants in the study was via postal invitation from Primary Care. This was achieved in collaboration with the Primary Care research group led by Professor Irwin Nazareth. In order to recruit participants with similar characteristics to the HIV positive study group, potentially suitable individuals were selected from Primary Care GP records at the Keats Practice and Hampstead Group Practice, London, matched by age, gender and smoking status. These individuals were then sent information

about the study by post. This matching and postal invitation was undertaken by research staff at the Keats Group Practice to maintain patient confidentiality. Limited demographic details of those who did not chose to participate were recorded.

4.4.2 Inclusion and exclusion criteria

The aim of the study was to evaluate a study sample population that was reflective of the UK HIV positive population we were aiming to study. Therefore, broad inclusion criteria were chosen.

Inclusion criteria for HIV-positive cohort participants

1. Willing to participate in study and able to return for review in the event of respiratory tract infections, and to participate for the duration of the study
2. 18 years or above

Inclusion criteria for HIV-negative participants

1. Willing to participate in study and able to return for review in the event of respiratory tract infections, and to participate for the duration of the study
2. 18 years or above
3. Consent to HIV testing and negative HIV test result

Exclusion criteria (for both study groups):

1. Unable to participate for the full duration of the study
2. Unable to return for review in the event of respiratory tract infection (for instance those living a long distance from the study site)
3. Current significant acute respiratory tract illness such as pulmonary tuberculosis, *Pneumocystis jirovecii* pneumonia or bacterial pneumonia

4.4.3 Data collection

Participants in the study were interviewed at recruitment and invited for review at all times of acute respiratory illness. Data were recorded on paper questionnaires and entered into a database under allocated study number.

Baseline data collection for all study participants consisted of:

1. Demographic details
2. HIV and non-HIV related medical history
3. History of tobacco and recreational drug use including use of electronic cigarettes
4. History of pneumococcal and influenza immunisation
5. Medication history
6. Social history: employment; educational attainment; young children within household

For HIV positive participants, additional data were collected from existing hospital records with written participant consent. This data included:

1. Nadir blood CD4 count
2. Duration of known HIV infection
3. Risk for HIV acquisition
4. Ever AIDS diagnosis
5. Blood CD4 count and HIV viral load at baseline

The presence of respiratory symptoms at baseline was assessed using the St Georges Respiratory Questionnaire (SGRQ) and MRC dyspnoea scores. General health status was measured by the EuroQoL 5-dimension (EQ-5D) (see Chapter 3 for further details of these instruments).

The following biological samples/measurements were collected at baseline:

1. Naso-pharyngeal swab for detection of respiratory viruses
2. Spontaneous or induced sputum collection for detection of respiratory viruses and bacteria
3. Spirometry (without bronchodilation)

As previously, spirometry was undertaken using a hand-held Micro I spirometer (Carevision, San Diego, USA). The Global Lung Function Initiative equations were used to calculate normal values. Airflow obstruction was defined as an FEV1/FVC ratio of <0.7 and restriction as FVC <80% predicted with FEV1/FVC ratio ≥0.7.

4.4.4 Detection of viral respiratory pathogens

The detection of respiratory viral pathogens from nasopharyngeal swabs at baseline and during an acute respiratory illness was undertaken using multiplex PCR testing. Oropharygneal swabs were collected using flocked swabs in viral culture medium (Copan UTM, Copan Diagnostics Inc., Corona, California, USA). This testing was performed in the Virology laboratory at the Royal Free London NHS Trust and conducted by biomedical scientists working within this laboratory, supervised by Dr Tabitha Mahungu. I did not undertake this laboratory analysis myself.

Analysis of samples was completed using standardised laboratory protocols. RNA and DNA were extracted from respiratory specimens using the EASYMAG® platform. In-house real-time RT-PCR assay was performed which was designed to simultaneously detect: rhinovirus, influenza (A & B), parainfluenza (1-4), RSV, adenovirus, enterovirus, coronavirus (NL63, HKU, 229E, OC43), parechovirus and human metapneumovirus. The procedure included contained a primer and probe pair that amplifies a DNA sequence present in all human cells (K-ras oncogene) and PDV (Phocine Distemper Virus) was added to samples prior to extraction acts as internal controls. PCR reaction was undertaken using the Applied Biosystems Prism 7500 Fast Real-Time PCR System.

4.4.5 Definition of acute respiratory illness

We defined an acute respiratory illness as the new occurrence (lasting more than 24 hours) of any of the following symptoms: cough, sore throat, blocked or runny nose with or without a sensation of facial pain or pressure, breathlessness, or pain on breathing. This definition sought to include both upper and lower respiratory tract illnesses. No assumption was made about whether an illness was caused by an infection, and fever was not included in the illness definition (although individuals were asked about a history of fever).

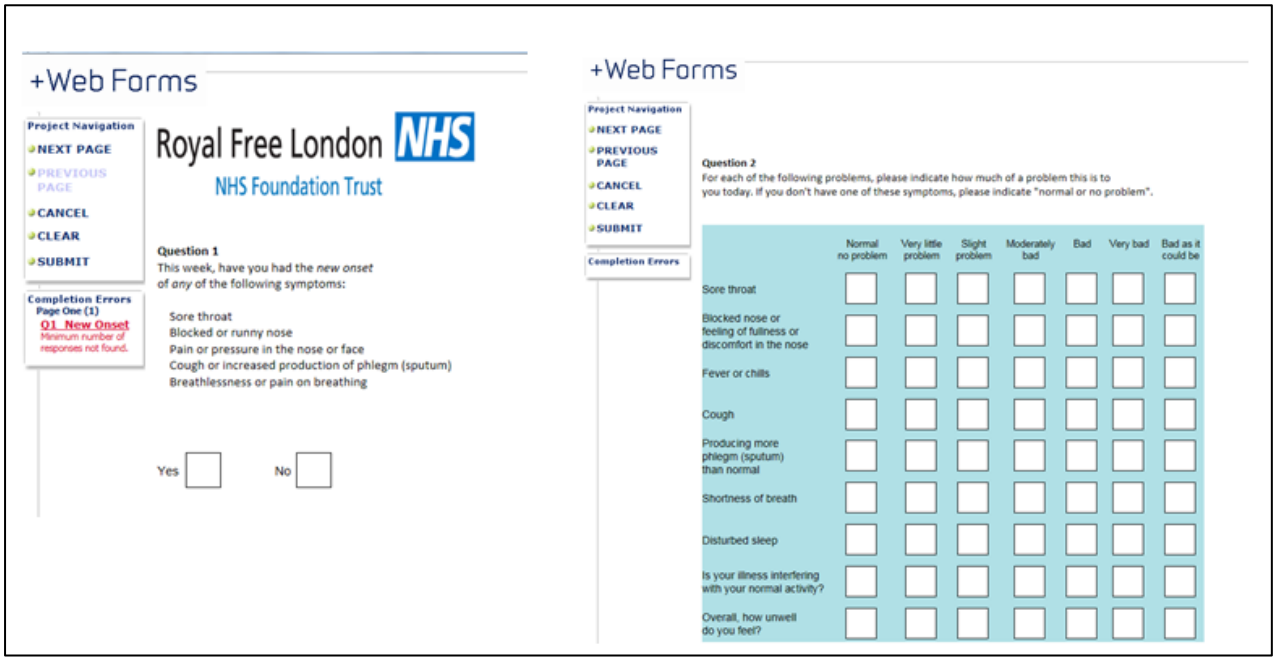
The definition of acute respiratory illness was chosen after review of the available literature. We did consider using an existing validated tool, however, none of these instruments fully met our requirements. For instance, validated tools exist to define acute upper respiratory illness such as the Wisconsin Upper Respiratory Symptom Survey (WURSS),(188) but these would not capture symptoms such as breathlessness. Definitions of Influenza Like Illness are often used in epidemiological studies,(189) but again we felt that such definitions would be too narrow to capture all acute respiratory illnesses, as we intended to do. Standard definitions of COPD and asthma exacerbations also exist,(190) but using these in a population without these conditions would be inappropriate without an extensive process of validation. I therefore decided to define an illness definition and set of symptoms to be scored that were specific to this project. The symptom definition necessarily was a compromise between including a broad enough definition to avoid missing significant respiratory illnesses and being brief enough for participants to read and follow easily. The symptom scores were then piloted with six individuals to ensure the feasibility of completion and use of the online reporting tool.

4.4.6 Follow-up of study participants and measurement of incidence of acute respiratory tract illness

Study subjects were followed for 12 months. During this time participants were requested to answer a weekly question asking about any new respiratory symptoms that had occurred over the previous week.

For the majority of study participants (190/215) this weekly contact was achieved by emailing a unique link to a web-based questionnaire (supplied by Formic Solutions, http://www.formic.com/). This enabled electronic capture of the response to the weekly respiratory symptom question, with supplementary questions asking about symptoms and healthcare resource utilisation if unwell. Responses to this electronic data capture were returned in Excel spreadsheet emailed each day. A screenshot of the question sent to participants is illustrated in Figure 10.

FIGURE 9 SCREENSHOT OF THE WEBFORM USED



For participants who preferred not to be contacted by email each week, the weekly symptom question could be completed by text message, or in written diary format. 20 (10%) participants preferred to be contacted by text messages and 2 completed written diaries. These methods of contact did not allow collection of supplementary data at the time of submission and participants simply answered “yes” or “no” to the question of whether they had new respiratory symptoms.

The intention of the diary cards was to ensure documentation of all episodes of acute respiratory illness and reduce recall bias that would be inevitable with retrospective documentation of these events.

The use of electronic submission of follow up data was intended to allow regular data collection from participants without imposing too great a demand on them, thus helping to optimise response rate during follow-up and minimise biases associated with recall and missing data.

4.4.7 Assessment at the duration and severity of acute respiratory illness

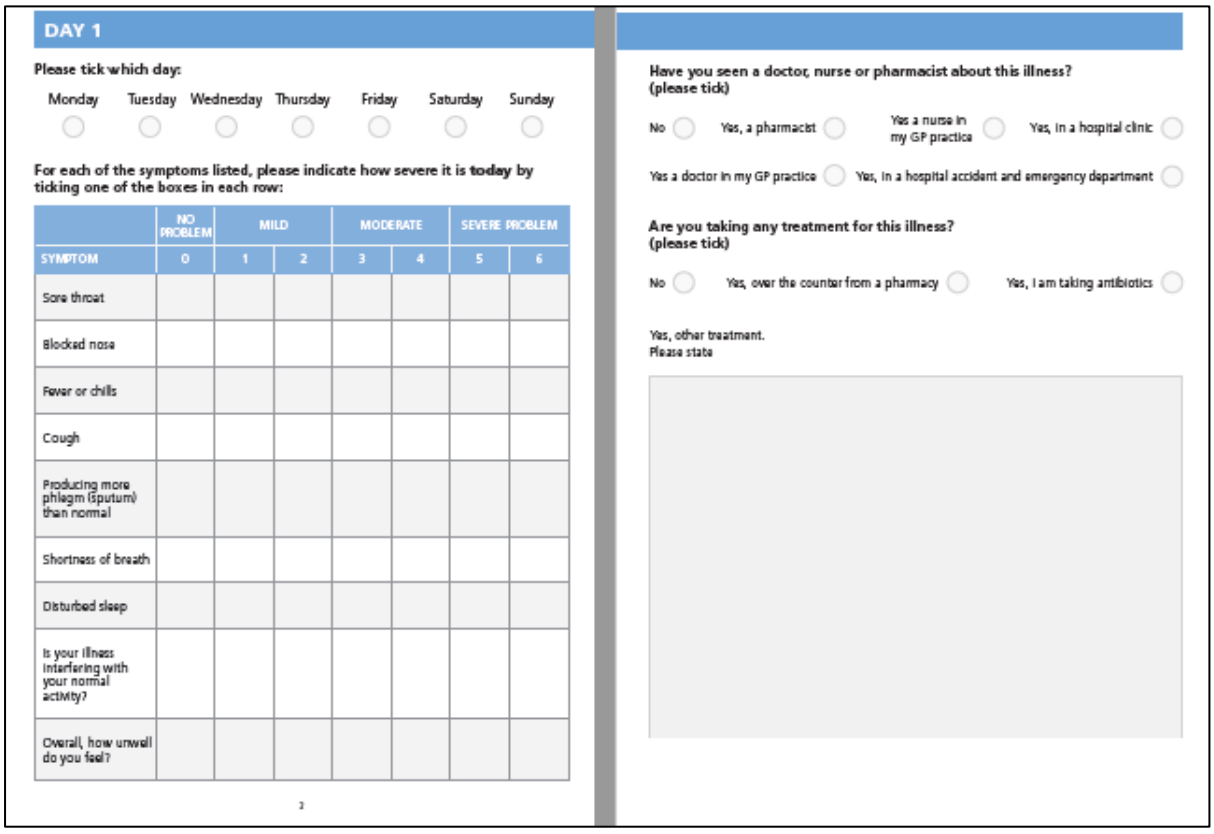
In addition to documenting the incidence of acute respiratory illness and any differences in this between HIV positive and negative participants, secondary objectives included measurement of the severity and duration of these illnesses. It was therefore necessary to collect data on the symptoms occurring each day during these illnesses, and how long they lasted.

Data on symptoms occurring were collected in two ways: all individuals reporting new respiratory symptoms on their weekly emailed symptom question were then asked a series of supplementary questions on the same web-based platform. These detailed several specific symptoms, each assessed on a 0-6 scale, plus general questions about ability to perform activities and how unwell they were feeling. These measures were specifically created for the study based on common symptoms of acute respiratory illness, as no previously validated similar score was available. Although this has the advantage of allowing specific questions to be tailored to the study aims, a disadvantage of this approach is that comparison with other populations and studies is difficult. Therefore, in addition to these “acute respiratory symptom” questions, we also included the more general and well validated health-status questions of the Euro-QoL 5D instrument in this web-based questionnaire.

4.4.8 Written symptom diaries

The web-based symptoms score was completed at the same time as reporting new symptoms. This therefore allowed high completion rate for these questions (as completion of this took only a few minutes). However, this permitted collection of data at a single time-point (whereas acute respiratory illnesses by definition are rapidly changing events). To try and capture this information, participants were asked to complete a written daily diary during these illnesses (Figure 11).

FIGURE 10 WRITTEN DAILY DIARY



DAY 1

Please tick which day:

Monday Tuesday Wednesday Thursday Friday Saturday Sunday

For each of the symptoms listed, please indicate how severe it is today by ticking one of the boxes in each row:

	NO PROBLEM	MILD		MODERATE		SEVERE PROBLEM	
SYMPTOM	0	1	2	3	4	5	6
Sore throat							
Blocked nose							
Fever or chills							
Cough							
Producing more phlegm (sputum) than normal							
Shortness of breath							
Disturbed sleep							
Is your illness interfering with your normal activity?							
Overall, how unwell do you feel?							

Have you seen a doctor, nurse or pharmacist about this illness? (please tick)

No Yes, a pharmacist Yes a nurse in my GP practice Yes, in a hospital clinic

Yes a doctor in my GP practice Yes, in a hospital accident and emergency department

Are you taking any treatment for this illness? (please tick)

No Yes, over the counter from a pharmacy Yes, I am taking antibiotics

Yes, other treatment. Please state

Although existing symptom scores have been used to detail daily symptoms of acute respiratory illness (for instance the COPD Assessment Test (CAT) score (191)), these have been developed for use in particular conditions (such as COPD or asthma) rather than in the general population. We therefore felt that it was appropriate to design a new symptom score rather than utilising an existing instrument.

This diary included details of 7 individual symptoms, plus an assessment of overall illness and impact on normal activity. Each of these items was scored by participants on a 0-6 Likert scale. In addition, questions detailed contact with healthcare services, and any treatment taken. These diaries were self-completed in paper booklets which were then returned to the investigators either in person or by post. Participants were requested to complete these diaries at the end of each day.

The sum of the responses to the 9 items detailing daily symptoms was used to assess daily symptom burden, giving a total daily score with a possible range from 0 to 54.

An illness was defined as having ended when participants answered “0” to the question “Overall, how unwell do you feel”. The duration of illness was then calculated as the number of days between illness onset and the first day with a response of “0”, or the number of days for which data were submitted. A total symptom score for each illness was calculated as the sum of the daily symptom scores.

4.4.9 Comparison with existing acute respiratory illness scores

Severity scores exist for acute upper respiratory tract illness – the most well characterised being the Wisconsin Upper Respiratory Symptom Survey (WURSS). This, however, specifically evaluates upper respiratory pathology (largely acute viral illness) and does not collect any data on breathlessness or lower respiratory symptoms. Similarly validated instruments for quantifying lower respiratory tract illness are lacking. Macfarlane *et al* used a standardised definition of acute lower respiratory tract illness: cough as the cardinal symptom with at least one of sputum production, dyspnoea, wheeze and chest discomfort / pain.(192) This, however, was based on physician definition of symptoms, and did not include any quantification of the severity of these symptoms, although Martineau *et al* subsequently scored these symptoms on a 0-3 scale for the purpose of quantifying severity of illness in a randomised trial.(193) Validation of the use of this scale in this way has not, however, been published, and the publication by Martineau *et al* was not available when our study was developed in 2014.

We therefore chose to create a symptom score specifically for this study – one aspect of the Macfarlane symptoms is that these were based on physician recognition of symptoms rather than participant self-report. What constitutes the symptom of “wheeze” is not defined and may be interpreted in several ways by individuals; and we therefore chose not to include wheeze in the symptoms recorded. However other than this, the symptoms documented in the 0-6 scale used included those in the Macfarlane definition. Once the score had been developed, it was piloted by four individuals to ensure feasibility of completion. No formal validation process was undertaken.

4.4.10 Assessment during acute respiratory illnesses

When participants reported an acute respiratory tract illness, they were requested to attend the hospital and have samples taken for the detection of bacterial and viral pathogens.

Samples collected from all participants who attended to be reviewed consisted of nasopharyngeal swabs for respiratory virus detection. Where possible a spontaneous or induced sputum sample was also collected for the detection. This was sent for routine bacterial culture; where sufficient residual sample was available this was retained and stored at -80°C for subsequent molecular microbiological analysis.

4.4.11 Statistical analysis

Statistical analysis of study results was undertaken to determine the significance of, and differences in, the primary and secondary study outcomes between the study groups. Baseline characteristics of the HIV positive and negative participants were compared by using t-test or Wilcoxon rank-sum test for normally and non-normally distributed continuous values. Chi-squared and Fisher's exact tests were used for categorical values.

4.4.12 Analysis of primary outcome

For the primary analysis, all participants who were recruited to the study and provided more than one week of follow-up data were included in the analysis.

An acute respiratory illness was defined as the new onset any of the following symptoms lasting more than 24 hours:

1. cough
2. sore throat
3. blocked or runny nose with or without a sensation of facial pain or pressure
4. breathlessness or pain on breathing

For an event to be labelled as a new illness, participants needed to have recorded at least one week without symptoms between events. Counting of independent events was undertaken by creating a graphical representation of the weeks of follow-up for each study participant, with the number of independent events manually counted.

The primary outcome of the study will be the annual incidence of acute respiratory illness in HIV infected adults compared to those without HIV infection. The number of events recorded in each group was divided by the number of weeks of follow up to give a rate of events per person-year of follow-up.

We analysed results using univariable Poisson regression analyses to determine the statistical significance of differences in frequency of acute respiratory illness according to baseline characteristics and to explore the effect of different patient characteristics (e.g. age, HIV status, smoking history, baseline respiratory symptoms and spirometric impairment) on frequency of illness.

For multivariable analyses, we compared the frequency of respiratory illness using Incidence Rate Ratios (IRR) and multivariable Poisson regression to adjust for potential confounders and explore the effect of participant characteristics on frequency of acute respiratory illness. Assumptions necessary for Poisson regression were checked and the analysis repeated using a negative binomial model to confirm that conclusions drawn were robust to the analysis method.

4.4.13 Analysis of duration of illness

Participants were asked to complete a daily diary recording their symptoms whilst unwell for as long as symptoms persisted, or up to 14 days. The written instructions provided with this diary asked participants to continue completing this until they felt “back to normal”. Therefore, the end of an illness was defined as the day that a participant stops entering data into the daily diary, or reports that they are no longer feeling unwell (defined as answering “0 - no problem” to the question “overall, how unwell do you feel?”). The total duration of illness (in days) for each illness was calculated and the median duration of illness in the HIV positive and negative groups compared.

4.4.14 Analysis of severity of illness

Total symptom scores for each day of illness were calculated by adding the scores for individual symptoms, with no weighting applied. These total scores were compared between HIV positive and negative groups by means of Wilcoxon rank-sum tests. The total scores were log-transformed to approximate a normal distribution and linear regression analyses of log-transformed total symptom scores were used to adjust for differences in baseline characteristics with appropriate adjustment for clustering. Factors considered for inclusion in these multivariable analyses were chosen *a priori* based on those with known or likely associations with acute respiratory illness (gender, tobacco smoking and use of recreational drugs, respiratory symptoms at baseline, presence of abnormal spirometry at baseline). Analyses were conducted using Stata v14 with appropriate adjustment for the clustered nature of the data (with multiple observations per participant).

The presence of chronic respiratory symptoms at baseline may affect reporting of symptoms during acute respiratory illness (e.g. individuals with chronic cough or breathlessness may be more likely to report these during acute illness). Therefore, in addition to comparing the whole HIV positive and negative groups I undertook a further analysis in which participants were stratified according to the presence of respiratory symptoms at baseline (measured by the MRC dyspnoea scale score or St Georges Respiratory Questionnaire score).

4.4.15 Healthcare utilisation during acute respiratory illness

Using the written diaries completed by participants, the proportion of illnesses for which participants sought treatment in GP practice, Hospital (outpatient clinic or A&E) or in-patient treatment was measured. To determine the statistical significance of differences in proportion of participant using these healthcare services, I performed logistic regression analyses with appropriate adjustment for repeated measures. The proportion of participants taking antibiotics and non-prescription medications was also compared.

4.4.16 Pre-planned sensitivity analyses

Sensitivity analyses were pre-specified in a data analysis plan prepared before study results were available and undertaken to evaluate the effect of missing responses on the primary outcome. The following sensitivity analyses were pre-specified:

a. Excluding participants with less than an 80% response rate to the weekly study contact.
b. Defining participants who stopped responding to weekly contacts after a point (e.g. 6 months) as having dropped out of the study. This therefore gave different durations of follow-up for some participants and I used this alternative offset value in the regression analysis to account for this.
c. Rather than analysing the primary outcome as a count variable of the number of events occurring during follow-up, I calculated the proportion responses from each participant in which they reported an acute respiratory illness. This therefore provided a continuous variable which could be explored using linear regression.

4.4.17 Calculation of required sample size

The primary study outcome was number of acute respiratory tract illnesses occurring over a one-year follow up period, this included both upper and lower respiratory tract illnesses. The Pulmonary Complications of HIV study, Multicentre AIDS Cohort Study and the Women's Interagency HIV study all found that HIV infected participants had rates of respiratory tract infections approximately 50% higher than in HIV uninfected individuals (22, 30).

The FluWatch study of the epidemiology of influenza in the UK provided data regarding the expected numbers of acute respiratory illnesses in our study; this was used to estimate the required samples size (194). This study also used weekly diaries of acute respiratory symptoms to determine the frequency of acute respiratory illness and reported that 44% of those without serological evidence of influenza infection experienced an acute respiratory illness during each influenza season (the Fluwatch study was only conducted during influenza seasons). Based on this, we conservatively estimated that at least 44% of the HIV negative participants would have an acute respiratory illness over a 12-month period. As both the Pulmonary Complications of HIV infection study and the Multicentre AIDS cohort study found around a 50% greater frequency of acute respiratory illnesses in People Living with HIV, we therefore predicted that 68% of the HIV infected individuals would develop an acute respiratory illness over a 12-month period.

We planned to have a 2:1 ratio between HIV infected participants and controls, as we anticipated that the HIV infected participants would be easier to recruit. To have an 80% power to detect this difference (i.e. 68% vs. 45%) with a type 1 error of 5% we estimated that we needed 119 HIV positive and 60 HIV negative participants in the cohort. We assumed up to a 20% drop out of subjects from the study and therefore aimed to recruit 140 individuals with HIV infection and 70 individuals without HIV infection. The choice of a 2:1 case-to-control ratio was made due to pragmatic considerations of our likely ability to recruit in a timely way within the two study populations. A 1:1 ratio may have been preferable, although this would have offered less ability to stratify within the HIV positive group, and so identify factors that may be contributing to any noted differences between HIV positive and negative participants.

4.4.18 Data management

Data were collected in participant-completed questionnaires and additional information extracted from hospital electronic medical records with participant consent. Data were stored identifiable by study number in a database held on secure hospital systems. The hard-copies of the questionnaires were stored in a locked filing cabinet on the hospital site. For statistical analyses, pseudo-anonymised data were exported into SPSS and Stata.

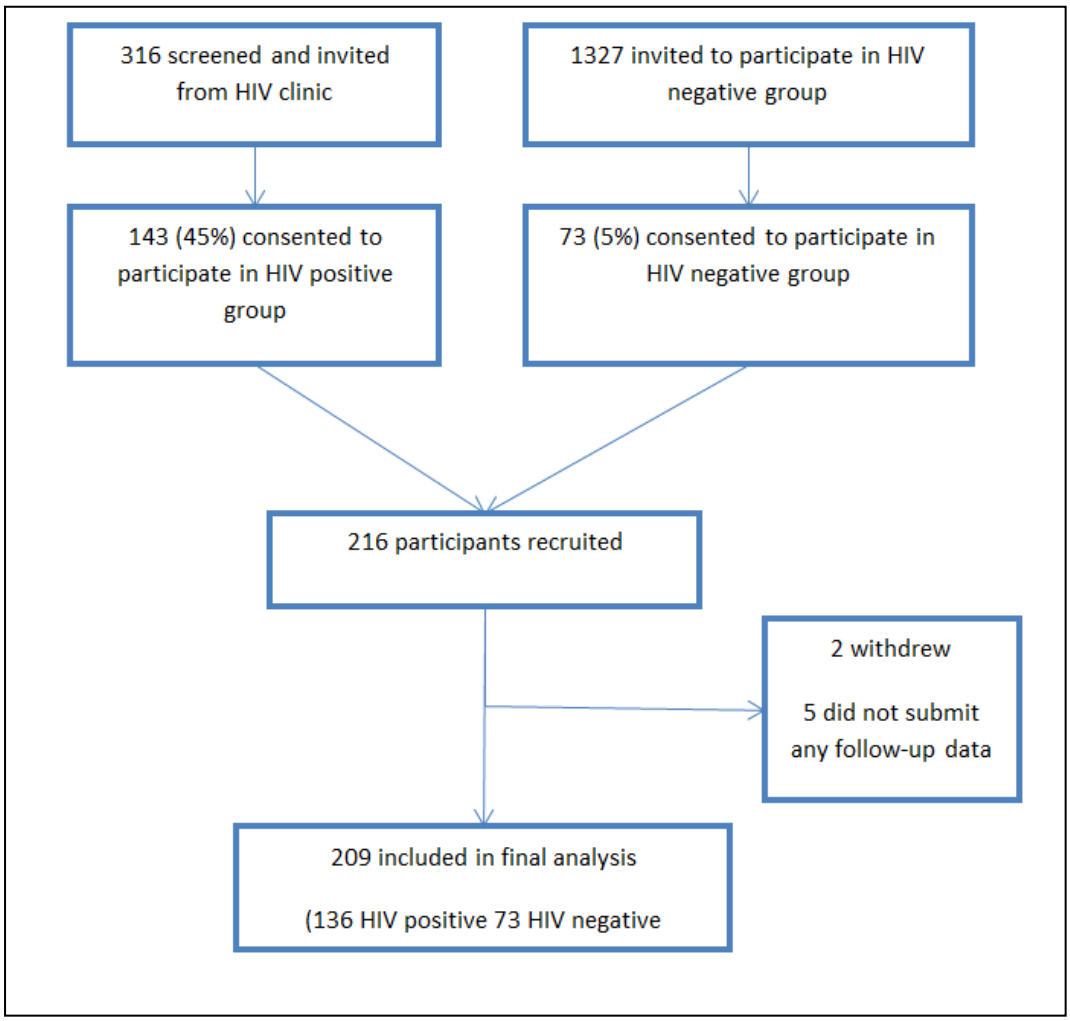
4.5 Results

4.5.1 Participant recruitment

We undertook recruitment between November 2015 and January 2017 (Figure 12). In total, we invited 317 HIV positive individuals to participate from HIV ambulatory care clinic, of whom 143 (45%) consented to participate and attended for their baseline clinic visit. There was no significant difference in the median age or gender of those who did and didn't consent to participate: (median (IQR) ages of 51 (46-55) vs 49 (41-54) years respectively); uptake was lower in women invited to participate (38% (30/78)) than men, in whom 48% consented to participate (106/221), although this difference was not statistically significant (p=0.15).

The response rate to postal invitations sent to HIV negative potential participants from Primary Care was 6%: 73 individuals reported that they would be interested in taking part in the study from 1,327 invited by post. Of the 77 who stated that they would be prepared to take part in the study, 63 attended for a baseline visit and were included in the cohort. The positive response rate was 4.5% for women (17 recruited from 376 invitations) and 5% in men (48 recruited from 951 invitations); 4.4% of those recorded as being current smokers in Primary Care records responded positively (20/451) compared to 5.7% of never smokers (42/725) and 1% of those recorded as being ex-smokers (2/151). Uptake was 5.6% in those aged 45-54 (59/1056) and 10.8% in those aged 65-75 (13/120).

FIGURE 11 STUDY RECRUITMENT FLOWCHART



4.5.2 Follow-up and completion of weekly responses

The median number of weeks for which each participant provided data was 44/52 weeks (85%); this was not significantly different between HIV positive and negative participants.

4.5.3 Baseline details of study participants

Demographic details of study participants are given in **Tables 21 and 22**. There were no significant differences in age or gender of the HIV positive and negative participants, but the PLWH were more likely to be current smokers (30% vs 15%, p=0.04) and more likely to report previous or current (within the last 3 months) use of recreational drugs. HIV positive participants had a median (IQR) CD4 count of 686 (458-848) cells/µL; 87% had an HIV load < 40 copies/ml.

Most participants had normal spirometry, with no significant difference in the proportion of HIV positive and negative people with obstructive spirometry (14 (14%) vs 11 (11%), p= 0.62). A greater proportion of HIV positive participants had restrictive spirometry (17 (17%) vs 2 (3%), p=0.01).

HIV positive participants had worse respiratory health status at baseline with higher scores on the St George's Respiratory Questionnaire (median SGRQ Total score 13 (IQR 5-28) vs 6 (2-9), p< 0.001) and were also more likely to report breathlessness using the MRC dyspnoea scale (59/138 43% vs 12/72 17% reporting MRC dyspnoea scale score of 2 or more, p= <0.001), representing at least "short of breath when hurrying or walking up a slight hill". HIV positive participants also reported a lower general health-related quality of life at baseline using the EQ5D-5L scale with UK with mean UK index scores of 0.84 (SD 0.21) and 0.95 (SD 0.08) in HIV positive and negative groups respectively (p<0.001) and Visual Analogue Scale scores of 75 (SD 19) vs 83 (SD 11) (p=0.001).

4.5.4 Detection of respiratory viral pathogens at baseline

We detected viral respiratory pathogens in 5 (2%) of baseline oropharyngeal swabs, all from HIV positive participants. These were: Parainfluenza 2 (two participants), Coronavirus OC43, Influenza A and Parechovirus. All other participants had negative baseline swab results.

TABLE 21: PARTICIPANT BASELINE CHARACTERISTICS

		HIV Positive N=136	HIV Negative N=73	p value
Gender	Female, n (%)	30 (22%)	18 (25%)	0.67*
	Male, n (%)	106 (78%)	55 (75%)	
Age, years, mean (SD)		50 (11)	52 (8)	0.11**
Ethnicity	Caucasian, n (%)	103 (76%)	70 (96%)	**<0.01** $^{\Delta}$
	Black African / Caribbean, n (%)	23 (17%)	0	
	South Asian, n (%)	2 (1%)	2 (3%)	
	Other, n (%)	8 (6%)	1 (1%)	
UK Born, n (%)		73 (54%)	54 (74%)	0.07*
Sexuality	Heterosexual, n (%)	37 (27%)	57 (78%)	<**0.001** $^{\Delta}$
	Homosexual, n (%)	86 (62%)	16 (22%)	
	Bisexual, n (%)	12 (9)	0	
	Not answered, n (%)	1 (1%)	0	
Educational attainment	No qualifications, n (%)	7 (5%)	2 (3%)	0.54 $^{\Delta}$
	GCSE or equivalent, n (%)	20 (15%)	11 (15%)	
	A level or equivalent, n (%)	24 (18%)	8 (11%)	
	University education, n (%)	72 (53%)	44 (60%)	
	Other qualifications, n (%)	13 (10%)	7 (10%)	
	Not answered, n (%)	0	1 (1%)	
Immunisations (self-report)	Influenza (last 12 months)	90 (66%)	21 (29%)	**<0.01***
	Pneumococcal (ever)	50 (37%)	9 (12%)	**<0.01***
Comorbid conditions (self-report)	Asthma	22 (16%)	7 (10%)	0.21 $^{\Delta}$
	COPD	3 (2%)	1 (1%)	0.56 $^{\Delta}$
	Heart disease	5 (4%)	2 (3%)	1 $^{\Delta}$
Previous history of respiratory opportunistic infection (HIV positive participants only)		9 (7%) $^{\#\#}$		
Use of inhaled medications	Any inhaled medication	25 (18%)	5 (7%)	0.07 $^{\Delta}$
	Inhaled corticosteroids	13 (10%)	1 (1%)	0.04 $^{\Delta}$
Tobacco smoking	Current smoker, n (%)	39 (29%)	12 (16%)	0.08*
	Ex-smoker, n (%)	46 (34%)	34 (47%)	
	Never smoker, n (%)	41 (37%)	27 (37%)	
Tobacco pack-years (median, IQR), $^{\lozenge}$		6 (2-12)	9 (3-15)	0.17 $^{\#}$
Recreational drug use (ever), n (%)		90 (66%)	37 (51%)	**<0.005***
Recreational drug use (last 3 months), n (%)		42 (31%)	9 (12%)	**0.007***

* Chi squared test, ** t –test # Mann Whitney Test, Δ Fisher's exact test

◊ calculated for smokers and ex-smokers only

consisting of PCP pneumonia (7 cases) tuberculosis (1 case) non-tuberculous mycobacterial infection (1 case)

TABLE 22 BASELINE RESPIRATORY HEALTH STATUS AND SPIROMETRY

			HIV Positive (N=136)	HIV Negative (N=73)	p value
Baseline SGRQ score	Symptoms, median (IQR)		30 (8-45)	11 (0-28)	<0.001#
	Activity, median (IQR)		18 (6-36)	6 (0-12)	<0.001#
	Impacts, median (IQR)		4 (0-16)	0 (0-2)	<0.001#
	Total score, median (IQR)		13 (6-29)	6 (2-9)	<0.001#
Baseline MRC Dyspnoea score	1: Not troubled by breathless except on strenuous exercise, n (%)		77 (57%)	58 (81%)	0.008Δ
	2: Short of breath when hurrying on a level or when walking up a slight hill, n (%)		43 (32%)	12 (17%)	
	3: Walks slower than most people on the level, stops after a mile or so, or stops after 15 minutes walking at own pace, n (%)		6 (4%)	0	
	4: Stops for breath after walking 100 yards, or after a few minutes on level ground, n (%)		4 (3%)	0	
	5: Too breathless to leave the house, or breathless when dressing/undressing, n (%)		1 (1%)	0	
	Not answered		3 (2%)	2 (3%)	
Spirometry*	FEV1, L, mean (SD)		3.22 (0.78)	3.53 (0.73)	0.01
	FEV1 % predicted, mean (SD)		91% (14%)	97% (11%)	0.005
	FVC, L, % predicted, mean (SD)		4.16 (1.01)	4.55 (0.98)	0.02
	FVC % predicted, mean (SD)		93 (14%)	99 (12%)	0.02
	Spirometry interpretation	Airflow obstruction, n%	13 (13%)	5 (8%)	0.004Δ
		Restriction, n%	18 (19%)	2 (3%)	
		Normal spirometry n%	65 (68%)	54(88%)	

* 96 HIV positive and 61 HIV negative participants had spirometry results meeting ATS/ERS quality criteria #Mann-Whitney test # chi squared test ~ t-test Δ Fisher's exact test

4.5.5 Results of prospective follow up: frequency of acute respiratory illness

There was no significant difference in the frequency of acute respiratory tract illness between HIV positive and negative participants. The incidence rate per person year of follow-up was 2.08 (95% CI 1.81-2.38) in HIV positive and 2.30 (1.94-2.70) in HIV negative participants; IRR 0.87 (0.70-1.07 p=0.18).

In univariable regression analyses, smoking status (being an ex-smoker), airflow obstruction (FEV1/FVC <0.7) and the presence of chronic respiratory symptoms (MRC score ≥2) at baseline were all associated with a significantly greater frequency of acute respiratory illness, and participants of black ethnicity reported a lower frequency of events than White participants (0.64 (0.42-0.96) p=0.03).

In a multivariable model including HIV status, age, gender, ethnicity and the presence of spirometric abnormality, there was no significant difference in the frequency of acute respiratory illness between HIV positive and negative participants (adjusted IRR 0.80 (0.61-1.06) p=0.13). Only tobacco smoking history (being an ex-smoker) and obstructive spirometry were independently associated with a greater frequency of acute respiratory illness in this model.

TABLE 23 RISK FACTORS FOR ACUTE RESPIRATORY ILLNESS AMONG COHORT PARTICIPANTS

Characteristic		N	Incidence rate of acute respiratory illness per person year of follow-up	Univariable analysis [b]		Multivariable analysis [b]	
				IRR (95% CI)	P value	IRR (95% CI)	P value
HIV status	HIV positive	136	2.08 (1.81-2.38)	0.87 (0.70-1.07)	0.18	0.81 (0.61-1.06)	0.13
	HIV negative	73	2.30 (1.94-2.70)	1		1	
Gender	Female	48	1.97 (1.56-2.48)	0.84 (0.54-1.23)	0.42	0.88 (0.65-1.21)	0.45
	Male	161	2.21 (1.95-2.47)	1		1	
Age (years)	65+	24	1.98 (1.42-2.68)	0.94 (0.67-1.32)	0.40	0.87 (0.58-1.31)	0.90
	55-65	35	2.19 (1.69-2.79)	1.04 (0.79-1.38)		1.03 (0.82-1.46)	
	45-55	104	2.19 (1.89-2.53)	1		1	
	<45	46	2.11 (1.64-2.67)	0.83 (0.63-1.09)		0.98 (0.68-1.43)	
Tobacco Smoking	Current smoker	51	2.25 (1.82-2.76)	1.12 (0.85-1.48)	0.08	1.28 (0.92-1.77)	0.04
	Ex-smoker	80	2.37 (2.01-2.77)	**1.29 (1.02-1.62)**		**1.45 (1.08-1.93)**	
	Never smoker	78	1.85 (1.53-2.23)	1		1	
Lung function	Obstructive	13	3.33 (2.42-4.47)	1.24 (0.90 1.72)	0.22	**1.69 (1.16-2.46)**	**0.01**
	Restrictive	18	1.71 (1.11-2.50)	0.81 (0.52-1.22)		0.83 (0.54-1.29)	
	Normal	65	2.07 (1.54-2.23)	1		1	
Baseline MRC breathlessness score	3-5	11	2.57 (1.63-3.86)	1.35 (0.82-2.22)	**0.01**		
	2	**55**	**2.64 (2.17-3.17)**	**1.37 (1.08-1.73)**			
	1	135	1.88 (1.63-2.15)	1			
Recreational drug use, ever	Yes	77	2.19 (1.92-2.50)	1.22 (0.85-1.76)	0.28		
	No	127	2.03 (1.68-2.42)	1			
Recreational drug use, last 3 months	Yes	51	2.11 (1.69-2.60)	0.92 (0.51-1.37)	0.67		
	No	153	2.18 (1.93-2.46)	1			
Ethnicity	Black African / Caribbean	**23**	**1.61 (1.06-2.37)**	**0.64 (0.42-0.96)**	**0.01**	1.07 (0.62-1.88)	0.25
	South Asian / other / mixed	13	3.01 (1.50-5.39)	1.39 (0.98-2.00)		1.40 (0.94-2.07	
	White	173	2.13 (1.90-2.38)	1		1	
Educational attainment	Other	20	2.27 (1.61-3.12)	0.88 (0.56-1.39)	0.91		
	Degree	116	2.38 (2.07-2.73)	0.94 (0.70-1.25)			
	A level	32	1.89 (1.41-2.47)	0.91 (0.63-1.31)			
	None/GCSE	40	1.64 (1.23-2.13)	1			
Baseline SGRQ	>20	42	2.72 (2.17-3.38)	1.39 (0.68-2.24)	0.14		
	10-20	28	2.52 (1.99-3.14)	1.41 (0.69-2.89)			
	<10	96	1.84 (1.58-2.11)	1			
Current CD4[a]	>500	94	1.98 (1.67-2.33)	0.73 (0.37-1.42)	0.27		
	350-500	27	1.86 (1.35-2.51)	0.58 (0.26-1.29)			
	<350	15	3.30 (2.23-4.72)	1			
Nadir CD4[a]	500+	17	1.86 (1.19-2.76)	0.75 (0.38-1.48)	0.28		
	350-500	20	2.05 (1.40-2.90)	0.84 (0.45-1.58)			
	200-350	36	1.84 (1.37-2.41)	0.93 (0.55-1.56)			
	<200	63	2.26 (1.78-2.72)	1			

a: calculated for HIV positive participants only b: Poisson regression

4.5.6 Severity and duration of illness

In response to the web-based symptom questions (completed by 90% of participants; 361 (97%) of illnesses), HIV positive participants reported a greater symptom severity score, with a median total score at the time of reporting illness of 14 points (IQR 8-23) in HIV positive and 9 (5-14) in HIV negative participants (fold change in score 1.61 (1.28-2.02), p<0.001) (Figure 13).

Participants recorded written diaries detailing daily symptoms during 166 (45%) acute respiratory illnesses. During these illnesses, HIV positive participants also reported greater symptoms scores with median total symptom scores per day of 9.36 (IQR 5.77-14.95) in HIV positive and 6.4 (4.74-9.82) in HIV negative participants (p=0.008). HIV positive participants also reported more days with at least mild symptoms with a median duration of 8 (IQR 5.1-10.5) vs 6 (4.25-9.5) days, but this difference was not statistically significant (p=0.18). The total symptom scores per day in HIV positive and negative participants are displayed in Figure 14.

FIGURE 12 PARTICIPANT-REPORTED SCORES FOR INDIVIDUAL SYMPTOMS DURING ACUTE RESPIRATORY ILLNESS

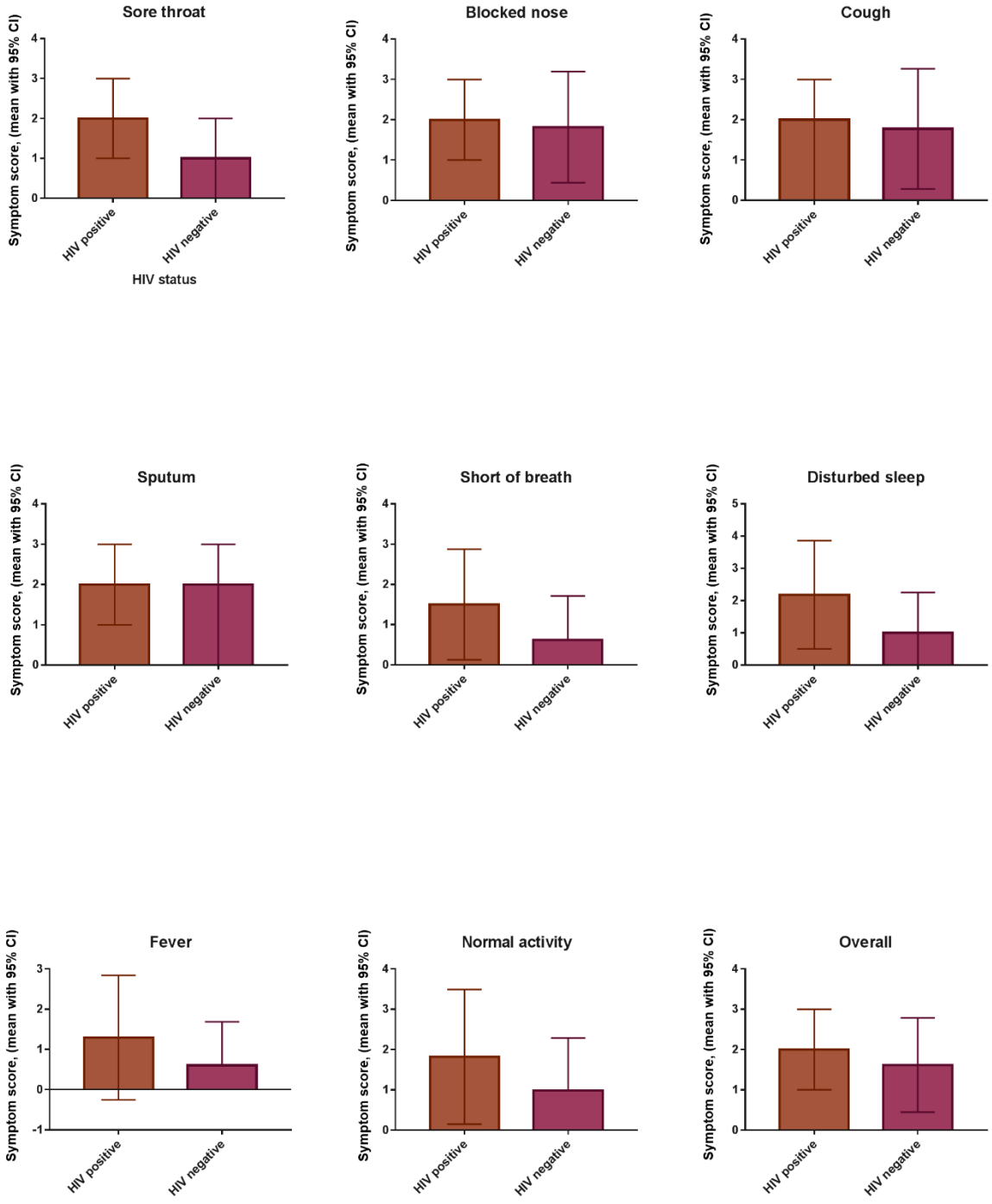
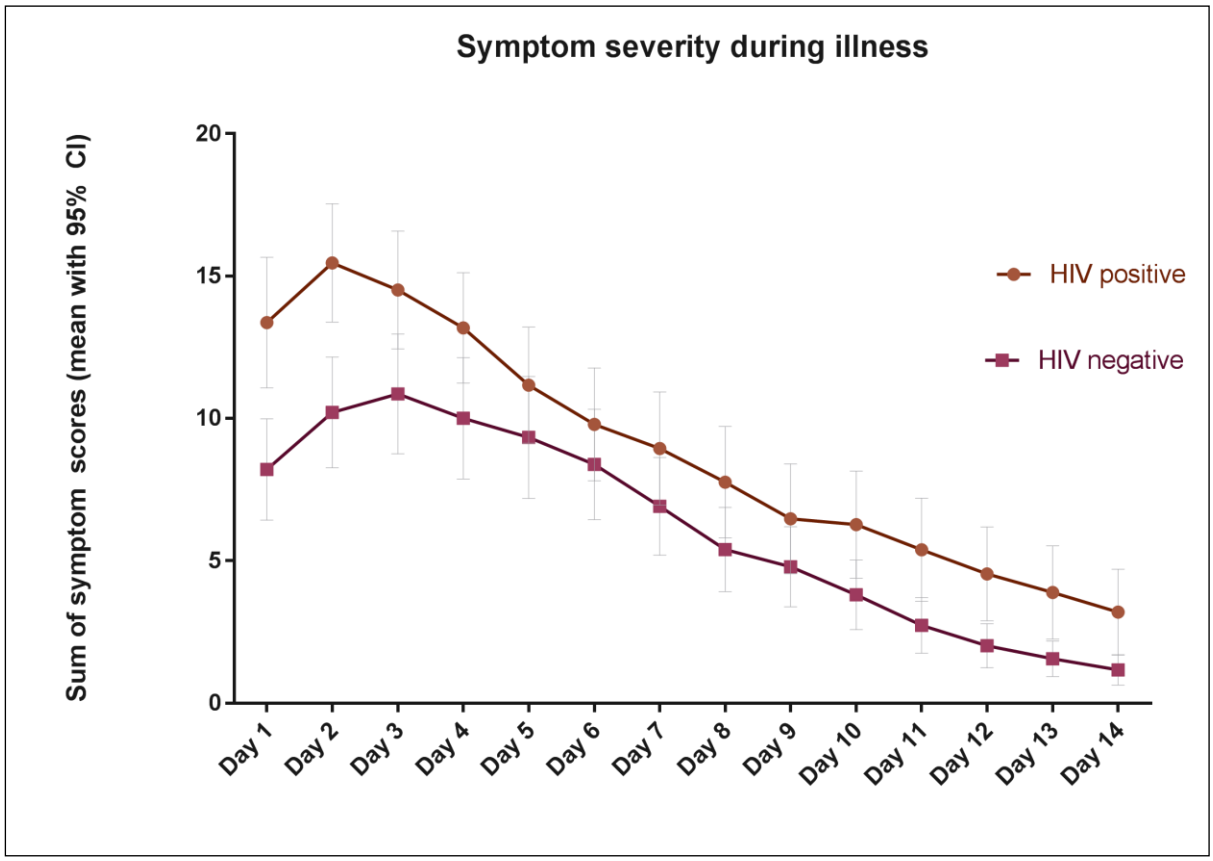


Figure 13 **Symptoms during acute respiratory illness**



4.5.7 Effect of participant baseline characteristics on symptom scores during acute respiratory illness

We explored the relationship between participant baseline characteristics and the severity of symptoms during acute respiratory illness. In univariable log-scale linear regression analyses, HIV status, airflow obstruction (FEV1/FVC <0.7) recent (within 3 months) use of recreational drugs and the presence of increased respiratory symptoms at baseline (MRC dyspnoea score or St George's Respiratory Questionnaire score) were associated with greater self-reported symptom severity during acute respiratory illness. HIV positive participants had a 61% greater symptom score in univariable analyses (fold change in symptom score 1.61, (95% CI 1.28-2.02, p <0.001).

In accordance with the statistical analysis plan, HIV status, gender, baseline spirometry, use of recreational drugs, tobacco smoking and baseline respiratory symptoms were adjusted for in multivariable analyses. These had been chosen *a priori* and no additional factors were added after univariable analysis. Baseline MRC dyspnoea scale score was not also included in the multivariable analysis (despite being significantly associated with symptom score in univariable analysis) due to collinearity with baseline SGRQ scores.

In a multivariable regression model including HIV status, spirometry, recent recreational drug use, SGRQ score, and tobacco smoking, only HIV status remained significantly associated with greater symptom scores during acute respiratory illness, with an adjusted fold-change in symptom score of 1.50 (1.14-1.97), p= 0.004.

TABLE 24 RELATIONSHIP BETWEEN PARTICIPANT BASELINE CHARACTERISTICS AND SYMPTOM SEVERITY AT TIME OF REPORTING ACUTE RESPIRATORY ILLNESS, LOG-SCALE LINEAR REGRESSION ANALYSES

Characteristic		**Median (IQR) symptom score**[#]	**Univariable analysis**		**Multivariable analysis**	
			Fold-change in total symptom score*	P value[#]	Fold-change in total symptom score	P value**
HIV Status	HIV Positive	14 (8-23)	1.61 (1.28-2.02)	**<0.001**	**1.50 (1.14-1.97)**	**0.02**
	HIV Negative	9 (5-14)	1		1	
Gender	Female	13 (7-22)	1.08 (0.77-1.66)	0.648	1.30 (0.92-1.83)	0.15
	Male	11 (6-20)	1		1	
Spirometry	Restrictive	12 (6-23)	1.00 (0.66-1.51)	0.04	0.96 (0.69-1.35)	0.56
	Obstructive	20 (10-23)	1.51 (1.10-2.07)		1.16 (0.80-1.67)	
	Normal	11 (6-19)	1		1	
Recreational drugs (ever)	Yes	12 (6-21)	1.64 (0.81-1.36)	0.71		
	No	12 (6-20)	1			
Recreational drugs, past 3 months	Yes	15 (8-21)	1.34 (1.07-1.67)	**0.01**	1.27 (0.95-1.72)	0.27
	No	11 (6-20)	1		1	
Ethnicity	Black African / Caribbean	8 (4-21)	0.83 (0.41-1.68)	0.47		
	South Asian Other/Mixed	13 (9-31)	1.08 (0.48-2.43)			
	White / Caucasian	12 (6-20)	1			
Qualifications / educational attainment	None	6 (5-24)	0.92 (0.69-1.22)	0.07		
	GCSE	8 (4-14)	0.75 (0.55-1.02)			
	A level	10 (7-17)	0.78 (0.54-1.14)			
	Other	13 (5-22)	1.06 (0.58-1.53)			
	University /degree	13 (6-21)	1			
Baseline St Georges Respiratory Questionnaire score	>20	14 (9-23)	1.68 (1.30-2.18)	**<0.001**	1.42 (1.11-1.83)	0.05
	10-20	14 (8-21)	1.48 (1.14-1.93)		1.43 (1.05-1.96)	
	<10	9 (4-18)	1		1	
Baseline MRC Dyspnoea score	3-5	14 (6-33)	1.75 (1.03-2.98)	**0.004**		
	2	13 (7-21)	1.22 (0.53-1.58)			
	1	10 (5-18)	1			
Tobacco smoking	Current smoker	11 (1-21)	1.12 (0.83-1.51)	0.22	0.91 (0.63-1.33)	0.96
	Ex-smoker	11 (5-20)	0.92(0.70-1.02)		1.06 (0.81-1.38)	
	Never smoker	12 (6-19)	1		1	

#Univariable log-scale linear regression analysis * univariable log-scale linear regression model ** multivariable log-scale linear regression including all factors with data in this column

4.5.8 Treatment and healthcare utilisation

Using the responses to the written diaries, HIV positive participants were more likely to seek advice from a healthcare professional during acute respiratory illness (42% vs 14%, p=0.003) and more likely to seek hospital outpatient assessment (for instance in an HIV ambulatory care service) **Table 25**. There was no significant difference in the proportion of illnesses for which participants took non-prescription medications (59% vs 54%). HIV positive participants took antibiotics during a numerically greater proportion of illnesses (22% vs 11.5%), but this difference was not statistically significant (OR (95% CI) 2.11 (0.75-5.94), p= 0.16).

TABLE 25 MEDICATION AND HEALTHCARE UTILISATION DURING ACUTE RESPIRATORY ILLNESSES

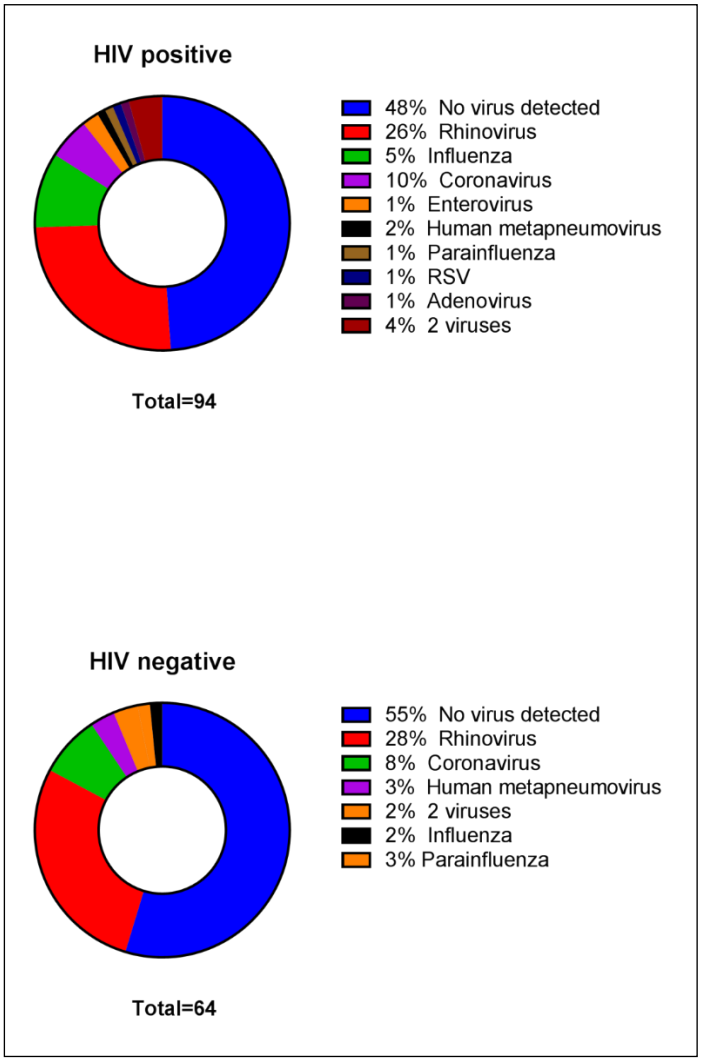
		HIV positive (88 illnesses)	HIV negative (78 illnesses)	Odds ratio[#]	P value
Healthcare utilisation	GP, (n%)	11 (12.5%)	8 (10%)	1.25 (0.40-3.68)	0.70
	Pharmacist, (n%)	7 (8%)	5 (6%)	1.26 (0.39-4.05)	0.696
	Primary care nurse, (n%)	6 (7%)	2 (3%)	2.78 (0.49-15.72)	0.247
	Hospital outpatient assessment, (n%)	**28 (32%)**	**7 (9%)**	**4.73 (1.91-11.69)**	**0.001**
	Emergency department, (n%)	5 (6%)	1 (1%)	4.63 (0.53-40.3)	0.164
	Any (excluding pharmacy), (n%)	**37 (42%)**	**14 (18%)**	**3.32 (1.48-7.39)**	**0.003**
Treatment	Over the counter medications, (n%)	52 (59%)	42 (54%)	1.23 (0.61-2.51)	0.55
	Antibiotics, (n%)	19 (22%)	9 (12%)	2.11 (0.75-5.94)	0.16

logistic regression analysis adjusting for clustered data using Generalised Estimating Equations

4.5.9 Isolation of viral and bacterial pathogens during acute respiratory illness

We collected 162 nasopharygeal swabs from study participants during acute respiratory illness. Viral pathogens were isolated in 77 (48%) of these samples, with no significant difference in the likelihood of isolation of respiratory viral pathogens between HIV positive and negative groups: a respiratory viral pathogen was identified during 51% of illnesses in HIV positive participants (48/95) compared to 43% of HIV negative participants (29/67), p=0.36. Rhinovirus was the predominant virus identified in both groups (**Figure 15**).

FIGURE 14 DETECTION OF RESPIRATORY VIRAL PATHOGENS FROM NASOPHARYNGEAL SWABS DURING ACUTE RESPIRATORY ILNESS



We obtained sputum samples for bacteriological culture during 70 illnesses from 37 participants (33 of whom were HIV positive). Bacterial pathogens were identified by routine bacteriological culture in 10 (19%) of these samples from 9 different participants, all of whom were HIV positive. The pathogens identified were: *Haemophilus influenzae* (5 isolates from 4 participants), *Streptococcus pneumoniae* (3 isolates each from different participants), *Moraxella catarrhalis* (2 isolates from different participants). The fungal pathogen Aspergillus *fumigatis* was identified from one sample.

Of the 10 samples from which bacterial pathogens were isolated, 5 had antimicrobial resistance to first line oral antibiotics detected: (2 of 5 samples with H*aemophilus influenzae* and all three isolates of S*treptococcus pneumoniae*). Table 26 contains details of the antimicrobial resistance detected.

TABLE 26 ANTIBIOTIC RESISTANCE PROFILES FROM SPUTUM SAMPLES DURING ACUTE ILLNESS

Streptococcus pneumoniae					
ISOLATE	Levofloxacin	Penicillin	Tetracycline	Erythromycin	Ceftriaxone
1	S	I	R	R	ND
2	S	I	R	R	S
3	S	R	R	R	ND

Haemophilus influenzae				
ISOLATE	Ampicillin	Clarithromycin / erythromycin	Tetracycline	Co-Amoxiclav
1	S	S	ND	ND
2	R	R	R	S
3	S	R	ND	S
4	S	ND	S	ND

4.5.10 Sensitivity analyses

In accordance with the analysis plan, I conducted several pre-specified sensitivity analyses to ensure that our findings were robust using different analytical methods. These included: a) excluding all participants with less than 80% response rate; b) defining the offset value for the regression analyses as the number of weeks between the first and last response to the weekly messages recorded (rather than the total number of responses) and c) defining the outcome as the proportion of weeks of follow up in which a new respiratory illness was reported (thus giving a continuous rather than count variable) and performing linear regression analyses. The findings of the main analysis (that frequency of acute respiratory illness did not differ with HIV status, but PLWH reported more severe symptoms when these illnesses occurred) were consistent in all of these sensitivity analyses.

To further explore the effect of baseline respiratory symptoms on illness frequency and severity, we stratified participants according to the presence of chronic respiratory symptoms, based on baseline MRC dysponea scale score (1 or 2-5). In this analysis, in the group with a baseline MRC dyspnoea score of 1, a similar magnitude of difference in the participant-reported symptom severity scores was found to the whole dataset (fold-change in online severity score 1.31 (95% CI 0.92-10.12) although this difference was not statistically significant (p= 0.06). In participants with an MRC dyspnoea scale score of 2 or more a greater effect of HIV status was found, with a fold-change in symptom score of 1.97 (1.32-2.94).

4.6 Conclusions

In this prospective cohort study, we found that the frequency of acute respiratory illness did not differ between HIV positive and negative individuals in a setting with a high uptake of antiretroviral therapy, but HIV positive individuals reported more severe and longer lasting illnesses. This greater symptom severity during acute respiratory illness was associated with an increased likelihood of seeking healthcare advice and a higher (although not statistically significant) proportion of illnesses treated with antibiotics.

As in other populations,(195) HIV positive participants were more likely to report chronic respiratory symptoms at baseline, and this was independently associated with the severity of acute respiratory illnesses during follow-up. However, the greater prevalence of baseline respiratory symptoms did not account for all of the difference between HIV positive and negative participants, as higher symptom scores in HIV positive participants persisted after adjustment for baseline symptoms.

As with the higher prevalence of respiratory symptoms found in the cross-sectional study described in Chapter 3, there are several possible explanations for the greater severity of self-reported symptoms during acute respiratory illness in PLWH. It is possible that impairments in lung function that were not measured by spirometry were present in the HIV positive group, as deficiencies in diffusing capacity,(94) or small airways disease, (196) may be more common among PLWH. Furthermore, an awareness among PLWH of the potential for more severe illness might lead to an increased perception or concern about physical symptoms. In addition, the higher prevalence of anxiety or depression (which impact on respiratory symptoms) in this population could contribute to the greater reporting of physical symptoms. (174, 197) Finally, there is evidence to suggest that despite immune reconstitution following antiretroviral therapy PLWH using ART have higher levels of immune activation than HIV negative individuals (107) – thus the heightened symptom burden during ARI could be a manifestation of a disordered immune response during these illnesses.

This study has several limitations: it aimed to document the frequency of all acute respiratory illness, and therefore it was not powered to measure less common but more severe events such as bacterial pneumonia. Epidemiological data suggest that pneumonia continues to be more common among PLWH, and remains a significant cause of mortality.(187) The lack of a difference in acute respiratory illness frequency in our data does not necessarily imply that severe respiratory infections such as bacterial pneumonia are not more common among PLWH. Whether HIV positive people with a good response to antiretroviral therapy continue to be at greater risk of severe bacterial pneumonia is a question that requires further attention. Our study was based on self-report of acute respiratory illness, and utilised participant reported outcomes to measure severity of respiratory symptoms, rather than having any objective measurement of illness severity based on physiological parameters or biomarkers in blood samples. Although the response rate to the weekly study contacts was very good (85%), written daily diaries during acute illnesses were only available for 46% of illnesses and biases in these responses could affect conclusions. Furthermore, as with any study evaluating a cohort

linked to HIV care services, these findings are not generalizable to settings where access to HIV care is poor, or to individuals who do not maintain linkage to HIV care.

For reasons discussed in the Methods section, we chose to specifically create a symptom scoring system for the study. This had the advantage of allowing us to define the symptoms of interest, and to create a score that could be easily completed by participants. However, limited validation of this questionnaire was performed,and consequently we cannot determine how well this score measured the severity of participant symptoms, or how it compared with existing scores. Use of pre-existing validated scores alongside this symptom score (such as the Jackson or WURSS scores for acute upper respiratory tract illness) would have enhanced the study by allowing comparison with these scores.

A further limitation of this study was the comparison of an HIV positive population with general population controls, and the different strategies used to recruit each group. There are likely to have been socioeconomic and behavioural differences between these two groups in ways that were not measured in the collected data (and thus could not be adjusted for in the analyses). These unmeasured differences could include confounding factors affecting the relationship between HIV status and outcomes. An important limitation of our study is the inability to draw causal conclusions about this. Our strategy for recruitment was chosen enable a comparator population with similar age and gender and exposure to the most important confounding effect (tobacco smoking) to be recruited within the time and resource limitations available. Alternative strategies of recruitment might have been possible, for instance recruitment of HIV negative individuals from Sexual Health clinics, or the healthcare workforce, peers or partners of HIV positive individuals. However, all of these presented their own challenges, such as slow recruitment of the HIV negative group, or further unaccounted for confounders occurring through unknown respiratory exposures (in particular in healthcare workers), which would have risked failure of the project as a whole.

The symptoms chosen to define acute respiratory illness included those arising from both the upper and lower respiratory tract and did not seek to differentiate between infective and non-infective causes. For instance, acute allergic rhinitis would fulfil the criteria for illness. The rationale for this was that distinguishing between infective and non-infective causes based on symptoms alone is not possible (for example, many infections will not result in a fever or sweats, and some non-infectious illnesses can do so). Furthermore, only seeking to evaluate infections might have introduced bias, as HIV positive participants might be more likely to interpret symptoms as a possible infection which may in fact be due to an non-infectious inflammatory response. Our aim therefore was to capture all acute respiratory illnesses regardless of underlying cause.

The greater severity of Acute Respiratory Illness symptoms identified suggests that we need a better understanding of the mechanisms underlying this finding. It may be result from organic processes such as impaired control of infections or dysregulated immune responses, though equally could reflect psychological processes such as greater anxiety or depression in the HIV positive population, or a

greater concern among people living with HIV about the potential significance of symptoms (and hence an increased likelihood of reporting any event to the researchers). Determining the relative importance of these processes is necessary to allow interventions to be designed that can effectively address this healthcare need.

Our data highlight the importance of identifying individuals with chronic respiratory symptoms who may particularly benefit from such interventions to reduce the risk of respiratory illness –these might include smoking cessation, immunisation against influenza and pneumococcus and diagnosis and treatment of underlying respiratory conditions.

In summary, in a population with a high uptake of antiretroviral therapy, the frequency of acute respiratory illness is not affected by HIV status; however, HIV positive individuals report more severe and longer lasting illnesses when these occur, with implications for healthcare resource utilisation and health-related quality of life.

Chapter 5: Effect of HIV status on the detection of pathogenic respiratory organisms in sputum

5.1 Background and rationale for study

HIV infection is associated with considerable morbidity and mortality caused by respiratory bacterial pathogens (187). For instance, Pneumococcal infection is estimated to be 20-100 fold times more frequent in untreated HIV infection (13). Although severe bacterial illness is more common at lower CD4 counts, a greater incidence of invasive pneumococcal disease appears to occur even in those with relatively preserved blood CD4 counts. Although antiretroviral therapy reduces the incidence of acute bacterial illness (10), PLWH continue to be at significantly higher risk than the general population: in an analysis of deaths among PLWH in England and Wales between 1997 and 2012, bacterial illness was significantly more common than occurred in the general population, with a standardised mortality ratio (compared to age-standardised general population data) of 10.8 (95% CI 9.8-12.0), with 358 observed deaths compared to the expected 33 (187). This was still present when individuals in the first year after HIV diagnosis (who were at considerably greater risk) were excluded. Standardised mortality rates for non-AIDS infections were 5.8 (95% CI 4.9-6.9) for men and 10.7 (8.2-13.6) for women. There is therefore a need to understand why the incidence and mortality from infections continues to be higher among PLWH despite the provision of antiretroviral therapy.

One reason for a greater susceptibility to severe respiratory infection might be that there are specific impairments in the immune response to particular pathogens, such as *Streptococcus pneumoniae*, which has been suggested by some authors (113, 198). Alternatively, impairment of mucosal immunity may allow a more generalised increase in bacterial proliferation or invasion. Studies in populations without access to ART have suggested a higher frequency of colonisation with *Streptococcus pneumoniae* in PLWH (199).

HIV infection is known to cause profound changes to the immune system at mucosal surfaces including the airways and the gut (200). A rapid disruption of gut mucosal immunity occurs soon after primary HIV infection: this has been well investigated, and a rapid loss of mucosal CD4 cells has been demonstrated, associated with an increase in bacterial translocation into the blood (117) (201). This loss of mucosal defence appears to be only partially reversed by ART (200).

As discussed in Chapter 1, whether similar effects occur in the lung and airway mucosa is less well established, in part due to the difficulty of obtaining adequate biopsy samples from these sites. HIV infection is associated with a loss of alveolar CD4 cells, often with an associated increase in (cytotoxic) CD8 T cells (which may be directed at HIV infected host cells themselves). Antiretroviral therapy appears to be able to reverse these changes (121). However, specific deficiencies in immune response may persist despite antiretroviral therapy, as demonstrated by Sepako *et al* who found that CD4 T cell responses to *Streptococcus pneumoniae* were still abnormal after 12 months of ART (198).

These changes in mucosal immunity could have the consequence of allowing increased bacterial colonisation, which might be one reason for the higher frequency of invasive bacterial disease seen in HIV.

5.1.1 Existing data regarding bacterial airway colonisation in HIV

Whether HIV infection is associated with changes in airway bacterial communities has been the subject of significant recent interest by several research groups. The technique of 16s ribosomal RNA sequencing allows a comprehensive evaluation of the bacterial communities present and has been used by multiple studies evaluating different clinical questions(202) (a full discussion of this methodology is beyond the remit of this thesis, and is reviewed by Dickinson *et al* (203)). Such culture independent techniques avoid the biases inherent to bacteriological culture, as many species are not easily cultured. These techniques have transformed our understanding of the bacterial communities present, particularly in the lungs (which were once thought to be essentially sterile). The clinical implications of changes in the "microbiome" are of particular current interest.

Two studies to date have used 16s rRNA sequencing to evaluate the effect of HIV status on the respiratory microbiome. Beck *et al* compared 86 HIV negative and 56 HIV positive individuals recruited at six centres in the US (204); the HIV positive group had relatively preserved immunity, with median CD4 counts of 618 cells/μL (range 208-1265 cells/μL) in those using antiretroviral therapy and 668 cells/μL (290-1192) cells/μL in those not on ART. Significant differences in age, gender and ethnicity were present between the HIV positive and negative groups, with HIV positive participants more likely to be male, of African American ethnicity and a greater proportion of HIV positive participants were current smokers, although this difference was not statistically significant. Both oral wash and broncho-alveolar lavage samples were analysed. Overall, Beck and colleagues did not find a difference in the number of Operational Taxonomic Units (related sequences identified by 16s sequencing analysis, broadly reflective of bacterial species), or diversity of bacteria present (Shannon diversity index) between HIV positive and negative individuals, although there were some differences in the microbial populations found in the oral wash samples between those with and without HIV.

A further study evaluating the respiratory microbiome in HIV was conducted by Twigg *et al (205)*. In contrast to the study by Beck *et al*, an HIV positive group with a greater level of immunocompromise was selected with significantly lower median CD4 counts (at 280 cells/μL before ART and 375 cells/μL after 3 years of ART exposure). Before antiretroviral therapy, the HIV positive lower airway microbiome was characterised by less richness (α diversity: diversity within individual samples) but greater β diversity (diversity between subjects) compared to HIV negative individuals. These differences were less marked when HIV positive participants using antiretroviral therapy were compared to HIV negative individuals, supporting the hypothesis that individuals with significant HIV-related immunocompromise have disordered microbial airway communities, and ART can allow the recovery of a "normal" respiratory microbial community.

Although studies using 16s rRNA sequencing can allow evaluations of entire microbial communities, these techniques do not enable specific pathogenic bacterial species or strains to be identified. As deficiencies in mucosal immunity may result from the loss of specific adaptive immunity to particular pathogens (such as *Streptococcus pneumoniae*) studies of this kind could miss important and clinically relevant differences between people with and without HIV, because pathogenic

Streptococcus pneumoniae cannot be differentiated from commensal streptococci. The use of targeted PCR techniques to evaluate specific pathogenic species can therefore provide information not available through 16s rRNA approaches.

Studies evaluating bacterial carriage using PCR typically demonstrate a significantly higher frequency of colonisation compared to culture-based techniques: for instance Desai *et al* identified at least one of four respiratory pathogens (*Haemophilus influenza, Moraxella catarrhalis, Streptococcus pneumoniae* or *Pseudomonas aeruginosa*) in 29% of sputum samples obtained from HIV negative individuals with COPD by PCR, compared to a positive culture result from only 8% of samples (206).

The carriage of specific bacterial pathogens in PLWH, and whether this differs from HIV negative people is a relatively unexplored question. Previous work from our group by Nimmo *et al* utilised targeted PCR for the presence of *Streptococcus pneumoniae, Haemophilus influenzae* and *Moraxella catarrhalis* in sputum samples obtained from HIV positive individuals (207). The samples were induced sputum, obtained from HIV positive individuals participating in a study evaluating the yield of testing for latent and active tuberculosis, and PCR analysis could only be performed where adequate quantities of residual sample were available. Overall, at least one of these bacteria was detected in 43% (23/53) samples, with *Streptococcus pneumoniae* being the most commonly detected pathogen.

5.1.2 Detection of pneumococcal carriage in the respiratory tract

Differing molecular and culture-based techniques can be used to evaluate the prevalence of respiratory pathogens, and these typically yield significantly different results. Culture of pathogenic organisms remains the gold standard for identification of bacteria, allowing phenotypic techniques for bacterial identification which have historically been the basis for species identification (for instance alpha-haemolytic colonies from a suitable medium which are optochin susceptible to identify *Streptococcus pneumoniae*). However, determining the presence of bacteria by culture of samples can lack sensitivity and may yield estimates of prevalence that are lower than when estimated by molecular techniques such as PCR. For example, van Deursen *et al* evaluated upper respiratory tract pneumococcal carriage in older adults in the Netherlands and found that although pneumococci was cultured from only 8% of participants, 20% were positive when tested by quantitative-PCR.(208) Although PCR techniques offer potentially greater sensitivity (allowing small bacterial populations to be detected by amplification of genetic material) this may come at the cost of lower specificity if the PCR target primer is not completely specific for the target. The identification of bacteria by PCR depends on the presence of genetic sequences that are unique and specific to the organism under evaluation. There is much debate, therefore, as to the degree by which PCR primers used are truly specific. This has been most thoroughly evaluated in the context of *Streptococcus pneumoniae*, where the specificity of some PCR assays for pneumococcal serotypes has been questioned. In particular, other oropharyngeal streptococci of the mitis group are closely related to *Streptococcus pneumoniae* and may possibly yield positive results to PCR primers that were previously felt to be specific for pneumococcus.(209, 210)

The potential insensitivity of culture and possible lack of specificity of PCR-based techniques represent a challenge when determining the frequency of bacterial carriage in the airways. These limitations of currently available techniques should be considered when interpreting results in this field.

5.2 Rationale for this study

The intention of this work was to compare the frequency of bacterial colonisation at a time when participants had no symptoms of an acute respiratory tract illness. We hypothesised that people living with HIV would be more likely to have pathogenic bacterial species detectable in sputum samples taken at baseline using culture-independent targeted PCR methods than HIV negative individuals.

To test this hypothesis, we used a targeted PCR technique to systematically evaluate baseline carriage of pathogenic respiratory bacteria in sputum samples

We chose to evaluate the presence of three bacterial airway pathogens: *Streptococcus pneumoniae*, *Haemophilus influenzae* and *Moraxella catarrhalis*. These were chosen because they represent common causes of acute bacterial respiratory illness, which previous work has demonstrated can be detected in airway bacterial flora at significant levels. Although this list of pathogens did not aim to provide a comprehensive evaluation of all respiratory pathogens present, significant differences in the frequency of identification of these pathogens could indicate differences in airway bacterial colonisation which could relate to baseline symptoms or susceptibility to subsequent acute respiratory illness.

Well-characterised PCR primers and laboratory protocols exist within the UCL Centre for Medical Microbiology allowing for rigorous quantitative evaluation of the presence of these bacteria.

The sputum samples analysed in this study were those obtained during baseline sampling within the acute respiratory illness cohort study. However, sputum samples represent a mixture of sputum from the lower respiratory tract as well as inevitable contamination from the upper respiratory tract and therefore comprise more than the microbial communities within the lower airway, a fact that must be considered when interpreting the results. Sputum is thought to primarily originate in the larger airways and the microbial communities in different parts of the lung (for instance the alveoli) are different from those in these larger airways – no single sample can therefore characterise the whole “lung” or “airway” microbiota. Sputum samples do not, therefore, represent a comprehensive assessment of the microbial populations present, nor are solely a lower respiratory tract sample.

The upper respiratory tract is often believed to be the origin of bacteria causing lower respiratory tract infections, and therefore assessment of carriage in the upper respiratory tract provides a useful insight into carriage and transmission of pathogenic organisms such as *Streptococus pneumoniae*, as will be discussed later. With these limitations accepted, however, the use of sputum samples as a “snapshot” of the microbial populations present in the airway can provide useful insights, and has the advantage of being a sample that is available without invasive sampling techniques (such as bronchoscopy) and with a high yield of bacterial species.

5.3 Methods

5.3.1 Sample collection and storage

All participants in the cohort study described in Chapter 4 were asked to expectorate a sputum sample during baseline sample collection. If this could not be done spontaneously, participants were asked if they would undergo induced sputum collection using 3.5% nebulised hypertonic saline. This was not a mandatory part of study participation and subjects could decline to do this if they preferred.

Once collected, samples were stored unprocessed at -80 degrees before processing for analysis.

5.3.2 DNA extraction from sputum samples

For processing and DNA extraction, I thawed samples and mixed with 0.1% Dithiothreitol (Sputasol, Oxoid Ltd, UK) at a 1:1 ratio in Category 3 Biosafety cabinet. This was Vortex-mixed and incubated at room temperature for 30 minutes. The sputum was transferred in 1ml aliquots and heat-killed by heating to 95° for 30 minutes.

Samples were then centrifuged for 12 minutes at 13,300 g and the supernatant discarded leaving a pellet. A quantity of glass beads (425-600µm) approximately equal in volume to the sample pellet was added to the tube. Ribolysis was then performed on a FastPrep 24 benchtop homogeniser (MP Biomedicals) for 45 seconds. Immediately following this 240µL of “extraction buffer 2” and 10 µL “Proteinase K” (both from Diasorin Ltd, Saluggia, Italy) were added to the sample. The sample was vortex mixed then incubated at 56°C for 10 minutes. DNA was extracted from sample lysates on the Diasorin IXT (Arrow) automated platform using DNA extraction cartridges eluting into 100µl. DNA concentration and purity was then assessed using a Nanodrop spectrophotometer (ThermoFisher Scientific).

5.3.3 Quantitative PCR for the detection of respiratory pathogens

I undertook quantitative real-time PCR to detect the presence of the following respiratory pathogens: *Haemophilus influenzae*, *Moraxella catarrahalis* and *Streptococcus pneumonae*. This procedure followed standard laboratory protocols developed by the UCL Centre for Medical Microbiology. The probe sets used have been previously evaluated and specificity for the bacteria targeted, confirmed by BLAST (Basic Local Alignment Search Tool) analysis by Sylvia Rofael who assisted with training for his aspect of this thesis (211). PCR probes and primers were obtained from Eurofins genomics, (Munich, Germany). Details and concentrations of probes and primers used are given in **Table 27.**

TABLE 27 PROBES AND PRIMERS USED FOR PCR ANALYSIS OF SPUTUM SAMPLES

Species	Probe/primer	Sequence (5'-3')	Gene target	Fluorescence detection colour
Streptococcus pneumoniae	Forward primer	AGT CGT TCC AAG GTA ACA AGT CT	*Streptococcus pneumoniae* Spn 9802 (gene fragment of unknown function (212, 213)	Green
	Reverse primer	ACC AAC TCG ACC ACC TCT TT		
	Probe*	[ROX] TAC ATG TAG GAA ACT ATT TTC CTC ACA AA [BHQ2]		
Haemophilus influenzae	Forward primer	CCG GGT GCG GTA GAA TTT AAT AA	Hel gene (encodes P4 lipoprotein)	Orange
	Reverse primer	CTG ATT TTT CAG TGC TGT CTT TGC		
	Probe	[CY5] TGC ACA AGC TAT GGA ACA CCA CGT [BBQ650]		
Moraxella catarrhalis	Forward primer	GTG AGT GCC GCT TTT ACA ACC	*Moraxella catarrhalis* copB (encodes outer membrane protein)	Yellow
	Reverse primer	TGT ATC GCC TGC CAA GAC AA		
	Probe	[JOE] TGC TTT TGC AGC TGT TAG CCA GCC TAA [BHQ1]		

* flourochromes and quenchers indicated in square brackets

5.3.4 Procedure for PCR experiments

A “mastermix” of reagents for each reaction was prepared in concentrations detailed in **Table 28**. Each sample was analysed in duplicate and also diluted 1:10 in PCR-grade distilled water (also in duplicate). This dilution was undertaken due to the possibility of inhibition of the PCR reaction by proteins persisting following the DNA extraction step (which could cause failure of the reaction for the undiluted sample).

PCR reactions were undertaken in 25µL with 20µL mastermix and 5µL sample; reactions were performed using a Rotor-gene Q (Qiagen) with cycling details optimised according to existing laboratory protocols. The maximum number of PCR cycles performed (CT value) before the result was declared to be negative was 30.

As an internal control to confirm the success of the PCR reaction and amplification of genetic material, 100µL of 10^{-8} concentration DNA from PhyB gene of potato (*Solanum tuberosum*) was included in the Mastermix, along with primers designed to amplify this material and probes allowing detection of the PCR reaction.

TABLE 28 REAGENTS AND CONCENTRATIONS USED FOR QPCR REACTIONS

Reagent	Conc.			Volume x1 reaction µL
Platinum qPCR Supermix	x1			12.5 µL
MgCl2	50mM			1.5 µL
Bacterial forward primers (x3 mixed)	50µM			0.225 µL
Bacterial reverse primers (x3 mixed	50µM			0.375 µL
Bacterial probes (x3 mixed)	50µM			0.375 µL
Internal Amplification Control (IAC) Forward primer	50µM			0.125 µL
IAC Reverse primer	50µM			0.125 µL
IAC Probe	50µM			0.1 µL
IAC Amplicon	100µM x10-8			1 µL
PCR Grade Water	NA			3.675 µL
TOTAL VOLUME PER REACTION				**20 µL**

5.3.5 Preparation of standard curves for quantitative PCR

To allow quantification of the amplicons arising from the PCR reactions, standard curves were created with known concentrations of the relevant organisms.

To create these standard curves, isolates of each pathogen were cultured from standard isolates held within the clinical microbiology laboratory (details of strains: *Streptococcus pneumoniae*: ATCC 49619; *Haemophilus influenzae* ATCC 8468; *Moraxella catarrhalis* sub-cultured laboratory sample). I incubated these isolates for 24 hours at 38° on chocolate agar (*Moraxella catarhallis* and *Haemophilus influenza*e) or blood agar (*Streptococcus pneumoniae*). Following incubation, a 2µL loop of bacteria was taken and a bacterial suspension created by vortex mixing. This suspension was then diluted into 10-fold serial dilutions from neat to 10^{-9}.

20 µL drops of these diluted suspensions were then placed on agar plates (3 drops for each concentration) and incubated for 24 hours. The concentration at which separate discrete colonies could be readily counted were then selected and the number of colonies manually counted and the mean number of colonies of the three samples calculated. From this, the total bacterial concentration of the neat suspension could be determined. This procedure is illustrated in **Figure 16**.

Alongside the serial dilution of the bacterial suspension for culture and colony counting, a separate series of serial dilutions were made for DNA extraction. These dilutions were then processed for DNA extraction using the same method as used for the sputum samples.

The DNA extracts resulting from this process were then used as standard solutions for the quantitative PCR reactions. Within each PCR run, an aliquot of standard solutions of a known concentration for each of the three pathogens was also included (in duplicate). These therefore contained a quantified concentration of bacterial DNA which could be used to adjust the quantitative results expressed in fluorescence intensity derived from the PCR reaction to yield a result that could be expressed in number of colony-forming units per ml (Figure 17).

In addition to the standard samples included in each run, a positive control including all three of the standards diluted 50-fold was also included as a positive control. This diluted sample approximated the lower limit of detection of the PCR reaction and therefore acted as a further assessment of the effectiveness of the PCR amplification and detection.

FIGURE 15: PROCESS FOR CREATION OF STANDARD CURVES

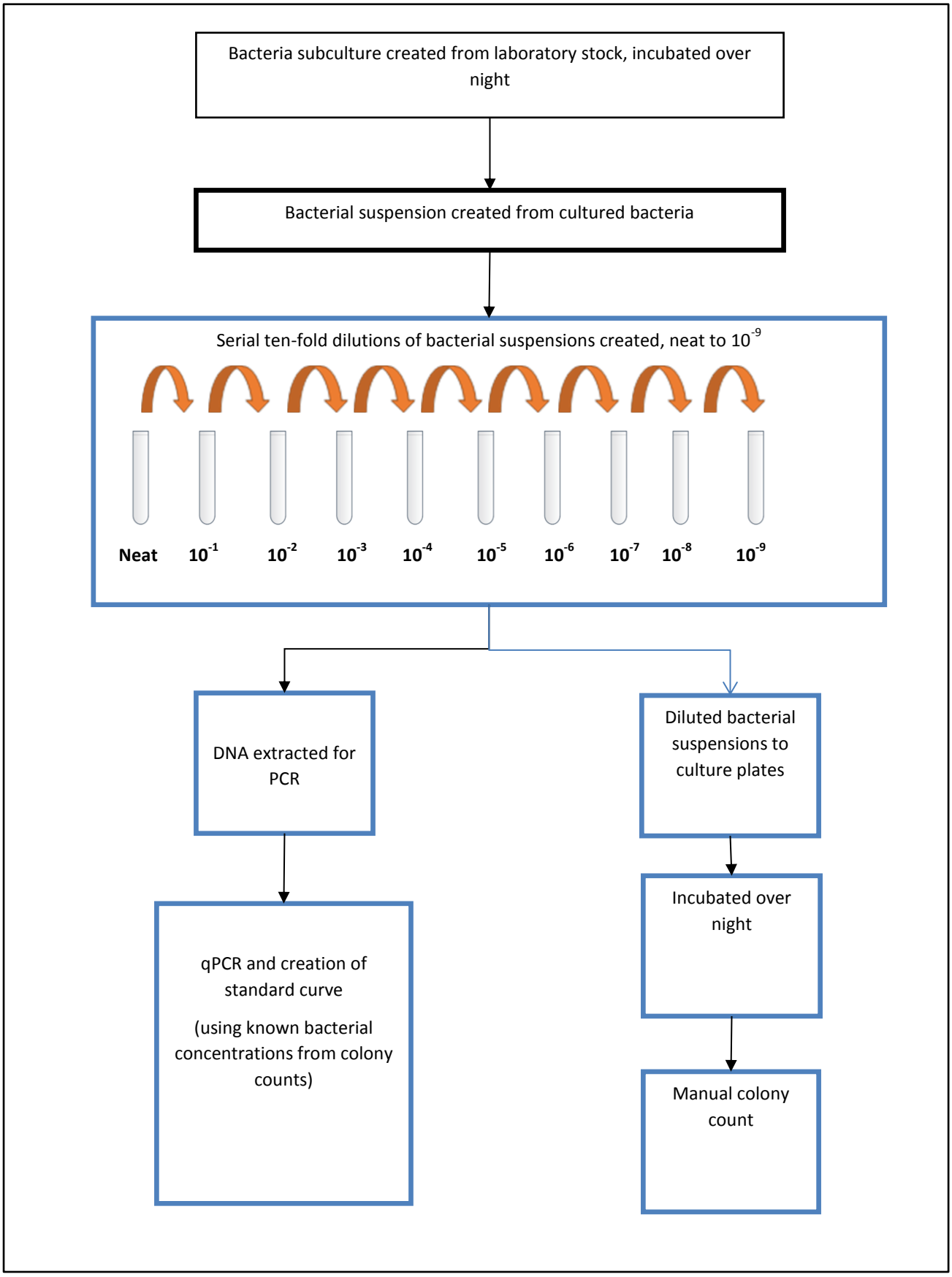
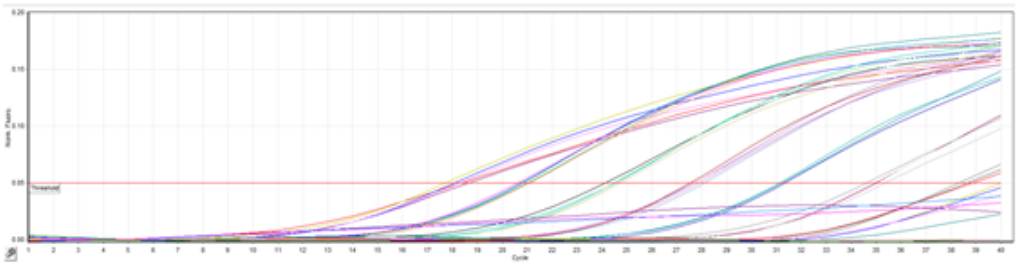
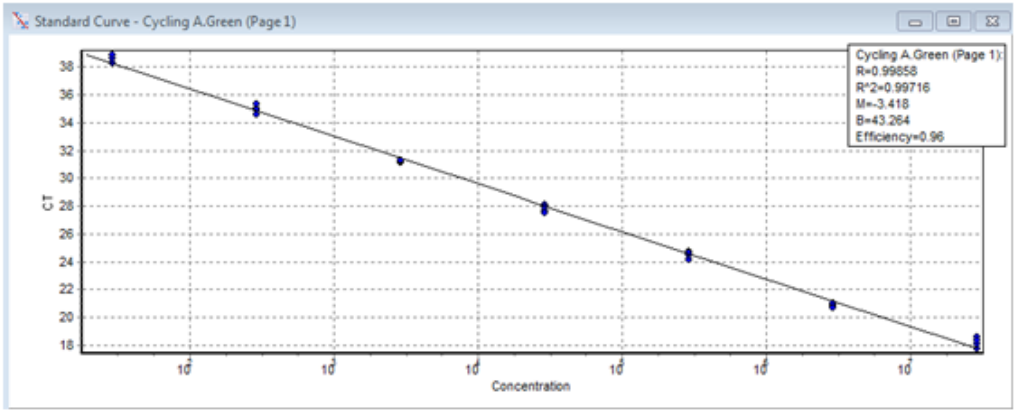


FIGURE 16 EXAMPLE OF QPCR FOR STANDARD CURVE CREATION.



Detection of target organism DNA at known concentrations using fluorescent dyes. PCR reactions undertaken using Rotorgene Q platform



Standard curve created allowing results expressed in CT values to be translated into equivalent concentrations

5.4 Results

5.4.1 Proportion of cohort participants with sputum samples

In total 101 of 217 (46%) of participants were able to provide a sputum sample at baseline. 91 of these samples were spontaneously expectorated and 10 were induced sputum. The details of participants who were and were not able to produce sputum samples are given in Table 29.

No significant differences were found in the gender, age, tobacco smoking history or HIV status of those who could, rather than could not, provide a sputum sample; nor did a greater proportion of those with airflow obstruction provide a sample. There was no significant difference in respiratory symptoms (defined using the St Georges Respiratory Questionnaire) between those with and without a sample.

TABLE 29 COMPARISON OF STUDY PARTICIPANTS WHO DID AND DID NOT PRODUCE A SPUTUM SAMPLE AT BASELINE

		Sputum produced N= 101 (47% cohort)	No sputum produced N = 115 (53% cohort)	P value
HIV status	**Positive, n (row %)**	65 (45%)	78 (54%)	0.59*
	Negative, n%	36 (49%)	37 (51%)	
Gender	**Male, n %**	84 (50%)	83 (50%)	0.07*
	Female, n%	17 (35%)	31 (64%)	
Age, years, median (IQR)		50 (11)	52 (9)	0.16#
Smoking status	**Current**	30 (60%)	20 (40%)	0.127*
	Previous	35 (43%)	47 (57%)	
	Never	34 (44%)	43 (56%)	
Participants with airflow obstruction, n (%)		8 (42%)	11 (58%)	0.34*
Respiratory symptoms (SGRQ Total score), median (IRQ)		10 (5-26)	8 (2-20)	0.16*
Blood CD4 count, cells /µL**		700 (471-848)	679 (435-852)	0.95#

* Chi squared test # T-Test **HIV participants only # Wilcoxon Rank Sum test

5.4.2 Quantitative PCR results

Overall, one or more of the three respiratory pathogens was detected in 71 (69%) of the 101 samples analysed: results are provided in Table 30. There were no significant differences in the proportion of samples from HIV positive and negative participants which were positive for any of the three pathogens*: Streptococcus pneumoniae* was detected in 55% of HIV positive and 44% of HIV negative samples (p=0.29); *Haemophilus influenzae* was detected in 43% of HIV positive and 56% of HIV negative samples (p=0.89) and *Moraxella catarrhalis* in 5% of HIV positive and 14% of HIV negative samples (p=0.32). Quantitative results were also similar in samples obtained from HIV positive and negative participants, with the exception of *Haemophilus influenzae* which was identified at lower concentrations in the HIV positive group: median (IQR) 7.71×10^2 (1.66×10^2 – 3.31×10^3) compared to 1.06×10^4 (1.92×10^3 – 7.20×10^4) cfu/ml (p=0.02).

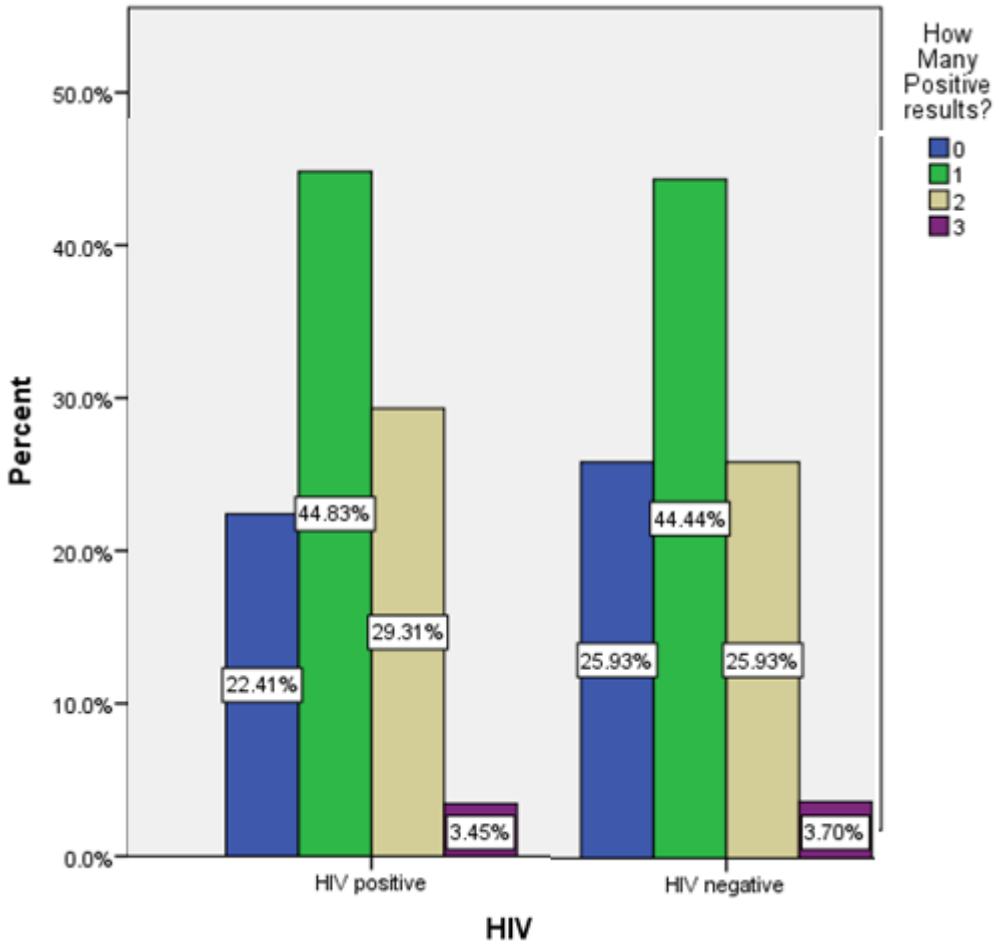
The proportion of samples with one, two or three of the pathogens identified also did not differ between HIV positive or negative groups (Figure 18)

TABLE 30 DETECTION OF RESPIRATORY PATHOGENS IN BASELINE SPUTUM SAMPLES BY QPCR

		HIV status		p value
		Positive (n=65)	Negative (n=36)	
Streptococcus pneumoniae	**Detected**	36 (55%)	16 (44%)	0.29*
	Quantitative count (cfu/ml) median, (IQR)	2 .00 x 10^5 (1.12 x 10^4 – 1.41 x10^6)	3.27 x 10^5 (8.79 x 10^3 – 8.22 x10^5)	0.69#
Haemophilus influenzae	**Detected**	28 (43%)	20 (56%)	0.89*
	Quantitative count (cfu/ml) median, (IQR)	7.71 x 10^2 (1.66 x 10^2 – 3.31 x10^3)	1.06 x 10^4 (1.92 x 10^3 – 7.20 x10^4)	**0.01#**
Moraxella catarrhalis	**Detected**	5 (8%)	5 (14%)	0.32*
	Quantitative count (cfu/ml) median, (IQR)	2.92 x 10^2 (8.55 x 10^1 – 4.51 x10^4)	1.12 x 10^3 (4.95 x 10^2 – 1.26 x10^6)	0.31#

* Chi squared test # Mann Whitney U test

FIGURE 17: NUMBER OF PATHOGENIC SPECIES IDENTIFIED IN HIV POSITIVE AND NEGATIVE GROUPS



5.4.3 Association between identification of respiratory bacterial pathogens and baseline participant characteristics

There were no significant differences in the age or gender of participants with and without pathogenic bacteria detected, nor were bacteria more likely to be identified from individuals with airflow obstruction. Baseline respiratory symptoms (using the St George's Respiratory Questionnaire) were not different in those with and without bacteria detected. It should be noted, however, that some of these comparisons were based on small numbers of participants, and this study was not designed to have sufficient statistical power to assess these associations.

TABLE 31 COMPARISON OF PARTICIPANT CHARACTERISTICS FROM WHICH BACTERIA WERE AND WERE NOT IDENTIFIED

		Bacteria identified (n=71)	No bacteria identified (N=30)	P value
HIV status	Positive, n (row %)	47 (72%)	18 (27%)	0.55*
	Negative, n (row)%	24 (67%)	12 (33%)	
Gender	Male, n (row %)	59 (70%)	25 (30%)	0.97*
	Female, n (row %)	12 (71%)	5 (29%)	
Age, years, median (IQR)		52 (9)	51 (10)	0.52**
Respiratory symptoms (SGRQ Total score), median (IQR)		9 (5-21)	10 (5-28)	0.89#
Smoking	Current smoker	15 (21%)	10 (33%)	0.48
	Ex-smoker	26 (37%)	9 (30%)	
	Never smoker	24 (34%)	10 (33%)	

* Proportions compared with Fisher's exact tests ** T test # Mann Whitney U Test

5.4.4 Sensitivity analysis including only spontaneously expectorated sputum samples

Although we had intended to increase the proportion of participants who could produce a sputum sample by using induced sputum collection, only seven of the sputum samples were obtained by this method (largely due to the additional time required from participants to complete this after the already considerable data collection and sampling already undertaken). These were analysed alongside the spontaneously produced samples, although arguably may have yielded different results due to the different sampling method (for instance dilution of the sample due to the hypertonic saline used for sputum induction). I therefore re-analysed the results including only spontaneously expectorated sputum samples (61 samples from HIV positive and 33 from HIV negative participants). The results were consistent with the main analysis, with no significant differences in the proportion of samples from HIV positive and negative participants with *Streptococcus pneumoniae* (57% vs 45% respectively (p=0.27) *Moraxella catarrhalis* (8% vs 15% respectively, (p= 0.30) or Haemophilus influenzae (46% vs 48% respectively, (p=0.81) detected, but again a slightly higher quantitative count of Haemophilus influenzae among samples from HIV negative participants in whom this bacteria was detected (median $1.06x10^4$ (IQR $1.02\ x10^2$ -$7.2\ x10^5$) in HIV negative compared to $7.7\ x10^2$ ($1.66\ x10^2$ – $3.3x10^4$) in HIV positive, p=0.014.

5.5 Conclusions

People living with HIV appear to remain at greater risk of severe bacterial respiratory infection despite using antiretroviral therapy. As the results of the cohort study described in chapter 4 demonstrate, although the frequency of acute respiratory illness does not differ between HIV positive and negative individuals, those with HIV are more likely to experience subjectively severe illness.

One possible reason for this could be a greater susceptibility to infection with bacterial respiratory pathogens. *Streptococcus pneumoniae* has been shown to cause severe or invasive infection at much greater frequencies in those with HIV, an effect which is reduced but not eliminated by use of ART (142).

One reason for this greater susceptibility to bacterial respiratory infection could be a failure to control such pathogens in the airways. Upper respiratory tract colonisation with *Streptococcus pneumoniae* is believed to be a necessary precursor of pneumococcal disease (214, 215).

The recruitment of the clinical cohort described in Chapter 4 provided the opportunity to test the hypothesis that PLWH are more likely to have respiratory pathogens present in their airways, or have such pathogens detectable in greater numbers, by using targeted PCR techniques to quantify the presence of these pathogens in sputum samples collected at baseline. Such molecular techniques are highly sensitive and specific and allow robust quantification of their targets.

To our knowledge, this is the first study to evaluate the presence of specific respiratory pathogens in sputum samples from HIV positive individuals using ART compared to an HIV negative comparator group. Our data demonstrate that this cohort of PLWH (with almost universal use of antiretroviral therapy) are no more likely to have pathogenic bacteria detectable in their airway flora than healthy controls, and when these bacteria are present there is no difference in their concentration.

These findings suggest that in a HIV positive population with good uptake of antiretroviral therapy and immune reconstitution (measured by blood CD4 counts and undetectable HIV viral load), any persisting deficiencies in airway mucosal immunity do not lead to the overgrowth of the pathogenic species studied when PLWH are free from acute respiratory illness. This suggests that there may not be significant differences in airway bacterial colonies in general between PLWH using ART and HIV negative individuals, if the populations of these bacteria are reflective of the wider bacterial populations present in these samples

Although this evaluation provides new data regarding the interaction between pathogenic bacteria and HIV positive individuals, there are significant limitations which should be considered: Sputum samples represent a mixture of lower airway products and saliva. We did not undertake sputum cell counts to determine the quality of sputum samples and exclude those with heavy oral contamination or attempt to separate mucoid sputum from saliva. I did not compare the samples with oral washes to evaluate differences between upper and lower airway bacterial populations. Unlike studies evaluating bronchoalveolar lavage samples, microbiological analyses of sputum samples cannot provide information on lower airway bacterial populations alone. Therefore, the results should be considered

as an indicator of “airway” bacterial populations, without knowledge of the origin of the isolate from the upper or lower airways.

A further limitation was that sputum samples were not available from all participants. This analysis was undertaken as a sub-study of the wider prospective cohort study. Sputum samples were only obtained from 46% of participants and the study therefore has limited power to evaluate some possible associations.

Although our data suggest that PLWH are not more likely to carry pathogenic bacteria in their airways when systematically well, other mechanisms might still result in a higher incidence of bacterial respiratory infection during acute illness. Deficiencies in immune response could mean that PLWH are more likely to develop invasive bacterial disease when acute illness occurs, or to acquire new bacterial species in their airway flora, either of which could lead to greater susceptibility to bacterial infection (as opposed to colonisation).

It should be noted that the carriage rate for pneumococci suggested by these data is higher than that estimated in most studies evaluating pneumococcal carriage. As previously discussed, differences in sampling technique, analysis and definitions of carriage can result in very different estimates of the prevalence of carriage. Pneumococcal carriage has been evaluated in many studies, so it is possible to contextualise these results. Multiple studies have evaluated the prevalence of pneumococcal carriage in the upper respiratory tract – these consistently find very high rates of carriage in infants and young children which then typically decrease with age. However, there is a wide variation in carriage rates between populations – a systematic review of pneumococcal carriage prevalence published in 2014 reported prevalence of carriage in adults between 0% and 55% (with typically higher prevalence reported in developing countries).(216) Studies in adults typically suggest carriage frequency in the region of 5-25%.(208, 217-219) It should be noted, however, that these results are usually based on sampling of the oropharynx or nasopharynx with swabs and detection of pneumococci by culture. These results are therefore not directly comparable to the detection of bacteria in sputum samples by PCR. Appreciating the need for standardisation of methods of detection of pneumococcal colonisation, the World Health Authority (WHO) has created standardised methods for the measurement of pneumococcal colonisation in epidemiological studies and vaccine trials.(220) As well as defining methods of sampling, these suggest that culture of pneumococci remains the standard means of identification. Therefore, most published estimates of pneumococcal carriage are based on culture from nasopharyngeal or oropharyngeal swabs with fewer comparable studies using different methods. As discussed above, samples based on molecular detection have yielded higher estimates of pneumococcal carriage than those by culture: Trumuto *et al* used LytA PCR to evaluate oropharyngeal swabs from adults and children in Italy and reported a high prevalence of pneumococci (56% in those aged 26-49; 61% in those aged over 50) - although this study was relatively small and the adults evaluated were all family members of young children.(221) Similarly, Van Deursen reported a prevalence of 18% in pharyngeal swabs taken from adults over 65 years old – a value higher than is typically suggested by surveys based on culture of swabs from individuals in this age group.(208)

Previous studies of pneumococcal carriage among PLWH have suggested that this group have very high levels of carriage. Furthermore, data currently available suggest that this persists after ART use, with no change in carriage after 24 months of ART despite good improvement in blood CD4 counts in a study undertaken in Malawi by Heinsbroek *et al*.(222)

Why might our findings apparently differ from those suggested elsewhere? Firstly, as discussed, the higher prevalence of pneumococci may be the result of the samples and analyses used, with most studies of pneumococcal carriage using pharyngeal swabs (as this is felt to be the primary site of colonisation and is the standard method recommended by the WHO). There are fewer studies reporting the detection of pneumococci in sputum samples from individuals without an acute respiratory illness. Garcha *et al* did so using sputum samples from people with COPD and found that around 10% were positive for pneumococci, (211) however, this group defined a threshold of 10^4 colony-forming units per ml as "positive". This was not the case with our data as we sought to determine colonisation by bacteria rather than define any particular population density of interest.

The higher prevalence of pneumococci found compared to most published estimates may relate to the use of sputum samples rather than pharyngeal swabs, or the greater sensitivity of PCR compared to culture, however we cannot also exclude the possibility that the potentially lower specificity of PCR for pneumococci (compared to other closely related streptococcal species) could increase the apparent prevalence of pneumococci, as this was not confirmed with culture. It is therefore a limitation of the data that should be noted.

Our findings are consistent with those of investigators who have used molecular techniques for the detection of similar pathogens in other (HIV negative) patient groups. For instance, Singh *et al,* using identical primer sets but applying a threshold of 10^4 cfu/ml to define "significant" bacterial colonisation found that one of the three pathogens could be detected in 35% of sputum samples from patients with COPD. The slightly higher proportion of samples with bacteria detected in our data may arise from differences in sample processing: in our study the whole sputum sample was heat-killed, combined with Dithiothreitol and mixed, with a 0.5-1ml aliquot of this homogenised sample used for PCR; in contrast, the protocols used by Singh *et al* and Garcha *et al* utilised individual sputum plugs derived from samples (211, 223).

These findings should be interpreted in the context of other recent studies examining airway bacterial populations in PLWH which have used 16s ribosomal RNA sequencing techniques to evaluate the respiratory microbiome. Although these have the advantage of potentially being able to assess the full microbial community present, these sequencing techniques do not allow determination of the presence of specific bacterial species, in contrast to the targeted PCR approach used here. These differing molecular techniques can therefore be seen as providing complementary information. Our findings, can therefore be interpreted as being in accordance with those of Beck *et al* who used 16s rRNA sequencing from oral washes and broncho-alveolar lavage and found that bacterial populations

were not significantly different between HIV positive and negative individuals, in a population where most PLWH were using ART with good levels of immune reconstitution.

In summary, my data suggest that PLWH using ART are no more likely to have asymptomatic colonisation of their respiratory tract with pathogenic bacteria. If, as several sources have suggested, a greater risk of severe bacterial respiratory illness persists despite ART,(10) (142) then other causes should be considered.

Chapter 6: Summary, conclusions and suggestions for future work

6.1 Summary of findings and contributions made in this thesis:

The findings discussed in this thesis contribute to the growing body of evidence regarding the health of HIV positive people since the introduction of effective antiretroviral therapy. The need for this new research is testament to the progress that has been made in treating HIV over recent years: antiretroviral therapy is now well tolerated and highly efficacious, allowing HIV positive people to enjoy full and productive lives.

However, there are several ways in which the health needs of people living with HIV remain different to the general population, and research is required to understand the epidemiology, mechanisms of disease and interventions that can improve the health of this population.

To summarise and discuss the findings of the work presented in this thesis:

Chapter 2 described the results of a systematic review summarising current knowledge regarding respiratory symptoms in PLWH. This demonstrated the significantly greater burden of chronic respiratory symptoms found among PLWH without access to ART and how this has reduced in populations using effective treatment. However, despite this improvement, PLWH remained about 50% more likely to report chronic respiratory symptoms such as breathlessness or cough. The review also highlighted areas where evidence is lacking – most importantly the almost complete absence of data from resource-limited populations with access to ART. As these populations (which represent the majority of the world's HIV positive population) are now much more likely to be able to obtain effective treatments, understanding the chronic comorbidities now affecting them, and how this may change in the future, is increasingly important.

The systematic review included data from 24 different studies containing over 12,000 HIV positive and 24,000 HIV negative participants. From this, I could investigate the association between HIV status and respiratory symptoms, and how this has changed since the introduction of widespread ART. However, the limitations of these findings must be appreciated – in particular many studies did not include data on important confounding factors (such as tobacco smoking), making the independent effect of HIV status difficult to confidently determine. Another important limitation is the difficulty in objectively measuring self-reported symptoms – many different definitions of, for instance, breathlessness or cough were used and relatively few studies used standardised instruments such as the MRC dyspnoea scale. The interpretation of, and response to, such symptom questionnaires may differ in different settings and be influenced by participant expectations of the responses – for instance PLWH may be more likely to report symptoms or be more concerned about the possible underlying causes and implications of symptoms.

Since the completion of this systematic review some new data have become available, such as a study from Denmark evaluating a cohort of over 1000 PLWH compared to the general population

controls.(76) This study supported the findings of the systematic review presented here and found that respiratory symptoms remained more common among PLWH, despite there being good access to care with 98% using antiretroviral therapy and 95% having an undetectable HIV load, although as with many other HIV positive populations, PLWH in this study were more likely to smoke than the HIV negative population they were compared with. Nonetheless, this study suggested that the higher prevalence of respiratory symptoms identified by the systematic review continues to be a problem despite the improving access to ART.

Chapter 3 presented the findings of a cross-sectional study evaluating the prevalence of respiratory illness among PLWH attending the Royal Free London NHS Foundation Trust. This represented the first published UK report of respiratory health among people living with HIV using antiretroviral therapy. This found a lower frequency of lung function impairment (assessed by spirometry) than studies in many other HIV positive populations but a significantly higher prevalence of respiratory symptoms in the HIV positive compared to negative participants. An important part of this work was the systematic evaluation of respiratory symptoms and health status impairment, compared to an HIV negative comparator group with similar risk factors for respiratory illness, although the limitations discussed above regarding the measurement of subjective symptoms apply equally here. We found that PLWH were significantly more likely to report chronic respiratory symptoms, a finding that persisted after adjustment for smoking, age, gender, body mass index and the presence of anxiety / depression. Indeed, mental health was found to have an important relationship with physical symptoms, such that the presence of anxiety or depression (by self-report or PHQ-9 score) was the factor most strongly related to respiratory symptoms. The study also highlighted the greater proportion of PLWH who smoke tobacco compared to the general population. Whilst this is not a new finding and has been demonstrated in the UK and other HIV positive populations previously, it is of great importance for the future health of PLWH and should be a focus for health improvement by HIV care services.

This study had the advantage of the systematic measurement of respiratory symptoms and spirometry, using well-validated respiratory and generic health-related quality of life scores. However, it should be noted that the relatively small sample size and recruitment of participants from a single site limits the confidence with which findings can be generalised to other HIV positive populations. A multi-site study would allow better evaluation of the wider UK HIV positive population and reinforce the generalisability of the findings. In addition, as with the prospective work described in chapter 4, more detailed physiological measurement would give a better idea as to whether the greater reported frequency of respiratory symptoms was primarily due to deficits in respiratory or cardiovascular function cause by objective physical impairments and how much resulted from psychological comorbidity (particularly anxiety and depression), heightened concern about symptoms among PLWH, or expectations about appropriate responses in this group. Studies involving measurements of lung function allow conclusions to be drawn regarding the cause of these symptoms. Such studies have been undertaken by other groups, for instance the work of Crothers *et al* reporting that HIV is

associated with reduced DLCO;(94) Triplette *et al* suggesting an association between HIV status and emphysema, (65) and Ronit *et al* reporting a greater frequency of airflow obstruction among PLWH(76, 196), however these still suffer from the potential for residual confounding and yield somewhat conflicting results.

The largest body of work within this thesis is presented in Chapter 4, which describes a prospective cohort study evaluating the frequency and severity of acute respiratory illness among PLWH compared to HIV negative individuals. Relatively few studies of respiratory health in PLWH using ART have been prospective in design, and those that were often rely on regular clinic reviews or collection of data utilising access to healthcare access to measure acute illness frequency. While this methodology is appropriate to record more severe disease (such as tuberculosis or bacterial pneumonia), most acute respiratory illnesses are relatively minor and usually self-limiting and would therefore be underestimated by these studies. Nonetheless, because they are so common, these acute respiratory illnesses can have an important effect on health-related quality of life. Furthermore, an increase in their severity or duration could contribute to the development of chronic lung disease, as has been suggested in COPD (224).

We therefore sought to carefully evaluate a defined cohort of participants, using active surveillance for new respiratory illness by weekly contact with email or telephone text message to allow documentation of all acute respiratory illnesses and their (self-reported) severity.

We hypothesised that PLWH would have a greater frequency of acute respiratory illness than HIV negative individuals. This was not what we found. The frequency of acute respiratory illness was not significantly different between people with and without HIV, however, when PLWH became unwell they reported more severe symptoms. This finding raises several further questions: one explanation could be that PWLH are no more likely to have an acute respiratory illness, but are at greater risk of more severe disease - after all, prior to the availability of ART, PLWH had around a 50% higher incidence of upper respiratory tract illness and bronchitis, but 8 fold higher frequency of bacterial pneumonia and perhaps 30 fold higher incidence of severe invasive pneumococcal disease.(13)

Again, the limitations of these data must be considered alongside these conclusions: this was a relatively small single centre study, and a larger and more diverse study would provide more robust results. The HIV negative comparator group, whilst central to the conclusions drawn, were not perfectly matched and (as with any observational study) residual confounding remains a concern. Nonetheless, this data represent a novel finding in the field of respiratory health and HIV, and one that has implications for clinical practice and future research.

Understanding why PLWH appear to be more susceptible to severe illness is an important question that should be addressed. The findings of our cohort study suggest that (in a population using antiretroviral therapy) acute respiratory symptoms occur at the same frequency in people with and

without HIV (probably reflecting similar exposure to respiratory viruses and other causes of acute symptoms) but there may still be differences in the PLWH's host response, which may lay behind the greater severity of symptoms reported and perhaps the apparent continued increase risk of more severe illness highlighted by epidemiological studies such as those of Sogaard *et al* and Croxford *et al*, evaluating populations in Denmark and England and Wales respectively (10, 187). Whether the greater reported symptom severity, based self-report, reflects underlying differences in immune response was not determined by this study as we were not able to undertake objective measurements of host inflammatory response during acute illness. A more detailed mechanistic study evaluating host immune response during acute respiratory illness in a systematic manner would provide insights into whether significant differences in inflammatory response to acute illness persist despite antiretroviral treatment.

Chapter 5 explored the hypothesis that possible impairments in mucosal immunity could lead to increased colonisation by bacterial pathogens, which might result in increased respiratory symptoms or acute illness. This was done using targeted PCR techniques to identify the presence and quantity of three bacterial pathogens (*Streptococcus pneumoniae, Haemophilus influenzae and Moraxella catarrhalis*) which represent important cases of respiratory infection. I analysed sputum samples collected at baseline (when participants did not have symptoms of acute respiratory illness) from 101 participants who were able to provide a sample. We found no difference in the proportion of individuals from whom we could detect these pathogens, or in the concentrations of bacteria detected in these samples. This suggests that a failure to control bacterial colonisation by pathogenic bacteria may not be the explanation for the greater frequency of more severe respiratory infection in this population.

These findings are consistent with recent research regarding the respiratory microbiome in PLWH – for instance the study by Beck *et al* which compared the respiratory microbiome in oral washes and broncho-alveolar lavage in an HIV positive cohort using ART and found no significant difference in the microbial populations compared to HIV negative comparators.(204) These findings were in contrast to a similar study evaluating HIV positive individuals prior to the initiation of ART, which reported significant differences compared to HIV negative individuals and a loss of diversity in the bacterial populations present. Such findings contrast, however, to evidence suggesting that colonisation with *Streptococcus pneumoniae* is common among PLWH and this high rate of carriage persisted after 24 months of ART. Although the findings based on sputum samples presented in this thesis add to this evidence, there were limitations to the approached used – in particular the assessment of bacterial colonisation might be better done using upper respiratory tract pharyngeal swabs rather than sputum; and despite the potential for greater sensitivity using PCR-based molecular techniques, the gold-standard for identification of bacteria such as pneumococci remains culture and phenotypic identification.

6.2 Discussion

A consistent finding throughout this thesis has been that PLWH report more respiratory symptoms than an HIV negative comparator group. In the systematic review described in Chapter 2, although ART use was clearly associated with a narrowing of the difference between people with and without HIV, PLWH continued to be about 50% more likely to report respiratory symptoms. Similar findings were present in the cross-sectional data described in Chapter 3 (where MRC dyspnoea scale and St George's Respiratory Questionnaire scores were higher among PLWH) as well as the prospective study discussed in Chapter 4, where HIV positive participants reported more severe and longer-lasting acute respiratory illnesses. These differences occurred despite having control groups with similar proportions of smokers and persisted after adjustment for differences in baseline characteristics in multivariable analyses.

Determining the cause of the higher burden of respiratory symptoms among people living with HIV goes beyond the data presented in this thesis, however available data (including the findings of other studies) do allow some discussion of possible causes. There is unlikely to be a single underlying cause, and multiple factors may play a part. Undiagnosed and sub-clinical lung disease might be relevant: where it has been evaluated, reductions in diffusing capacity for carbon monoxide (DLCO) have been found to be common among PLWH, possibly indicating underlying alveolar or pulmonary vascular changes. For example, the report by Ronit *et al* using multiple breath washout techniques suggest that small airways disease (not fully evaluated by spirometry) may be present among PLWH, even in never smokers (196). More detailed physiological assessments of lung function among PLWH who report breathlessness may therefore yield insights here, although (as with many of the studies discussed) comparing PLWH with appropriate HIV negative comparator groups is essential if the effect of HIV status is to be determined.

The small (although statistically significant) reduction in lung volumes seen in the cohorts of patients studied here may be of importance: a similar finding has been reported by Ronit *et al* from data arising from a large Danish cohort study in which 1083 HIV positive individuals were compared with a large general population control group (76). Here, the proportion with airflow obstruction (defined as FEV1/FVC less than the lower limit of normal) did not differ between HIV positive and negative participants, but those with HIV had concurrent reduction in *both* FEV1 and FVC (in a multivariable regression model, HIV was associated with a 197 ml lower FEV1 and 328 ml lower FVC). This resulted in significantly more HIV positive participants who meet the diagnostic criteria for COPD if defined as FEV1/FVC <70% *and* FEV1-predicted <80%. This reduction in lung volumes might be a consequence of previous immunocompromise, as lower nadir CD4 counts were associated with lower FEV1 or FVC, although other explanations such as the confounding effect of differences in socioeconomic status on both lung development and nadir CD4 could also potentially explain this. Within the data presented in this thesis, the suggestion that previous levels of immunocompromise are important to the understanding of current respiratory symptoms is supported by the trend towards greater impairments of respiratory health (as measured by the St George's Respiratory

Questionnaire) in those with a lower nadir CD4 count, longer time since HIV diagnosis and longer period between HIV diagnosis and starting ART in the cross-sectional data described in Chapter 4. However, this data-set did not have sufficient power to explore these associations further, and we had limited data regarding previous opportunistic respiratory infections which might affect current respiratory health.

Cardiovascular disease may also contribute to respiratory symptoms in some individuals; ischaemic heart disease appears to be more common among PLWH, due to the higher prevalence of risk factors such as smoking and possible ART and direct HIV-mediated effects. It has also been suggested that PLWH have a higher prevalence of pulmonary hypertension and cardiomyopathies.(225-228)

A significant finding of the cross-sectional study discussed in Chapter 3 is the influence of mental health on the reporting of physical symptoms, as anxiety and depression were associated with respiratory complaints. This is intuitive but nonetheless important, as the presence of anxiety or depression (measured by the PHQ-9 scale) was the factor with the strongest relationship to respiratory symptoms in multivariable analyses. The interaction between these two outcomes is likely to be complex and bi-directional. However, this demonstrates how factors other than HIV status *per se* may have an important influence on the health of this population.

The findings presented in this thesis in some ways contradict those from other studies evaluating chronic respiratory disease among PLWH. Drummond *et al* in the US,(66) Madeddu *et al* in Italy (67) and Makinson *et al* in France (75) all suggested that obstructive lung disease may be significantly more common among people with HIV. There are several possible explanations for these different conclusions. The good access to health services, uptake of antiretroviral therapy and retention in care seen in the UK may mean that some HIV-related effects are less pronounced than in other populations where access to treatment is more limited. Although a higher proportion of PLWH in the UK smoke and use recreational drugs than the general population, this difference is less marked than in several HIV positive populations evaluated in the US, where extremely high levels of smoking are seen (for instance only 27% of the MACS cohort are recorded as being never smokers compared with 41% in this work). Finally, the choice of comparator groups used in studies evaluating PLWH may have magnified the apparent effect of HIV status in studies which used “controls” with significantly different socioeconomic backgrounds, as it is possible that confounding effects were not adequately controlled for.

6.3 Understanding the mechanisms lying behind the association between HIV status and respiratory health impairment

Much of the data presented in this thesis have concerned the relationship between HIV status and respiratory health, and the extent to which HIV infection causes respiratory impairment despite antiretroviral therapy. As has been discussed, there are several possible mechanisms by which this could occur, including both direct effects of HIV on the lung (for instance HIV directly causing the development of emphysema) and indirect mechanisms mediated by other processes – for instance, HIV causing respiratory infection which then impairs subsequent respiratory health. A key question of course is the extent to which the observed association between HIV status and respiratory health impairment is the result of these direct or indirect mechanisms, and the extent to which this association is the result of confounding by factors related to both the exposure of interest (HIV infection) and the outcome (respiratory health). The simplest example of this is tobacco smoking – PLWH in the UK are more likely to smoke than the general population and therefore this will confound the relationship between HIV and respiratory health unless properly controlled for. This raises further difficulties – it is hard to accurately quantify smoking exposure,and the linear regression techniques that I have used in this thesis, assume a linear relationship between smoking exposure and respiratory health. Such difficulties are inevitable in any observational research, and careful consideration of which factors should be controlled for, and how these confounding measures can be accurately quantified, is necessary in order to attempt to determine a causal relationship between HV status and outcome.

6.4 Suggestions for future work

The findings of my research within this thesis encourages several avenues that future work could explore.

6.4.1 Respiratory symptoms among PLWH

The finding that PLWH are more likely to report respiratory symptoms, even after adjustment for confounding effects such as tobacco smoking, and anxiety or depression is important and requires further investigation, particularly as these symptoms may become more manifest as PLWH age. These symptoms are likely to arise from multiple aetiologies, and the relative importance of respiratory illness, cardiovascular impairment, muscle strength, and mental health needs to be determined for health services to better manage this potentially increasing problem.

The strong association between poor mental health (in the form of anxiety and depression) and physical symptoms identified in chapter 3 is worthy of further exploration. Interventions to improve mental health may allow individuals to tolerate physical symptoms more easily and could therefore significantly improve quality of life. In addition, improved metal health may allow individuals to take up lifestyle changes such as smoking cessation and increased physical exercise which could directly reduce physical illness in the long term.

6.4.2 Interventions to address tobacco smoking

One important message of the data presented here is the high prevalence of tobacco smoking and significant burden of respiratory ill-health. This should become an important focus of HIV services (as smoking now represents the leading cause of lost life-years for PLWH). How best to deliver smoking cessation services requires operational research into effective strategies and health economic evaluations of the cost benefit of different interventions. At present, the provision of smoking cessation services depends of local commissioning decisions, and services in different areas appear to be highly variable – making the provision of a comprehensive service for PLWH and other "high risk for cardiovascular and pulmonary disease" populations difficult. Targeted interventions specific to particular groups have been effective in people with peripheral vascular disease (229) or diabetes(230). The introduction and evaluation of specifically-targeted services to improve sustained smoking cessation for PLWH would be an important first step towards testing this out.

6.4.3 Frequency and severity of bacterial pneumonia

Although the data presented in Chapter 4 regarding the frequency of acute respiratory illness suggest that where antiretroviral therapy is readily available these are no more common in PLWH, the greater severity of symptoms reported requires further evaluation. My study was too small to allow evaluation of relatively infrequent events such as bacterial pneumonia, and (as discussed in the Introduction) there is epidemiological evidence to suggest that PLWH continue to have a higher frequency of death from pneumonia compared to the general population. Obtaining a clearer picture of why this may be would ideally be performed within large, prospectively observed cohorts, with data collected on potential risk factors and accurate determination of the occurrence and cause of respiratory symptoms and infections documented.

6.4.4 Completeness of immune reconstitution

Although the success of antiretroviral therapy in suppressing viral replication and allowing immune recovery is clear, the extent to which the immune system can completely recover and return to a state equivalent to that seen in HIV uninfected people is unknown. Clinical assessment of immune reconstitution primarily focusses on the CD4 count in peripheral blood. However, this single measure of immune health may miss important persisting changes, either in other areas of the immune system, or more distant body sites. Persisting deficiencies in specific groups of immune cells might be one mechanism for ongoing increased susceptibility to infection (and other conditions where immune changes are implicated in pathogenesis, such as some cancers) despite antiretroviral therapy.

It is possible that changes to the immune system occurring soon after primary HIV infection have a long-term impact on the immune response that are not fully corrected by antiretroviral therapy. Early HIV infection is associated with a rapid loss of CD4+ lymphocytes, particularly at mucosal surfaces such as the gut. Although antiretroviral therapy allows recovery of CD4 lymphocyte numbers, this reconstituted immune system may remain deficient in some aspects of immune function – an example

of this is that the repertoire of T cell receptors becomes significantly less diverse in people living with HIV who are not using antiretroviral therapy, and this appears not to improve after short-term antiretroviral therapy.(145)

A better understanding of immune changes despite ART could have important implications for the future care of people living with HIV – for instance through helping to explain why some infections (and non-infectious conditions in which immune responses could play a part, for instance some cancers) continue to be more common in HIV positive individuals using antiretroviral therapy. It could also allow individuals who may be at particular risk of such conditions to be identified prior to disease onset – and so receive preventive therapy.

Interventions to improve immune recovery in PLWH using ART may have the potential to yield important health benefits, these might include more comprehensive use of immunisations, although individuals at greatest risk of infection might respond least well to immunisations,(231) and hence strategies to boost immunisation response may be required.

6.5 Summary

The respiratory health of people living with HIV has been transformed by antiretroviral therapy, but challenges remain which should be addressed if the health of this population is to be maintained and improved. HIV infection is now a manageable chronic condition, and HIV positive people using antiretroviral therapy can have a life expectancy equalling that of the HIV uninfected. Sustaining health within this aging HIV positive population requires a shift in the emphasis of HIV care towards long-term reduction of risk factors such as tobacco smoking.

Although the finding of a significantly higher prevalence of chronic lung disease such as COPD suggested by some researchers has not been borne out in more recent studies, people living with HIV continue to represent a high-risk group for respiratory illness both due to risk factors such as smoking and HIV-related factors. As highlighted in this thesis, PLWH report a greater prevalence of respiratory symptoms, both in cross-sectional analyses and during acute illnesses. This difference persists even after adjustment for known confounding effects such as smoking. The reasons for this are unclear – it may be due to residual confounding, either by unmeasured additional factors, or incomplete or inaccurate measurement of known confounders such as mental health, socioeconomic status or tobacco and drug use. The very fact that PLWH are aware of their HIV status means that they may devote greater attention or concern to physical symptoms, which could result in a greater reported symptom burden when measured with patient reported outcome measures. However, it may also be that these symptoms are the result of impairments in respiratory function, either as a direct consequence of HIV infection or as a result of previous infection or immunocompromise. Understanding the relative importance of these effects would help to guide interventions that could improve the health-related quality of life of this population.

As uptake of antiretroviral therapy improves, there will be a growing global population of people with stable chronic HIV infection. In the absence of a cure for HIV, understanding the health needs of this group, and how they differ from those without HIV, will be central to future, long-term HIV care.

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Appendix 1: Participant questionnaire for cross-sectional study reported in chapter 3.

Royal Free London NHS
NHS Foundation Trust

HEALTH PERCEPTIONS SMOKING AND HEALTH STATUS QUESTIONNAIRE

We very much appreciate your help with this study.

The following questionnaire has been designed to look at people's beliefs about health and smoking. The results will be used to help us understand how to reduce the risk of heart attacks, strokes and lung disease and help people quit smoking.

At the end, there are some questions about how you feel. We would also like to check your breathing with a machine called a spirometer, which works out how much air you can blow out of your lungs and is a measure of lung function.

The questionnaire should not take more than 20 minutes to complete, then about 5 minutes to do the spirometry.

If you do not want to answer any specific questions or sections, simply leave these blank and move on to the next section. Your care in this clinic will not be affected in any way if you chose not to complete this questionnaire, or any particular questions within it.

Please tick the boxes where appropriate. There are no right or wrong answers so just select the answer that best suits you. We have also left some space for additional comments.

Many thanks for taking the time to help us with our study.

Your responses will be treated in confidence.

Study No: Date:

Section A. General Information:

(tick boxes as appropriate)

A1) What is your gender?:

Male ☐ Female ☐ Transgender ☐

A2) What is your age (years) ☐

A3) Which ethnic group best describes you? (please tick only one)

- Asian – Indian/ Pakistani/ Bangladeshi ☐
- Black African ☐
- Black Caribbean ☐
- Black other ☐
- Mixed white and black ☐
- Mixed other ☐
- Chinese ☐
- White British ☐
- White Irish ☐
- White other ☐
- Other (please state) : ..... ☐

A4) Were you born in the UK?

- Yes ☐
- No ☐

A5) How would you describe your sexuality?:

- Heterosexual / straight ☐
- Homosexual / gay ☐
- Bisexual ☐
- Other (please state) : ☐

A6) What is your current level of education? (please tick ONE ONLY)

- Finished education with no qualifications
- O levels / GCSE (or equivalent qualifications at age 16)
- A levels (or equivalent qualifications at age 18)
- University degree or above
- Other qualifications (please specify)

A7) What is your current work situation? (please tick ONE ONLY)

- Employed or self-employed FULL TIME (at least 30 hours per week)
- Employed or self-employed PART-TIME (less than 30 hours per week)
- Full time student / education / training
- Unemployed
- Permanently sick / disabled (for 3 months or more)
- Temporarily sick / disabled (for less than 3 months)
- Looking after home / family / dependants full time
- Retired
- Other (please specify)

A8) Do you have enough money to cover your basic needs (e.g. food, heating)

- Yes, all of the time
- Yes, most of the time
- Yes, some of the time
- No

Section B. General health:

If unknown, please ask the person who gave you your questionnaire for assistance

B1) Were you exposed to cigarette smoke at home as a child?

Yes ☐ No ☐

B2) Are you currently living with someone else who smokes?

Yes ☐ No ☐

B3) Have you EVER been told by a doctor that you have any of the following conditions?

- Asthma ☐
- Chronic Obstructive Pulmonary Disease or emphysema ☐
- Cancer ☐
- Heart disease / Coronary artery disease (e.g. heart attack, angina) ☐
- Stroke ☐
- Diabetes ☐
- Any other major condition (please specify) ☐

B4) Are you currently receiving treatment (medicine or other therapy) for depression?

Yes ☐ No ☐

B5) Did you have an influenza (flu) vaccine last winter 2013/14 (approximately between Oct 2013 and January 2014)?

Yes ☐ No ☐

If so, where?

- At a hospital ☐
- At my local GP surgery ☐
- At a pharmacy/supermarket ☐
- Other (please specify) ☐
- Don't know ☐

B6) Have you ever had a pneumonia (pneumococcal) vaccine – otherwise called Pneumovax or Prevenar?

Yes ☐ No ☐

If so, when was this? ☐

If so, where?

At a hospital ☐

At my local GP surgery ☐

At a pharmacy/supermarket ☐

Other (please specify) ☐

Don't know ☐

B7) A. Do you use an inhaler/inhalers for your breathing?

Yes ☐ No ☐

If so, please tick any that you take regularly (more than 1x/week):
(blue inhalers are usually relievers, brown/purple/red/pink are usually preventers)

Ventolin (blue) ☐ Terbutaline (blue) ☐

Beclomethasone (brown) ☐ Pulmicort (brown) ☐

Qvar (brown) ☐ Clenil (brown) ☐

Seretide (purple) ☐ Symbicort (red/white) ☐

Fostair (pink) ☐ Tiotropium/Spiriva (grey) ☐

Atrovent (white/green) ☐

Other (please name) ☐

B8) Recreational drug use. The answers to these questions are confidential, however if you prefer not to answer these questions this is fine, and please move on to the next section.

Have you ever used any of the following drugs?

Yes ☐ No ☐

If YES, which drugs have you used?

Cannabis (marijuana, grass), smoked	☐	Ketamine	☐
Crack / cocaine smoked	☐	Mephedrone	☐
Cocaine (sniffed or rubbed in gums)	☐	Crystal meth	☐
Ecstasy or GHB	☐	Heroin (injected)	☐
Heroin (smoked)	☐		
Others (please specify)			

B9) Have you used any recreational drugs in the last 3 months?

Yes ☐ No ☐

If YES, which drugs have you used?

Cannabis (marijuana, grass), smoked	☐	Ketamine	☐
Crack / cocaine smoked	☐	Mephedrone	☐
Cocaine (sniffed or rubbed in gums)	☐	Crystal meth	☐
Ecstasy or GHB	☐	Heroin (injected)	☐
Heroin (smoked)	☐		
Others (please specify)			

Section C. Your respiratory health and fitness:

C1) Do you get breathless when you walk?
Please tick the most appropriate statement (ONE ONLY)

1. Not troubled by breathlessness except on strenuous exercise ☐
2. Short of breath when hurrying or walking up a slight hill ☐
3. Walk slower than contemporaries on level ground or have to stop for breath when walking at your own pace ☐
4. Stop for breath after walking about 100m or after a few minutes on level ground ☐
5. Too breathless to leave the house, or breathless when dressing/undressing ☐

C2) In the past 12 months, have you had any of the following illnesses?
(tick box)

Sinusitis	☐	Pneumonia	☐
Bronchitis	☐	Asthma	☐
Chest infection	☐	Pleurisy	☐
Cold or Flu serious enough to miss work or stop normal activities			☐

C3) Do you undertake physical activity regularly?
(at least once per week)

Yes ☐ No ☐ If so: times/wk: _____

Type of physical activity (tick any that apply below):

Run	☐	Cycle	☐
Swim	☐	Gym - weight training	☐
Other	_____		

Section D. Health and Wellbeing

Under each heading, please tick the ONE box that best describes your health TODAY

MOBILITY

- I have no problems in walking about ☐
- I have slight problems in walking about ☐
- I have moderate problems in walking about ☐
- I have severe problems in walking about ☐
- I am unable to walk about ☐

SELF-CARE

- I have no problems washing or dressing myself ☐
- I have slight problems washing or dressing myself ☐
- I have moderate problems washing or dressing myself ☐
- I have severe problems washing or dressing myself ☐
- I am unable to wash or dress myself ☐

USUAL ACTIVITIES (e.g. work, study, housework, family or leisure activities)

- I have no problems doing my usual activities ☐
- I have slight problems doing my usual activities ☐
- I have moderate problems doing my usual activities ☐
- I have severe problems doing my usual activities ☐
- I am unable to do my usual activities ☐

PAIN / DISCOMFORT

I have no pain or discomfort ☐

I have slight pain or discomfort ☐

I have moderate pain or discomfort ☐

I have severe pain or discomfort ☐

I have extreme pain or discomfort ☐

ANXIETY / DEPRESSION

I am not anxious or depressed ☐

I am slightly anxious or depressed ☐

I am moderately anxious or depressed ☐

I am severely anxious or depressed ☐

I am extremely anxious or depressed ☐

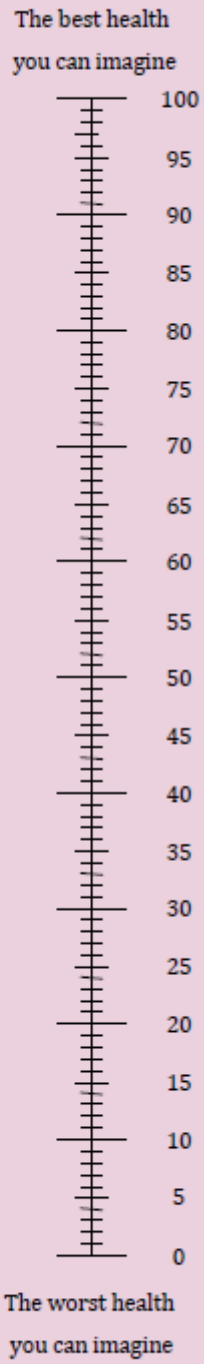
We would like to know how good or bad your health is TODAY.

This scale is numbered from **0** to **100**.
100 means the best health you can imagine.
0 means the worst health you can imagine.

Mark an **X** on the scale to indicate how your health is **TODAY**.

Now, please write the number you marked on the scale in the box below.

YOUR HEALTH TODAY = ____



Section E: Anxiety and resilience

Over the PAST 2 WEEKS, how often have you been bothered by any of the following problems? Please tick one box in each row.

	Not at all	Several days	More than half the days	Nearly every day
1) Little interest or pleasure in doing things	☐	☐	☐	☐
2) Feeling down, depressed, or hopeless	☐	☐	☐	☐
3) Feeling sad	☐	☐	☐	☐
4) Feeling nervous, anxious or on edge	☐	☐	☐	☐
5) Not being able to stop or control worrying	☐	☐	☐	☐
6) Worrying too much about different things	☐	☐	☐	☐
7) Becoming easily annoyed or irritable	☐	☐	☐	☐
8) Trouble relaxing	☐	☐	☐	☐
9) Being so restless that it is hard to sit still	☐	☐	☐	☐
10) Feeling afraid as if something awful might happen	☐	☐	☐	☐
11) Trouble falling or staying asleep, or sleeping too much	☐	☐	☐	☐
12) Feeling tired or having little energy	☐	☐	☐	☐
13) Poor appetite or overeating	☐	☐	☐	☐
14) Feeling bad about yourself—or that you are a failure or have let yourself or your family down	☐	☐	☐	☐
15) Trouble concentrating on things, such as reading the newspaper or watching television	☐	☐	☐	☐
16) Moving or speaking so slowly that other people could have noticed.	☐	☐	☐	☐
17) Thoughts that you would be better off dead, or of hurting yourself in some way	☐	☐	☐	☐
If you were bothered by any of these problems, how difficult have they made it for you to do your work, take care of things at home, or get along with other people?	☐ Not at all difficult ☐ Somewhat difficult ☐ Very difficult ☐ Extremely difficult			

Please read the following statements. To the right of each you will find seven numbers, ranging from "1" (Strongly Disagree) on the left to "7" (Strongly Agree) on the right. Tick the box below the number which best indicates your feelings about that statement. For example, if you strongly disagree with a statement, click the circle below "1". If you are neutral, click "4", and if you strongly agree, click "7", etc. You must answer every question to submit the test for scoring.

		Strongly disagree						Strongly agree
1.	I usually manage one way or another.	1	2	3	4	5	6	7
2.	I feel proud that I have accomplished things in life.	1	2	3	4	5	6	7
3.	I usually take things in stride.	1	2	3	4	5	6	7
4.	I am friends with myself.	1	2	3	4	5	6	7
5.	I feel that I can handle many things at a time.	1	2	3	4	5	6	7
6.	I am determined.	1	2	3	4	5	6	7
7.	I can get through difficult times because I've experienced difficulty before.	1	2	3	4	5	6	7
8.	I have self-discipline.	1	2	3	4	5	6	7
9.	I keep interested in things.	1	2	3	4	5	6	7
10.	I can usually find something to laugh about.	1	2	3	4	5	6	7
11.	My belief in myself gets me through hard times.	1	2	3	4	5	6	7
12.	In an emergency, I'm someone people can generally rely on.	1	2	3	4	5	6	7
13.	My life has meaning.	1	2	3	4	5	6	7
14.	When I'm in a difficult situation, I can usually find my way out of it.	1	2	3	4	5	6	7

Section F. Smoking:

F1) Which of the following best applies to you?

a. I have never been a smoker (i.e. smoked for a year or more) ☐

b. I smoke cigarettes (or hand-rolled) every day ☐

c. I smoke cigarettes (or hand-rolled), but not every day ☐

d. I do not smoke cigarettes at all, but I do smoke tobacco of some kind (eg. pipe/cigar) ☐

e. I used to smoke but have stopped smoking completely ☐

f. If you are an ex-smoker how old were you when you stopped smoking? (If you cannot remember the exact age, please provide an estimate)

_____ years old

If you have never smoked, please move on to section G.

If you are a current or ex-smoker, please answer these questions:

F2) What age did you start smoking? _____ years old

When you smoked the most, how many cigarettes did you smoke a day? _____

F3) If you have tried or managed to quit smoking: which, if any, of the following smoking cessation aids did you use?
(please tick all that apply)

Nicotine replacement product (eg. patches/gum/inhaler) ☐

Zyban (bupropion) or Champix (varenicline) ☐

Attended a Stop Smoking group or support session ☐

Used alternative or complementary therapies (e.g. hypnotherapy or acupuncture) ☐

Used Electronic cigarettes ☐

Other (please specify) ☐

None ☐

Never tried to quit ☐

F4) At your most recent serious quit attempt did you cut down before trying to stop?

- Cut down first ☐
- Stopped without cutting down ☐
- Don't know/can't remember ☐
- Never tried to quit ☐

F5) Do you use electronic cigarettes?

YES ☐ NO ☐

If no, please go to F7

F6) If you do use electronic cigarettes is this: (please tick all that apply)

- Because I have stopped smoking and use it as a substitute for cigarettes ☐
- As well as tobacco cigarettes ☐
- To help me give up tobacco cigarettes ☐
- In places where tobacco smoking is not allowed ☐
- Because I prefer electronic cigarettes ☐
- Other reason (please state)

F7) If you have ever quit and restarted, why did you start smoking again? (e.g. stressful life event, just started again one night in the pub…)

Please give more than one reason if you've restarted more than once).

If you are a current smoker, please answer these questions.
If you do not currently smoke please move on to section G:

F8) If you currently smoke, how many cigarettes per day do you usually smoke?

Cigarettes ☐ how many are hand rolled? ☐

How much money do you think you spend on tobacco in a week? £ ☐

F9) Which of the following best describes you?

I want to stop smoking and intend to do so soon ☐

I want to stop smoking but haven't thought about when ☐

I don't want to stop smoking ☐

Don't know ☐

F10) Thinking about stopping smoking now:

a. How important is it for you to stop smoking at this time? (circle one number)

Not at all 1 2 3 4 5 6 7 8 9 10 Very Much

b. How much do you intend to stop smoking?

Not at all 1 2 3 4 5 6 7 8 9 10 Very Much

c. How confident are you that you will be able to stop smoking?

Not at all 1 2 3 4 5 6 7 8 9 10 Very Much

F11) Has your GP advised you to stop smoking in the past year (i.e. last 12 months)?

YES ☐ NO ☐ UNSURE ☐

F12) If your GP spoken to you about smoking in the past year, which of these best described what happened?

My GP raised the topic of smoking and advised me to stop smoking ☐

My GP raised the topic of smoking together with the offer of a prescriptionor help from a stop-smoking advisor ☐

Neither of these ☐

Don't know ☐

Other (please state) ☐

F13) If you have tried to stop smoking, how many serious attempts to stop smoking have you made in the last 12 months?
(By serious attempt, you decided that you would try to make sure you never smoked again. Please include any attempt that you are currently making and please include any successful attempt made within the last year)

Section G. Respiratory symptoms:

St George's Respiratory Questionnaire

This questionnaire is designed to help us learn much more about how your breathing is troubling you and how it affects your life. We are using it to find out which aspects of your illness cause you most problems, rather than what the doctors and nurses think your problems are.

Please read the instructions carefully and ask if you do not understand anything. Do not spend too long deciding about your answers.

Before completing the rest of the questionnaire:

Please tick in one box to show how you describe your current health:

Very good ☐ Good ☐ Fair ☐ Poor ☐ Very poor ☐

Questions about how much chest trouble you have had over the past 3 months.

Please tick one box for each question:

1. **Over the past 3 months, I have coughed:**

most days a week	☐	only with chest infections	☐
several days a week	☐	not at all	☐
a few days a month	☐		

2. **Over the past 3 months, I have brought up phlegm (sputum):**

most days a week	☐	only with chest infections	☐
several days a week	☐	not at all	☐
a few days a month	☐		

3. Over the past 3 months, I have had shortness of breath:

most days a week	☐	only with chest infections	☐
several days a week	☐	not at all	☐
a few days a month	☐		

4. Over the past 3 months, I have had attacks of wheezing:

most days a week	☐	only with chest infections	☐
several days a week	☐	not at all	☐
a few days a month	☐		

5. During the past 3 months how many severe or very unpleasant attacks of chest trouble have you had? Please tick one:

more than 3 attacks	☐	1 attack	☐
3 attacks	☐	no attacks	☐
2 attacks	☐		

6. How long did the worst attack of chest trouble last?
(Go to question 7 if you had no severe attacks)

Please tick one:

a week or more	☐	1 or 2 days	☐
3 or more days	☐	less than a day	☐

7. Over the past 3 months, in an average week, how many good days (with little chest trouble) have you had?

Please tick one:

no good days	☐	nearly every day is good	☐
1 or 2 good days	☐	every day is good	☐
3 or 4 good days	☐		

8. If you have a wheeze, is it worse in the morning?
Please tick one:

No	☐	Yes	☐

These questions ask about chest problems. If you have not had any chest problems please still answer every question and simply tick the appropriate boxes (e.g. "False" or "Causes no problem").

Section 1

How would you describe your chest condition?

Please tick one:

- The most important problem I have ☐
- Causes me quite a lot of problems ☐
- Causes me a few problems ☐
- Causes no problem ☐

If you have ever had paid employment.

Please tick one:

- My chest trouble made me stop work altogether ☐
- My chest trouble interferes with my work or made me change my work ☐
- My chest trouble does not affect my work ☐

Section 2

Questions about what activities usually make you feel breathless these days.

Please tick in each box that applies to you these days:

	True	False
Sitting or lying still	☐	☐
Getting washed or dressed	☐	☐
Walking around the home	☐	☐
Walking outside on the level	☐	☐
Walking up a flight of stairs	☐	☐
Walking up hills	☐	☐
Playing sports or games	☐	☐

Section 3

Some more questions about your cough and breathlessness these days.
Please tick in each box that applies to you these days:

	True	False
My cough hurts	☐	☐
My cough makes me tired	☐	☐
I am breathless when I talk	☐	☐
I am breathless when I bend over	☐	☐
My cough or breathing disturbs my sleep	☐	☐
I get exhausted easily	☐	☐

Section 4

Questions about other effects that your chest trouble may have on you these days.
Please tick in each box that applies to you these days:

	True	False
My cough or breathing is embarrassing in public	☐	☐
My chest trouble is a nuisance to my family, friends or neighbours	☐	☐
I get afraid or panic when I cannot get my breath	☐	☐
I feel that I am not in control of my chest problem	☐	☐
I do not expect my chest to get any better	☐	☐
I have become frail or an invalid because of my chest	☐	☐
Exercise is not safe for me	☐	☐
Everything seems too much of an effort	☐	☐

Section 5

Questions about your medication, if you are receiving no medication go straight to section 6.

Please tick in each box that applies to you these days:

	True	False
My medication does not help me very much	☐	☐
I get embarrassed using my medication in public	☐	☐
I have unpleasant side effects from my medication	☐	☐
My medication interferes with my life a lot	☐	☐

Section 6

These are questions about how your activities might be affected by your breathing.

Please tick in each box that applies to you because of your breathing:

	True	False
I take a long time to get washed or dressed	☐	☐
I cannot take a bath or shower, or I take a long time	☐	☐
I walk slower than other people, or I stop for rests	☐	☐
Jobs such as housework take a long time, or I have to stop for rests	☐	☐
If I walk up one flight of stairs, I have to go slowly or stop	☐	☐
If I hurry or walk fast, I have to stop or slow down	☐	☐

	True	False
My breathing makes it difficult to do things such as walk up hills, carrying things up stairs, light gardening such as weeding, dance, play bowls or play golf	☐	☐
My breathing makes it difficult to do things such as carry heavy loads, dig the garden or shovel snow, jog or walk at 5 miles per hour, play tennis or swim	☐	☐
My breathing makes it difficult to do things such as very heavy manual work, run, cycle, swim fast or play competitive sports	☐	☐

Section 7

We would like to know how your chest usually affects your daily life.

Please tick in each box that applies to you because of your chest trouble:

	True	False
I cannot play sports or games	☐	☐
I cannot go out for entertainment or recreation	☐	☐
I cannot go out of the house to do the shopping	☐	☐
I cannot do housework	☐	☐
I cannot move far from my bed or chair	☐	☐

Here is a list of other activities that your chest trouble may prevent you doing. (You do not have to tick these, they are just to remind you of ways in which your breathlessness may affect you):

Going for walks or walking the dog

Doing things at home or in the garden

Sexual intercourse

Going out to church, pub, club or place of entertainment

Going out in bad weather or into smoky rooms

Visiting family or friends or playing with children

Please write in any other important activities that your chest trouble may stop you doing:

Now would you tick in the box (one only) which you think best describes how your chest affects you:

- It does not stop me doing anything I would like to do ☐
- It stops me doing one or two things I would like to do ☐
- It stops me doing most of the things I would like to do ☐
- It stops me doing everything I would like to do ☐

Section H. Thoughts about lung health:

Please read the following statements and indicate, by ticking the most appropriate box, whether you agree with the statement:

H1) Most smokers will develop lung disease

- Strongly agree ☐
- Tend to agree ☐
- Unsure ☐
- Tend to disagree ☐
- Strongly disagree ☐

H2) Smoking increases the risk of heart disease

- Strongly agree ☐
- Tend to agree ☐
- Unsure ☐
- Tend to disagree ☐
- Strongly disagree ☐

H3) Only people who are old or overweight or pregnant die of the 'flu

- Strongly agree ☐
- Tend to agree ☐
- Unsure ☐
- Tend to disagree ☐
- Strongly disagree ☐

H4) Having the 'flu vaccine:

Means I can't catch the 'flu at all this year:

- Strongly agree ☐
- Tend to agree ☐
- Unsure ☐
- Tend to disagree ☐
- Strongly disagree ☐

Means I can still get the 'flu, but it might be less severe:

- Strongly agree ☐
- Tend to agree ☐
- Unsure ☐
- Tend to disagree ☐
- Strongly disagree ☐

Will make me feel terrible:

- Strongly agree ☐
- Tend to agree ☐
- Unsure ☐
- Tend to disagree ☐
- Strongly disagree ☐

H5) Having the pneumococcal vaccine (Pneumovax or Previnar):

Means I won't ever get pneumonia in the future:

- Strongly agree ☐
- Tend to agree ☐
- Unsure ☐
- Tend to disagree ☐
- Strongly disagree ☐

Will give me some protection from pneumonia for a few years:

- Strongly agree ☐
- Tend to agree ☐
- Unsure ☐
- Tend to disagree ☐
- Strongly disagree ☐

Will make me feel terrible

- Strongly agree ☐
- Tend to agree ☐
- Unsure ☐
- Tend to disagree ☐
- Strongly disagree ☐

Appendix 2: Publications arising from this thesis.

The following published manuscripts and presented conference abstracts are reproduced here:

Published manuscripts:

1. Brown J, Roy A, Harris R, Filson S, Johnson M, Abubakar I, Lipman M Respiratory symptoms in people living with HIV and the effect of antiretroviral therapy: a systematic review and meta-analysis. Thorax. 2017 Apr;72(4):355-366.

2. Brown J, McGowan J, Chouial H, Capocci S, Smith C, Ivens D, Johnson M, Sathia L, Shah R, Lampe FC, Rodger A, Lipman M Respiratory health status is impaired in UK HIV-positive adults with virologically suppressed HIV infection. HIV Med. 2017 Sep;18(8):604-612

3. Pickett E, Brown J, van Schalkwyk M, Hunter A, Edwards K, Edwards S, Marshall N, Swaden L, Burns F, Johnson M, Lipman M Access to influenza immunisation services by HIV-positive patients in the UK. Influenza Other Respir Viruses. 2018 Jul;12(4):544-546

Conference abstracts:

1. JP Brown, J McGowan, H Chouial, S Capocci, C Smith, D Ivens, F Lampe, M Johnson, L Sathia, A Rodger, M Lipman P226 Impaired respiratory health status in the UK HIV infected population despite the use of antiretroviral therapy Thorax Dec 2015, 70 (Suppl 3) A191; presented at British Thoracic Society winter meeting 2015

2. C Kyriacou, N Stewart, A Melville, J Brown, K Edwards, R Lloyd, M Johnson, J Flint, A Rodger, M Lipman S81 Feasibility and uptake of enhanced smoking cessation services within ambulatory HIV care Thorax Dec 2015, 70 (Suppl 3) A47-A48; presented at British Thoracic Society winter meeting 2015
3. James Brown, Jennifer McGowan, Hende Choual, Santino Capocci, Colette Smith, Daniel Ive ns, Fiona Lampe, Margaret Johnson, Alison Rodger, Marc Lipman Attitudes to smoking and quitting in UK HIV positive adults: Would more education help? European Respiratory Journal 2016 48: PA4603

4. Elisha Pickett, May Van Schalkwyk,
James Brown, Kelly Edwards, Neal Marshall, Sarah Edwards, Leenah Sathia, Maragaret Johnson, Marc Lipman Provision of influenza immunisation for UK HIV positive adults European Respiratory Journal Sep 2016, 48 (suppl 60) PA2606;

5. James Brown, Elisha Pickett, Santino Capocci, Sara Madge, Mike Youle, Lucy Brookes, Marg aret Johnson, Swapna Mandal, John Hurst, Marc Lipman High frequency of unexplained breathlessness among UK HIV positive adults European Respiratory Journal Sep 2017, 50 (suppl 61) PA2601

6. J Brown, E Pickett, C Smith, T Mahungu, D Lowe, S Madge, M Youle, M Sachikonye, M Johnson, J Hurst, T McHugh, I Abubakar and M Lipman Is there a difference in the frequency or severity of acute respiratory illness between HIV-positive and –negative individuals? British HIV Association conference, April 2018

7. J Brown, S Rofael, E Pickett, C Smith, I Abubakar, T McHugh, M Lipman: P191 No difference in the detection of pathogenic respiratory pathogens between HIV-positive individuals using ART and matched negative individuals: British HIV Association conference, April 2018

8. J Brown, E Pickett, C Smith, S Rofael, T Mahungu, D Lowe, S Madge, M Youle, M Johnson, JR Hurst, T McHugh, I Abubakar, M Lipman The Effect of HIV Status on the Frequency of Acute Respiratory Illness: A longitudinal Cohort Study: American Thoracic Society conference, May 2018.

OPEN ACCESS

ORIGINAL ARTICLE

Respiratory symptoms in people living with HIV and the effect of antiretroviral therapy: a systematic review and meta-analysis

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► Additional material is published online only. To view please visit the journal online (http://dx.doi.org/10.1136/thoraxjnl-2016-208657).

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ABSTRACT

Background Antiretroviral therapy (ART) has significantly altered the pattern of acute and chronic HIV-related disease. However, it is not clear what this means in terms of respiratory symptoms. We sought to investigate the association between HIV status and respiratory symptoms and how these have changed with the availability of ART.

Methods We searched Cochrane, Medline and Embase databases for studies published between 1946 and August 2015 comparing the prevalence of respiratory symptoms in populations with and without HIV infection. We undertook random effects meta-analysis of the main symptoms reported. We studied heterogeneity and completed sensitivity analyses and funnel plots.

Results From 5788 unique references identified, 24 papers provided relevant data: 18 documented the prevalence of cough and 11 examined the prevalence of breathlessness among other symptoms reported. Compared with the HIV negative, people living with HIV (PLWH) were more likely to have respiratory symptoms with pooled ORs for the prevalence of cough of 3.05 (95% CI 2.24 to 4.16) in resource-limited populations without access to ART; 2.18 (1.56 to 3.18) in resource-rich populations without access to ART and 1.11 (0.99 to 1.24) in resource-rich populations with access to ART. In resource-rich settings, although the availability of ART was associated with a reduction in the difference between HIV-positive and HIV-negative individuals, PLWH were more likely to report breathlessness, OR 1.39 (95% CI 1.11 to 1.73).

Conclusions Respiratory symptoms are more common in PLWH than controls. This association persists although at a reduced level in populations with access to ART.

Key messages

What is the key question?
- Are respiratory symptoms more common in people living with HIV, and what effect has antiretroviral therapy had on this?

What is the bottom line?
- Our study suggests that respiratory impairment remains more common in HIV-positive adults despite the use of antiretroviral therapy.

Why read on?
- This is the first systematic evaluation of existing evidence regarding respiratory symptoms in people living with HIV, summarising the current evidence base and highlighting where deficiencies in this exist.

INTRODUCTION

Antiretroviral therapy (ART) has altered the natural history of HIV-associated respiratory illness such that acute, life-threatening opportunistic respiratory infections (eg, Pneumocystis pneumonia) are much less common. This has reduced the incidence of AIDS-related respiratory pathology, for instance, there is clear evidence of a reduction in the incidence of TB after ART in all CD4 count strata.[1] This means that HIV infection can be a manageable chronic illness, with people on ART having a life expectancy approaching that of the general population.[2] However, HIV-positive people appear to continue to have an increased frequency of respiratory illness compared with the general population.[3] This is important given that respiratory conditions are likely to become more common as populations age.

There is evidence from multiple sources of lung function impairment in people with long-standing HIV infection.[4 5] Although most current evidence comes from cohorts in the USA or Europe and less is known about populations in other settings, higher rates of respiratory pathology despite antiretroviral treatment have been a consistent finding of studies in HIV-positive populations.[6–8] There are likely to be several reasons for this, including higher rates of smoking and recreational drug use, lung injury occurring prior to antiretroviral treatment and possible consequences of the ongoing immune activation and disordered immune responses associated with HIV.[9]

Although objective impairment of lung function has received considerable recent attention, less attention has been paid to the symptoms that such individuals may experience. This is important, as lung function impairment can correlate poorly with symptoms and functional status.[10] We sought, therefore, to provide a comprehensive analysis of existing data regarding respiratory symptoms in people living with HIV with the aim of understanding how this might have changed with ART, and where deficiencies in the current evidence base exist.

To cite: Brown J, Roy A, Harris R, *et al*. *Thorax* 2017;**72**:355–366.

We provide a systematic review of the evidence regarding respiratory symptoms from population-based studies directly comparing HIV-positive and HIV-negative individuals with or without ART in both resource-limited and resource-rich settings, and a meta-analysis of the effect of HIV infection on the prevalence of these symptoms.

METHODS

Search strategy

Our aim was to evaluate evidence regarding the relative frequency of respiratory symptoms in HIV-positive and HIV-negative populations. We chose search terms that would capture any respiratory symptom (excluding those arising from pathology of the ear, or of obstructive sleep apnoea). We did not seek to set rigid definitions, for instance, regarding the duration of symptoms required to distinguish chronic from acute cough, and instead used those provided by the investigators of the primary studies. This review was registered with the PROSPERO database and details of the protocol are available (PROSPERO 2015:CRD42015013762).

We completed a systematic literature search of publications in the MEDLINE, EMBASE, Cochrane and CINAHL databases between 1946 and August 2015, as well as search of abstracts from the Conference of Retroviruses and Opportunistic Infections and International AIDS Society and American Thoracic Society conferences since 1982 where available. Searches were limited to humans and there was no language limitation.

Search terms included 'HIV' OR 'Human Immunodeficiency virus' OR 'AIDS' AND 'Signs and symptoms, respiratory' (MeSH heading) OR 'cough' OR 'breathless' OR 'dyspnea' OR 'haemoptysis' OR 'short of breath' (see online supplementary appendix 1).

Study selection

Studies were included if they were published in peer-reviewed journals and included both HIV-positive and HIV-negative participants, measured respiratory symptoms in each group and provided quantitative data. All age groups were included in the review and there were no language restrictions.

Exclusion criteria were as follows: studies reporting symptoms of obstructive sleep apnoea (such as hypersomnolence); studies reporting symptoms arising from the ear (such as ear pain, discharge or hearing change); studies that did not include HIV-negative individuals; and studies reporting data with the intention of describing a specific condition (eg, TB, bacterial or *Pneumocystis jirovecii* pneumonia) rather than the occurrence of symptoms within the general or HIV-positive population. This included studies evaluating hospital in-patients alone, as these would not be representative of the population as a whole. We did not exclude population-based studies if, by chance, they contained people with prevalent respiratory conditions as part of the general (HIV-positive or HIV-negative) population under test.

Data extraction

All abstracts were independently reviewed by two members of the review team. Shortlisted full-text articles were then jointly reviewed by two reviewers based on the inclusion/exclusion criteria. Where necessary, discrepancies were resolved by discussion with a senior member of the team.

Data were extracted on the following study characteristics: study design, date of study, age and gender of participants, prevalence of tobacco smoking, risk of acquisition of HIV, geographical location(s) of study, number of HIV-positive and HIV-negative participants, frequency of respiratory symptoms, description of respiratory symptoms/tool used to evaluate, availability of antiretroviral treatment, treatment provided for the respiratory illness, level of immunocompromise in the HIV-positive group (eg, median blood CD4 count).

The risk of bias in observational studies was assessed using a modified Newcastle Ottawa Scale (see online supplementary appendix 2);[11] the version used by Herzog *et al* for the evaluation of cross-sectional studies was modified with criteria selected appropriate to this field.[12] This scale awarded up to eight points to each study: three points for the adequate selection of participants, three points for comparability of the HIV-positive and HIV-negative participants included in the study and two points for the assessment of symptoms and reporting of statistical tests. In this scale, stars are awarded for higher scores in each criterion, giving a total score out of a possible 8 stars. We defined studies of high quality as those that scored 6–8 stars, moderate quality 5 or 6 stars and low quality those with 0–3 stars.

Statistical analysis

The relative prevalence of respiratory symptoms in HIV-positive and HIV-negative groups was compared using ORs: although different definitions of symptoms were used by the investigators of the primary studies (meaning that the absolute prevalence of particular symptoms varied considerably), the relative frequency of symptoms in HIV-positive and HIV-negative participants could be compared by means of ORs, thus allowing estimation of the effect of HIV infection on the likelihood of having these symptoms.

Studies of adults were stratified by location of study into resource-limited and resource-rich settings (which also equated with high and low TB prevalence and high and low HIV prevalence settings) and availability of ART (as reported by the authors). This stratification was undertaken because the aetiology and determinants of respiratory illness were likely to differ between these settings. Studies evaluating children were not included in the quantitative synthesis as these represent a unique population and these studies were highly heterogeneous in methodology and outcome. Meta-analysis was conducted using RevMan V5.3 (The Cochrane Collaboration, 2014).

Pooled ORs for the presence of specific respiratory symptoms were calculated within each stratum wherever more than one study provided data. The DerSimonian and Laird random effects model was used to account for between-study heterogeneity. Statistical heterogeneity was assessed using the Cochran χ^2 test and the I^2 statistic was used to summarise the degree of variation; causes of heterogeneity were further explored by meta-regression. A priori factors chosen for meta-regression were the proportion of HIV-positive individuals with undetectable viral load and the log OR of smoking in HIV-positive versus HIV-negative groups. The possibility that small studies have more extreme results (due to reporting bias) was assessed by the visual inspection of funnel plots.

RESULTS

A total of 5788 publications were identified by our database search after removal of duplicate publications. Of these, 5596 were excluded after review of the article title and abstract. One hundred and ninety-two potentially relevant articles were reviewed in full text, of which 154 were excluded; reasons for exclusion are given in figure 1. Twenty-four publications were included in the final list for review (table 1). Using a modified Newcastle Ottawa Scale, 6 studies were rated as low quality, 14 as moderate quality and 4 as high quality (see online supplementary appendix 3).

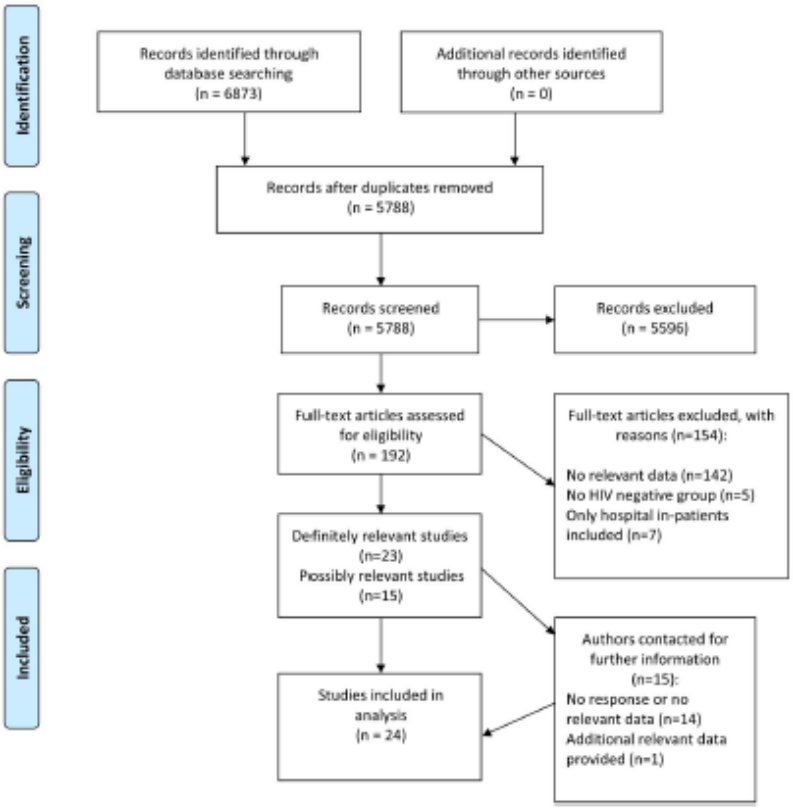

Figure 1 Flow chart of study selection.

Characteristics of included studies and data presented

All studies included were observational studies, although two papers investigated cohorts recruited as part of randomised trials.[20 24] With the exception of five studies following birth cohorts over time, all studies were either cross-sectional in nature or reported data arising from prospective cohorts at a single time point.

The earliest study recruited patients from 1985,[15] with the most recent provided data collected in 2012.[32] Eight articles reported research conducted in the USA (detailing 9 separate studies), with 12 studies conducted in Africa, 2 in Asia and 2 in Europe (both from Italy). Nineteen studies included adult patients and five evaluated infants only.

The presence of cough and breathlessness were the most common symptoms evaluated: data on cough were presented by 17 of 19 studies in adults. Definitions of cough varied, with the most common being the presence of cough for >2 weeks (tables 2–6). Frequency of breathlessness was reported by 11 studies. Usually this was defined only as the presence of 'dyspnoea', 'breathlessness' or 'shortness of breath', though four studies (all evaluating adults with access to ART) used the Medical Research Council (MRC) dyspnoea score as a standardised measure.[35]

Studies that examined respiratory symptoms in HIV-positive adults without access to ART

Twelve studies provided data regarding respiratory symptoms in HIV-positive adults without access to ART (tables 2 and 3). All of these demonstrated a greater proportion of HIV-positive participants with respiratory symptoms. Five studies reported prevalence of breathlessness (two from the USA, others from Nigeria, Zimbabwe and India).

Three studies provided data on respiratory symptoms in HIV-positive populations in the first decade of the HIV epidemic in the USA. The Multicenter AIDS Cohort Study collected data from a cohort of MSM at four sites from 1985 onwards; el Sadr *et al* studied a cohort of injection drug users in New York and Diaz *et al* evaluated a cohort of predominantly white men in Ohio. Data on respiratory symptoms in these populations are presented in table 3.

Nine studies reported the prevalence of respiratory symptoms in HIV-positive and HIV-negative individuals without access to ART in resource-limited settings, and seven of these were in sub-Saharan African populations. The earliest of these used data from Rwanda collected in 1988, and the most recent was from Antwal *et al* in 2014. Only two studies evaluated populations outside of the USA and Africa (Kheaw-on *et al* in Cambodia and Antwal *et al* in India).

Studies that examined symptoms in HIV-positive children

Five studies reported the prevalence of respiratory symptoms in HIV-positive and HIV-negative children (table 4). All of these evaluated cohorts of vertically infected infants followed up from birth, and no studies assessed older children. The earliest was conducted in Rwanda from 1988 with subsequent work in

Table 1 Studies included in this review

First author and reference	Year of publication	Design of study	Risk for HIV	Study location	Total number of participants	% HIV+	Respiratory data collected	Quality rating*
Lepage[13]	1991	Cross-sectional	General population	Rwanda	431	50	Persistent cough >1 month	Moderate
el Sadr[14] Multicentre AIDS Cohort Study	1992	Prospective cohort†	IDU‡	USA	223	56	Standardised symptom checklist (SCS22)	Moderate
Hoover[15] Multicentre AIDS Cohort Study Visit 3	1993	Prospective cohort†	MSM	USA	2854	29	New or unusual cough	Moderate
Hoover—visit 7	1993	Prospective cohort†	MSM	USA	2627	29	New or unusual cough	
Norrgren[16]	1998	Cross-sectional	General population	Guinea-Bissau	2215	9	Cough >1 month	Moderate
Spira[17]	1999	Prospective (birth) cohort	Vertical transmission	Rwanda	401	13	Persistent cough	Moderate
Olayinka[18]	1999	Prospective (birth) cohort	Vertical transmission	Zimbabwe	272	16	Cough since last review	Low
Nilses[19]	2000	Cross-sectional	General population	Zimbabwe	1213	22	Dyspnoea, cough	Moderate
Taha[20]	2000	Prospective (birth) cohort	Vertical transmission	Malawi	808	24	History of wheezy illness collected at clinic appointments	Moderate
Diaz[21]	2003	Cross-sectional	MSM	USA	379	86	ATS-DLD-78 questionnaire‡	Moderate
Galli[22]	2003	Prospective (birth) cohort	Vertical transmission	Italy	2060	4	Retraction of intercostal muscles, wheezes, cough, active nasal flaring or rales at follow-up visits	Low
Lewis[23]	2009	Cross-sectional	General population	South Africa	1955	29	New or worsening cough (>2 weeks or >3 weeks) Haemoptysis	Moderate
Reid[24]	2009	Prospective (birth) cohort	Vertical transmission	Malawi, Tanzania, Zambia	1317	6	Chronic cough (>14 days)	Moderate
Khaw-On[25]	2009	Cross-sectional	General population	Thailand	210	50	History of chronic cough	Low
Corbett[26]	2010	Cross-sectional	General population	Zimbabwe	8979	21	Cough <2 weeks. Cough >3 weeks, haemoptysis	Low
Drummond[27] ALIVE cohort	2010	Prospective cohort†	IDU	USA	974	30	Modified ATS-DLD-78 MRC dyspnoea scale	High
Onyedum[28]	2010	Cross-sectional	General population	Nigeria	200	50	Cough, cough with sputum, breathlessness, wheezing	Low
Gounder[29]	2011	Cross-sectional	General population	South Africa	3937	37	Cough >2 weeks. Sputum production	Moderate
Crothers[8] Lung HIV Study	2013	Prospective cohort†	Mixed	USA	589	51	Usual cough/phlegm, wheezing (ATS-DLD-78) MRC dyspnoea scale	High
Fitzpatrick[7] WHIS	2013	Prospective cohort†	Mixed	USA	99	64	ATS-DLD questionnaire	High
Madeddu[30]	2013	Cross-sectional	MSM	Italy	176	63	Symptom questionnaire MRC dyspnoea scale	Moderate
Antwal[31]	2014	Cross-sectional	Not detailed	India	1546	35	Breathlessness, cough with sputum	Moderate

Continued

Table 1 Continued

First author and reference	Year of publication	Design of study	Risk for HIV	Study location	Total number of participants	% HIV+	Respiratory data collected	Quality rating*
Campo[32] EXHALE substudy, VACS cohort	2014	Prospective cohort†	Mixed	USA	340	53	Chronic cough (for most days for 3 months or more per year for >1 year), chronic phlegm, wheeze, MRC dyspnoea scale	Moderate
Gingo[33] (MACS)	2014	Prospective cohort†	MSM	USA	1896	48	ATS-DLD-78 questionnaire	High
Gingo (WIHS)	2014	Prospective cohort†	Mixed	USA	1976	71	ATS-DLD-78 questionnaire	
Telisinghe[34] Prison cohort	2014	Cross-sectional	General population	South Africa	846	25	Chronic cough (>14 days)	Low

*Modified Newcastle Ottawa Scale.
†Data arising from prospective cohort studies; however, data used in quantitative analyses for this review from single time point.
‡American Thoracic Society Division of Lung Disease questionnaire.
IDU, injection drug user; MACS, Multi-Centre AIDS Cohort Study; MSM, men who have sex with men; VACS, Veterans Aging Cohort Study; WIHS, Women's Interagency HIV Study.

Table 2 Respiratory symptoms in resource-limited settings without access to ART

First author	Year of publication	Study location	Total N	% HIV+	HIV+ on ART (%)	Mean/median blood CD4*	% HIV+ Smokers	% HIV− smokers	Definition of cough used	% HIV+ with cough	% HIV− with cough	Definition of breathlessness	% HIV+ with breathlessness	% HIV− with breathlessness
Lepage[13]	1991	Rwanda	431	50	0	24% CD4:CD8 <0.5	NR	NR	Persistent cough >1 month	14	7			
Norrgren[16]	1998	Guinea-Bissau	2215	9	0	715 (men) 430 (women)	NR	NR	Cough >1 month	3	1			
Nilses[19]	2000	Zimbabwe	1213	21	0	NS	NR	NR	Cough	4	2	Dyspnoea	1	1
Khaw-On[25]	2009	Thailand	210	50	NR	261	36	15	History of chronic cough	57	31			
Lewis[23]	2009	South Africa	1955	29	NR	NR	NR	NR	New or worsening cough, any duration	4	2			
									>2 weeks	2	1			
									>3 weeks	2	1			
Corbett[26]	2010	Zimbabwe	8979	21	NR	NR	NR	NR	Cough for 2 weeks or more	8	2			
Onyedum[28]	2010	Nigeria	200	50	0	71% CD4 <200	0	0	Cough	48	8	Breathlessness	32	0
Gounder[29]	2011	South Africa	3937	37	NR	333	NR	NR	Cough for 2 weeks or longer	7	4			
Antwel[31]	2014	India	1546	35	0	241	NR	NR	Cough with sputum	10	2	Breathlessness	4	1

*Cells per microlitre unless otherwise stated.
ART, antiretroviral therapy; NR, not reported.

Table 3 Studies in resource-rich settings without access to ART

First author	Year	Study location	Total number of participants	% HIV+	Risk for HIV	HIV+ on ART (%)	Mean/ median blood CD4*	% HIV+ Smokers	% HIV− smokers	Definition of cough used	% HIV+ with cough	% HIV− with cough	Definition of breathlessness	% HIV+ with breathlessness	% HIV− with breathlessness
el Sadr[14]	1992	USA	223	56	IDU	0	200–500	NR	NR	Persistent cough	17	7			
Hoover visit 3[15]	1993	USA	2854	33	MSM	0	619	NR	NR	New or unusual cough lasting >2 weeks	5	2	Persistent shortness of breath	1	2
Hoover—visit 7	1993	USA	2627	29	MSM	0	552	NR	NR		5	2		3	2
Diaz[21]	2003	USA	379	86	MSM	0	370	54	50%	Do you usually have a cough?	40	25	Are you troubled by shortness of breath when hurrying on the level or walking up a slight hill?	42	8
										Do you usually cough like this on most days For ≥3 consecutive months of the year?	24	12	Do you have to walk slower than most people of your own age on the level ground because of breathlessness?	20	0

*Cells per microlitre.
ART, antiretroviral therapy; IDU, injection drug user; MSM, men who have sex with men.

Table 4 Studies evaluating children (cohorts followed up from birth)

First author	Year of publication	Study location	Total number of participants	% HIV+	% HIV+ on ART	Duration of follow-up (from birth)	Respiratory data collected	Notes/comments
Spira[17]	1999	Rwanda	401	13	0	Median 27 months (HIV-positive group), 51 months (HIV-negative group)	Chronic cough (>14 days)	Measured cumulative incidence of developing a cough lasting >14 days
Olayinka[18]	1999	Zimbabwe	272	16	0	24 months	Persistent cough	
Taha[20]	2000	Malawi	689	19	0	Median 18 months	Cough since last review	2 HIV-negative groups—exposed uninfected infants and unexposed. Rate of cough the same in each.
Galli[22]	2003	Italy	280	75	25	24 months	History of wheezy illness collected at clinic appointments	Presents proportion of infants with wheezy illness in first 2 years of life
Reid[24]	2009	Malawi, Tanzania, Zambia	1317	84	0	12 months	Retraction of intercostal muscles, wheezes, cough, active nasal flaring or rales at follow-up visits	HPTN 024 study cohort, studied 2001–2004

ART, antiretroviral therapy; HPTN, The HIV Prevention Trials Network.

Table 5 Studies evaluating populations in resource-rich settings with access to ART

Author	Year	Study location	Total number of participants	% HIV+	HIV+ on ART (%)	% undetectable HIV plasma load	Mean/ median blood CD4	% HIV+ smokers	% HIV− smokers	Definition of cough	% HIV+ with cough	% HIV− with cough	Definition of breathlessness	% HIV+ with breathlessness	% HIV− with breathlessness
Drummond[27] ALIVE cohort	2010	USA	974	30	54	16.5	320	83	86	Cough present	3	29	MRC dyspnoea score ≥2	35	28
										Morning cough ≥4 days per week	19	19			
										Morning cough ≥3 months	15	15			
Crothers[8] Lung HIV Study	2013	USA	589	51	89	84	493	47	35	Unusual cough	28	20	MRC dyspnoea score ≥2	15	11
Fitzpatrick[7] WIHS	2013	USA	99	64	81	NS	426	46	50				Dyspnoea	35	31
Madeddu[30]	2013	Italy	176	63	78	71	541	57	58	Cough	32	14	MRC dyspnoea score ≥2	31	15
Campo[32] EXHALE substudy, VACS cohort	2014	USA	340	53	89	NS	431	64	58	Chronic cough	25	16	MRC dyspnoea score ≥2	25	16
Gingo[33] MACS	2014	USA	1896	48	77	70	572	31	23	Cough	42	38	Dyspnoea	14	9
Gingo WIHS	2014	USA	1976	71	76	56	502	39	52	Cough	54	58	Dyspnoea	36	37

ART, antiretroviral therapy; MACS, Multi-Centre AIDS Cohort Study; VACS, Veterans Aging Cohort Study; WIHS, Women's Interagency HIV Study.

Malawi and Zimbabwe. None of these study populations had access to ART.

One study evaluated symptoms in HIV-positive infants born in Chieti, Italy; this suggested a lower frequency of 'wheezy respiratory illness' in HIV-positive infants despite more maternal smoking, premature delivery, low birth rate and formula feeding. However, the design of the study (based on routine clinic records) did not allow appraisal of the possibility of differences in health-seeking behaviour or reporting of acute respiratory illness, which could bias the comparison of respiratory symptoms between the different groups.

Studies that examined respiratory symptoms in HIV-positive adults with access to ART

Six studies (using data from seven cohorts) reported on adults with access to ART. All were from resource-rich settings other than one from South Africa. Data on respiratory symptoms are presented in tables 5 and 6.

All contained a high proportion of current smokers in both the HIV-positive and HIV-negative groups and, where reported, often had high rates of former or ongoing recreational drug use.

The first systematic comparison of respiratory symptoms in a HIV-positive population with access to ART with HIV-negative comparators is provided by the AIDS Linked to the IntraVenous Experience (ALIVE) cohort of former and current injection drug users in Baltimore, Maryland, USA.[27] This found no difference in frequency of current cough, although a history of 'wheezing' was reported by 50% of HIV-positive and 37% of HIV-negative participants.

Several cohort studies have addressed respiratory symptoms in HIV-positive individuals with access to ART in the USA: the Women's Interagency HIV Study (WIHS); Multicentre AIDS Cohort Study and the EXHALE substudy of the Veterans Aging Cohort Study.[32 36] Tobacco use was high in these cohorts, and smoking was more frequent in the HIV-positive than HIV-negative groups (reported in 39%–64% and 23%–58%, respectively). The prevalence of respiratory symptoms was high in these populations, for instance Fitzpatrick *et al*[7] described breathlessness in 35% of HIV-positive and 31% HIV-negative participants in the WIHS study and Campo *et al* [32] reported cough in 25% of HIV-positive and 16% in HIV-negative participants in the EXHALE VACS substudy.

There is little information comparing respiratory symptoms in HIV-negative and HIV-positive individuals with access to ART outside of North America. The only study providing data from Europe documented the presence of any respiratory symptom in 47% HIV-positive participants and 23% of HIV-negative participants, with 32% vs 14% reporting cough (p=0.006) and 31% vs 15% breathlessness (p=0.02).[30] Telisinghe *et al*[34] evaluated a prison population in South Africa (with a 25% HIV prevalence) in which cough lasting 2 weeks or more was present in 13% of HIV-positive and 8% of HIV-negative individuals. 36% of the HIV-positive participants reported the use of ART.

The prevalence of tobacco smoking was only consistently reported in studies undertaken in resource-rich settings with access to ART. In all of these studies, a large proportion of both HIV-positive and HIV-negative participants were current smokers (see table 5), and this was similar across these groups.

Quantitative data synthesis

Data were available on 12 075 HIV-positive individuals compared with 24 450 individuals without HIV infection. The symptoms for which sufficient data were available to allow quantitative synthesis were cough and breathlessness. Figures 2 and 3 demonstrate the ORs for the presence of cough and breathlessness in those with and without access to ART and in resource-rich and resource-limited settings.

Meta-anaysis suggests that the availability of ART is associated with a reduction in the difference in prevalence of respiratory symptoms between HIV-positive and HIV-negative individuals, yet these remain more common in people living with HIV. There is little evidence regarding respiratory symptoms in HIV-positive populations in resource-limited settings. The pooled OR for the presence of cough in such populations without access to ART was 3.05 (95% CI 2.24 to 4.16, I^2=68%); in resource-rich populations without access to ART, it was 2.18 (1.55 to 3.04 I^2=0%), and in those from resource-rich settings with access to ART, the OR for the presence of cough was 1.26 (0.97 to 1.63) —although there was a substantial degree of heterogeneity in this analysis (I^2=74%). Only one study reported frequency of cough in a high-prevalence setting with access to ART.[34]

Breathlessness was also assessed, although fewer studies reported this. In resource-limited settings without access to ART, the OR for the presence of breathlessness was 7.5 (0.92 to 61.31, I^2=83%). Only two studies were available from resource-rich settings without access to ART, and meta-analysis of these data provides an OR of 2.60 (0.25 to 26.93, I^2=93%). In resource-rich settings with access to ART, there was good evidence for a higher rate of breathlessness in HIV-positive individuals with an OR of 1.39 (1.11 to 1.73, I^2=52%).

Funnel plots were inspected to evaluate the possibility of reporting or publication bias where sufficient studies were available within each stratum of ART availability and location to make this useful. For the most part these did not suggest the presence of significant reporting bias (eg, see online supplementary figure S1) although this was possible for the apparent association between breathlessness in HIV-positive populations with access to ART (see online supplementary figure S2). As there were fewer than 10 studies within each stratum, quantitative tests of reporting bias were not used, in accordance with the Cochrane Handbook guidance.[37]

Sensitivity analyses

Two studies (Onyedum 2010 and Antwal 2014) included in the analysis of respiratory symptoms in populations without access to ART were judged to be at high risk of bias due to the inclusion of significant numbers of participants presenting for care with acute respiratory illnesses due to their methodology (which involved recruitment within acute care services).[32 33] Exclusion of these studies reduced somewhat the effect size for the prevalence of cough to an OR of 2.84 (2.45 to 3.30) as well as the heterogeneity in this analysis (I^2=55%).

Four studies (all in populations with access to ART) used the MRC dyspnoea scale to measure breathlessness (see table 5). A meta-analysis including only studies using the MRC dyspnoea scale (with breathlessness defined as a score of ≥2) did not significantly change the effect size (compared with the analysis of all studies reporting the prevalence of breathlessness in populations with access to ART) but reduced heterogeneity significantly (OR for having MRC dyspnoea ≥2=1.50, 95% CI 1.21 to 1.85, I^2=0%, online supplementary figure S3).

The true effect of HIV status on respiratory symptoms may be exaggerated by the higher proportion of HIV-positive subjects who were current smokers in several of the included studies, as few studies provided data adjusted for smoking status. Meta-regression confirmed an association between higher rates of respiratory symptoms in studies with a greater imbalance of smokers versus non-smokers in the HIV-positive

Table 6 Studies evaluating populations in resource-limited settings with access to ART

Author	Year	Study location	Total number of participants	% HIV+	HIV+ on ART (%)	% Undetectable HIV plasma load	Mean/ median blood CD4	% HIV+ smokers	% HIV− smokers	Definition of cough	% HIV+ with cough	% HIV− with cough
Telisinghe[24] Prison cohort	2014	South Africa	846	25	41	NS	NS	59	59	Cough >2 weeks	13	8

ART, antiretroviral therapy.

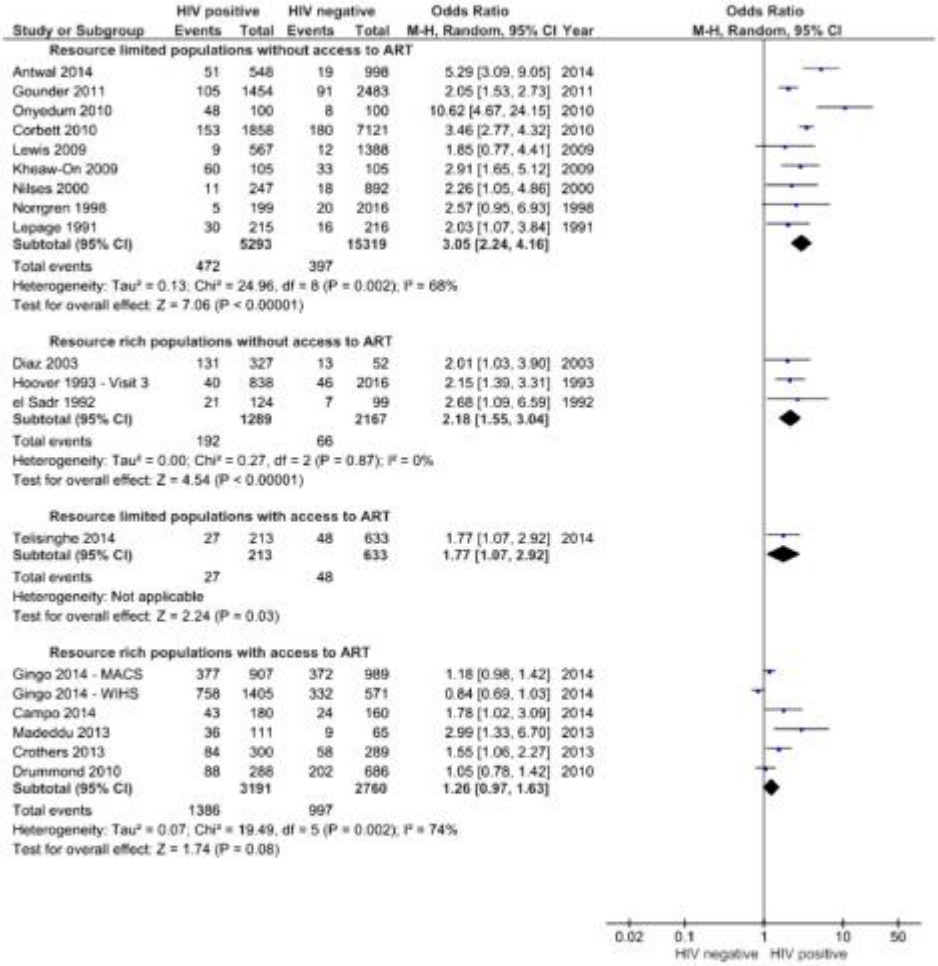

Figure 2 Forest plot of ORs for presence of cough, stratified by availability of ART and location. ART, antiretroviral therapy; MACS, Multicentre AIDS Cohort Study; WIHS, Women's Interagency HIV Study.

compared with HIV-negative group (data not shown). However, this had little impact on the estimated effect of HIV infection on the frequency of cough or breathlessness.

DISCUSSION

This systematic review and meta-analysis demonstrates that despite the availability of ART, HIV-positive populations continue to experience more respiratory symptoms than comparable HIV-negative groups, at least in resource-rich settings for which there are sufficient data to draw conclusions. It also highlights several research needs: in particular the lack of rigorous data concerning respiratory symptoms in HIV-positive populations in low-income and middle-income settings where the majority of the world's HIV-positive individuals live.

The successful provision of ART means that HIV-positive populations can expect considerable improvements in life expectancy;[38] however, ageing HIV-positive populations have more comorbidities and respiratory illness is likely to become increasingly important over time.[39 40] An improved understanding of the health needs of ageing HIV-positive populations is required,

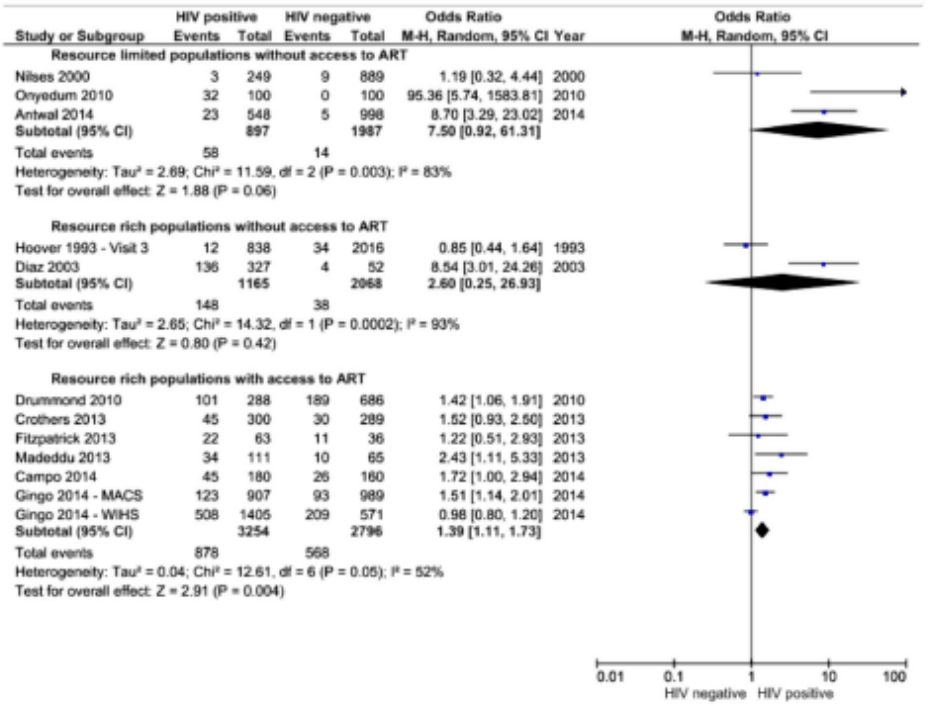

Figure 3 OR for the presence of breathlessness, random effects meta-analysis stratified by location and availability of antiretroviral therapy.

as even in resource-rich settings, the impact of respiratory illness on individuals (measured by instruments designed to evaluate health-related quality of life) or at a societal health-economic level is evaluated infrequently in HIV-positive populations.

In resource-rich settings, HIV-positive populations often have greater cigarette and recreational drug use than the general population.[41 42] Interventions to reduce this are therefore important, given that there is accumulating evidence of increased rates of both cardiovascular and respiratory illness. In some high HIV prevalence settings, tobacco smoking is rising fast,[43] and attention should be given to the possibility that HIV-positive individuals may be particularly susceptible to the harmful effects of tobacco smoking.[44]

Strengths of this analysis include a comprehensive search strategy and robust methods for study selection. Limitations include the lack of standardised assessments of respiratory symptoms in the primary studies, and incomplete reporting of important confounding factors (eg, smoking and recreational drug use), which may influence the effect sizes found. The incomplete reporting of exposures such as tobacco smoking and drug use in the original papers means that the extent to which the association identified between HIV status and respiratory symptoms is a direct result of the HIV virus is uncertain as we were not able to adjust for differing exposures to these possible confounding factors between HIV-positive and HIV-negative individuals. Another important limitation is that the data included in the quantitative meta-analyses were cross-sectional in nature and we cannot therefore infer causation from such studies. Bias or confounding in the primary studies may be one cause of the association between HIV status and respiratory symptoms found, and the strength of conclusions that can be drawn is therefore limited by the paucity of evidence in many areas.

One difficulty when evaluating respiratory symptoms is the challenge of standardising assessments: The wide range in the reported frequency of respiratory symptoms arises in part from the diversity of methods used to evaluate them: the definitions used for symptoms such as cough or breathlessness were often not stated, and a minority of studies used validated tools such as the St George's Respiratory Questionnaire,[45] American Thoracic Society Division of Lung Disease questionnaire[46] or the MRC dyspnoea scale (see tables 2–6 for details of data collected in each study).[35] Furthermore, results were presented as the proportion/number with respiratory symptoms, with no study reporting estimates adjusted for potential confounders.

It is important to note that although populations may have access to ART, most studies include a significant proportion of participants who were either not using ART, or who do not have an undetectable viral load. We cannot therefore estimate the effect of HIV infection at an individual level in those with a favourable virological and immunological response to ART. Furthermore, most of the data regarding populations with access to ART derive from the USA and there is limited information from other settings. Exposures of importance that could lead to respiratory disease may differ in other locations, and the findings of the studies included in this review may not be generalisable—for instance, to HIV-positive African populations using ART.

Our analysis suggests that despite the availability of ART, HIV-positive individuals are more likely to experience respiratory symptoms than those without HIV. Part of the reason for this higher burden of symptoms is likely to be the greater prevalence of tobacco smoking and recreational drug use in many

HIV-positive populations, particularly in resource-rich countries. However, direct HIV-related mechanisms may also play a role: although ART treatment achieves virological suppression for the majority of people treated, derangements of immune function persist despite treatment, which include evidence of ongoing immunological activation, which may promote the development of comorbid disease.[47] There is growing evidence that this is clinically important for the development of cardiovascular disease[48] and may also be relevant for respiratory pathology—for instance, Attia *et al* demonstrated an association between soluble CD14 (a marker of immune activation) and radiological emphysema in HIV-positive adults.[4]

Interventions are required to address this health need. For populations with high levels of tobacco smoking, smoking cessation services should be prioritised. Earlier and more successful ART treatment may yield better lung health in the long term, although no significant differences in respiratory symptoms or lung function change were seen in HIV-positive adults with CD4 counts above 500 cells/μL randomised to early compared with deferred ART treatment in the recent START trial.[49]

Furthermore, as highlighted in this review, there is little available evidence regarding the determinants of respiratory illness in HIV-positive individuals in resource-limited settings, where the majority of the world's HIV-positive individuals live.

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Contributors JB, AR, MJ, ML and IA planned the study, and JB is the guarantor of the work. JB, AR and SF undertook the literature review and selection of studies for inclusion in analysis. JB and AR undertook appraisal of the studies. JB and RH undertook statistical analysis. JB drafted the paper, and all authors revised and approved the final draft paper.

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ORIGINAL RESEARCH

Respiratory health status is impaired in UK HIV-positive adults with virologically suppressed HIV infection

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Objectives

We sought to evaluate whether people living with HIV (PLWH) using effective antiretroviral therapy (ART) have worse respiratory health status than similar HIV-negative individuals.

Methods

We recruited 197 HIV-positive and 93 HIV-negative adults from HIV and sexual health clinics. They completed a questionnaire regarding risk factors for respiratory illness. Respiratory health status was assessed using the St George's Respiratory Questionnaire (SGRQ) and the Medical Research Council (MRC) breathlessness scale. Subjects underwent spirometry without bronchodilation.

Results

PLWH had worse respiratory health status: the median SGRQ Total score was 12 [interquartile range (IQR) 6–25] in HIV-positive subjects *vs.* 6 (IQR 2–14) in HIV-negative subjects ($P < 0.001$); breathlessness was common in the HIV-positive group, where 47% compared with 24% had an MRC breathlessness score ≥ 2 ($P = 0.001$). Eighteen (11%) HIV-positive and seven (9%) HIV-negative participants had airflow obstruction. In multivariable analyses (adjusted for age, gender, smoking, body mass index and depression), HIV infection remained associated with higher SGRQ and MRC scores, with an adjusted fold-change in SGRQ Total score of 1.54 [95% confidence interval (CI) 1.14–2.09; $P = 0.005$] and adjusted odds ratio of having an MRC score of ≥ 2 of 2.45 (95% CI 1.15–5.20; $P = 0.02$). Similar findings were obtained when analyses were repeated including only HIV-positive participants with a viral load < 40 HIV-1 RNA copies/mL.

Conclusions

Despite effective ART, impaired respiratory health appears more common in HIV-positive adults, and has a significant impact on health-related quality of life.

Keywords: lung, patient reported outcome, quality of life, respiratory, smoking

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Introduction

Antiretroviral therapy (ART) has transformed HIV infection into a manageable chronic condition [1]. Despite this, people living with HIV (PLWH) continue to have higher rates of comorbidities such as cardiovascular [2] and renal disease [3], as well as some malignancies [4]. There is also evidence that, despite ART, PLWH may have a higher prevalence of chronic respiratory illness [5]. For instance, in the large US Veterans Aging Cohort Study, after adjustment for smoking status and other characteristics, chronic obstructive pulmonary disease (COPD) was 50–60% more common in PLWH than in HIV-negative participants [6]. The development of persistent, noncommunicable respiratory disease such as COPD in HIV-positive individuals may impact significantly on their quality of life [7].

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There are, however, limitations to the currently available data concerning chronic respiratory disease in HIV-positive people on ART. In particular, most reported studies have included significant proportions of participants without virological suppression. Also, results may be influenced by residual confounding factors such as tobacco smoking and recreational drug use. Furthermore, most studies assessing respiratory health in HIV-positive populations have focused on objective measurements of lung function (such as spirometry) rather than the subjective impact of respiratory impairment on health-related quality of life [5, 8]. This is an important issue, as respiratory symptoms can correlate poorly with objectively measured lung function [9], yet may have a significant effect on quality of life. There is a need, therefore, to better understand the degree to which PLWH with access to effective ART have worse respiratory health than HIV-negative individuals (with similar risk factors), and the impact of this on their quality of life [10].

The use of validated patient-reported outcome measures allows the systematic evaluation of the impact of pathologies on health-related quality of life, and enables direct comparisons to be made between different groups and populations. These instruments can attempt to quantify general health status (such as the EuroQoL 5D 5L (EQ5D)) or may be disease or organ specific [e.g. the St George's Respiratory Questionnaire (SGRQ)]. In this study, we used these measures to provide an assessment of overall respiratory health (rather than an assessment specific to one condition such as COPD) – which we refer to as "respiratory health status".

We sought to evaluate whether respiratory health status was impaired in a contemporary (on ART) HIV-infected population and to test the hypothesis that HIV-positive adults have worse respiratory health than HIV-negative people with similar risk factors; to assess whether impaired respiratory health correlated with spirometric impairment in this population, and to explore the effect of potential confounding factors such as smoking, recreational drug use, and physical and mental comorbidities. In addition, for those with HIV infection, we describe the relationship between HIV-related factors such as blood CD4 count, HIV load and duration of HIV infection and respiratory health status.

Methods

Study population

We conducted a cross-sectional observational study in the HIV ambulatory care service and sexual health clinics (at Royal Free and Barnet Hospital sites) of the Royal Free London NHS Trust, London, UK from February to July 2015. Consecutive clinic patients were invited to take part in the study when they attended routine care appointments. Subjects provided written informed consent. London sexual health clinics were chosen as the site for recruitment of HIV-negative participants as this population was anticipated to have similar lifestyle characteristics, including smoking behaviours, to the HIV clinic population. As service users in the sexual health clinics were significantly younger than those attending clinics for HIV care, recruitment in sexual health clinics was restricted to those over the age of 35 years to achieve a sample that approximated the age of the HIV-positive participants. There were no exclusion criteria for the HIV-positive group. Ethical approval was granted by the London – Camden and Islington Research Ethics Committee (14/LO/1646).

Procedures

Participants completed a questionnaire including items on risk factors for respiratory illness, smoking and recreational drug use and health-related quality of life (using the EQ5D) [11,12,13]. As depression might affect the experience and expression of respiratory symptoms (and therefore act as an important confounding factor), symptoms of depression were evaluated using the Patient Health Questionnaire – 9 (PHQ-9) (a scale providing scores of 0–27, where scores of ≥ 10 suggest the presence of moderate or severe depressive symptoms [14]). Respiratory health status was measured using (a) the SGRQ and (b) the Medical Research Council dyspnoea (breathlessness) scale, a scale with scores from 1 to 5 recording the severity of breathlessness on exertion [15, 16].

The SGRQ is a patient-reported outcome measure which quantifies respiratory health using a 50-item self-completed questionnaire [15]. Responses are translated onto a scale from 0 to 100 in which higher scores indicate worse respiratory health status, with domains assessing symptoms, activities and impacts as well as a total score. Although initially developed for use in asthma and COPD, it is not disease-specific and has been widely used in other respiratory conditions.

Subjects underwent spirometry without bronchodilation (Carevision Micro I spirometer, Beckton Dickinson, New Jersey, USA) and had their height and weight measured. Normal values for spirometry were calculated using the Global Lung Function Initiative equations [17]; airflow obstruction was defined as an Forced Expiratory Volume in one second (FEV1)/ Forced Vital Capacity (FVC) of < 0.7. For current and past blood test results, data were obtained from hospital databases with participant consent. Self-

reported HIV status was noted and not independently confirmed in the sexual health clinic population.

Statistical analysis

Data were recorded and analysed in EXCEL (Microsoft) and SPSS version 22 (IBM, New York, USA). Univariable comparisons between HIV-positive and -negative participants were undertaken using χ^2 and Fisher's exact tests for categorical variables and unpaired *t*-tests or Mann–Whitney *U*-tests for continuous variables, as appropriate. To adjust for participant characteristics and to assess the independent associations of factors with respiratory measures, multivariable regression analyses were performed. SGRQ Total scores were log-transformed to normalize their distribution for these analyses. Multivariable linear regression analyses were used to assess factors independently associated with log-transformed SGRQ Total scores and estimates were then back-transformed to derive adjusted fold-changes in SGRQ score for covariates of interest. Multivariable logistic regression models were used to assess factors independently associated with having an MRC dyspnoea score of ≥ 2. For the multivariable analyses, a core set of variables of importance were selected *a priori* (age, tobacco smoking, gender and HIV status) and, following univariable analysis, additional variables found to be significantly associated at the 5% level with respiratory health status were added to the multivariable model.

Table 1 Comparison of demographic characteristics between HIV-positive and HIV-negative participants

	HIV-positive ($n = 197$)	HIV-negative ($n = 93$)	*P*-value
Gender			
Male [*n* (%)]	158 (80)	64 (71)	0.09*
Age (years) [median (IQR)]	50 (42–55)	43 (38–52)	0.025†
BMI (kg/m^2) [mean (SD)]	25.84 (5.04)	25.36 (4.23)	0.434‡
Race/ethnicity [*n* (%)]			
White	143 (72)	54 (60)	0.003§
Black	37 (20)	15 (17)	
Other	15 (8)	24 (26)	
Born in UK [*n* (%)]	121 (62)	49 (54)	0.24*
Gender/sexuality [*n* (%)]			
MSM	131 (66)	25 (27)	< 0.001§
MSW	27 (14)	37 (40)	
Female	37 (20)	24 (28)	
Not stated	0	5 (5)	
Highest educational attainment [*n* (%)]			
None	24 (12)	10 (12)	0.64§
GCSE or equivalent	28 (14)	7 (8)	
A level or equivalent	31 (16)	14 (16)	
University degree or higher	106 (54)	50 (59)	
Other	6 (3)	4 (5)	
Employment [*n* (%)]			
Full-time	92 (47)	59 (67)	0.015§
Part-time	22 (11)	11 (12)	
Unemployed	25 (13)	5 (6)	
Retired	18 (9)	4 (4)	
Student	5 (3)	1 (1)	
Not working because of ill health	28 (14)	4 (5)	
Other	6 (3)	5 (5)	

BMI, body mass index; GCSE, General Certificate of Secondary Education; IQR, interquartile range; MSM, men who have sex with men; MSW, men who have sex with women; SD, standard deviation.
*χ^2 test.
†Mann–Whitney *U*-test.
§Fisher's exact test.
‡Independent samples *t*-test.

Results

Study participants

Of 402 individuals invited to participate, 290 (72%) agreed: 197 HIV positive and 93 HIV negative. Recruitment of HIV-negative individuals was lower as fewer eligible individuals attended these clinics over the study period – however, the response rate was similar, being 75% among HIV-positive individuals and 73% among HIV-negative individuals. Demographics and details of comorbid conditions are listed in Tables 1 and 2.

The median blood CD4 count of HIV-positive participants was 627 cells/μL [interquartile range (IQR) 456–838 cells/μL]; 171 (94%) of PLWH reported using ART, with a median duration of treatment of 7 years. Eighty-nine per cent of all PLWH and 93% of those using ART had an undetectable plasma HIV load (< 40 HIV-1 RNA copies/mL) at their last clinic visit. The median nadir CD4 count of this cohort was 250 cells/μL (IQR 122–365 cells/μL). No significant differences were found in gender, educational attainment or being non-UK born between HIV-positive and -negative participants, but PLWH were more often ethnically white (72% *vs.* 60%, respectively; $P = 0.001$). HIV-negative participants had a lower median age than those recruited from the HIV ambulatory care clinic (43 *vs.* 50 years, respectively; $P = 0.05$) and were more likely to be heterosexual (71% *vs.* 32%, respectively; $P < 0.01$).

The HIV-positive and -negative groups had similar reported prevalences of a range of physical comorbidities (asthma, COPD, diabetes, heart disease and stroke). However, symptoms of depression were more common in the HIV-positive group: 39 (20%) of the HIV-positive group and 13 (14%) of the HIV-negative group had PHQ-9 scores of ≥ 10, indicating moderate/severe depression ($P = 0.64$).

Smoking and recreational drug use

Sixty (30%) HIV-positive and 31 (33%) HIV-negative participants were current smokers; 54 (28%) and 22 (25%), respectively, were ex-smokers (Table 3). In smokers, PLWH reported more intensive smoking than HIV-negative individuals, with

Table 2 Comparison of comorbidities and their management between HIV-positive and HIV-negative participants

	HIV-positive (n = 197)	HIV-negative (n = 93)	P-value
Diagnosis of comorbid conditions (self-report), ever [n (%)]			
Asthma	36 (18)	21 (23)	0.34□
COPD/emphysema	9 (5)	1 (1)	0.18□
Cancer (any)	9 (5)	3 (3)	0.76□
Heart disease	11 (6)	4 (4)	0.78□
Stroke	3 (1)	1 (1)	1□
Diabetes	10 (5)	6 (7)	0.59□
Currently receiving treatment for depression [n (%)]	40 (20)	8 (9)	0.02*
Patient Health Questionnaire – 9 score [median (IQR)]	4 (1–8)	2 (0–6)	0.002#
Patient Health Questionnaire – 9 score ≥ 10 [n (%)]	39 (20)	13 (14)	0.254□
Self-reported history of immunization against [n (%)]			
Influenza in past 12 months	138 (70)	27 (30)	< 0.01□
Streptococcus pneumonia (ever)	50 (26)	6 (7)	< 0.01□
Use of inhaled medications (any) [n (%)]	24 (12)	15 (17)	0.30*
Undertakes physical activity at least once per week [n (%)]	115 (59)	63 (71)	0.05*
History of acute respiratory illness in past year [n (%)]			
Sinusitis	23 (12)	7 (8)	0.41□
Bronchitis	6 (3)	3 (3)	1.0□
Chest infection	39 (20)	11 (12)	0.132□
Cold or flu serious enough to miss work or stop normal activities	54 (28)	19 (21)	0.307□
Pneumonia	3 (1.5)	0	0.55□
Any acute respiratory illness	93 (47)	30 (32)	0.02*

COPD, chronic obstructive pulmonary disease; IQR, interquartile range.
□Fisher's exact test.
*χ^2 test.
#Mann–Whitney U-test.

Table 3 Smoking and recreational drug use

	HIV-positive (n = 197)	HIV-negative (n = 93)	P-value
Smoking [n (%)]			
Current smoker	60 (30)	31 (33)	0.81□
Ex-smoker	54 (27)	22 (24)	
Never smoker	80 (41)	37 (40)	
Not stated	3 (1.5)	3 (3)	
Cigarettes smoked per day (current smokers only) [median (IQR)]	15 (8–20)	10 (5–13)	< 0.01#
Most cigarettes smoked per day in the past [median (IQR)]	20 (15–30)	12.5 (7.5–20)	0.04#
Currently using electronic cigarettes [n (%)]	20 (18)	8 (16)	0.83*
History of recreational drugs use, ever [n (%)]			
Any	118 (61)	41 (48)	0.07□
Cannabis	98 (51)	35 (41)	0.15□
Cocaine (smoked)	23 (12)	3 (3)	0.03□
Cocaine (sniffed or rubbed in gums)	71 (37)	21 (25)	0.05□
Ecstasy/Gamma-hydroxybutyrate/ ketamine/ crystal meth	72 (36)	13 (14)	< 0.001□
Heroin (smoked)	13 (7)	1 (1)	0.07□
Heroin (injected)	7 (4)	1 (1)	0.44□
History of recreational drug use in last 3 months [n (%)]			
Any	58 (30)	18 (20.7)	0.15□
Cannabis	39 (20)	14 (16.1)	0.51□
Cocaine (smoked)	3 (1)	0	0.55□
Cocaine (sniffed or rubbed in gums)	13 (7)	8 (9)	0.47□
Ecstasy/Gamma-hydroxybutyrate/ ketamine/crystal meth	22 (11)	3 (3)	0.025□
Heroin (smoked)	3 (1)	0	0.55□
Heroin (injected)	0	0	–

IQR, interquartile range.
□Fisher's exact test.
#Mann–Whitney U-test.
*χ^2 test.

a median of 15 (IQR 8–20) *vs.* 10 (5–13) cigarettes per day for current smokers, respectively (P < 0.001).

Past recreational drug use was more often reported in those with HIV infection, with 60% indicating drug use ever compared with 48% of HIV-negative participants (P = 0.05). No significant differences were found in the proportion of participants indicating any recreational drug use in the past 3 months.

Spirometry, respiratory symptoms and health-related quality of life

Spirometry was within normal limits in most people: 18 (11%) HIV-positive and seven (9%) HIV-negative participants had evidence of airflow obstruction (FEV_1/FVC < 0.7) (P = 0.55).

The MRC dyspnoea and SGRQ scores suggested a higher prevalence of breathlessness and respiratory health status impairment in PLWH (Table 4). SGRQ scores were higher in the HIV-positive group for all domains, with median SGRQ Total scores of 12 in the PLWH and 6 in HIV-negative participants (P < 0.01). Breathlessness was more common in the HIV-positive group, with 47% having an MRC dyspnoea score ≥ 2 (on a scale of 1–5), suggesting at least moderate breathlessness, compared with 25% of the HIV-negative participants (P = 0.001); 13% of HIV-positive *vs.* 1% of HIV-negative participants had MRC dyspnoea scores of ≥ 3 (P = 0.001).

There was no significant difference in general health-related quality of life scores between the HIV-positive and HIV-negative groups, with median EQ5D (UK) index values of 0.88 and 0.85 (P = 0.06) and median Visual Analogue Scale scores of 78 and 72, respectively (P = 0.46).

Factors associated with respiratory health status impairment in univariable analyses

In addition to HIV status, we explored other possible contributors to impaired respiratory health in the whole

Table 4 Respiratory symptoms and health status

	HIV-positive ($n = 197$)	HIV-negative ($n = 93$)	P-value
FEV_1			
Mean (SD) (L)	3.49 (0.87)*	3.25 (0.76)*	0.56[A]
% predicted	93	91	
FVC			
Mean (SD) (L)	4.29 (1.05)*	3.95 (0.91)*	0.23[a]
% predicted	91	89	
$FEV_1/FVC < 0.7$ [n (%)]	18 (11)*	7 (9)*	0.50[□]
MRC dyspnoea scale [n (%)]			
1. Not troubled by breathlessness except on strenuous exercise	99 (53)	61 (75)	0.02[□]
2. Short of breath when hurrying or walking up a slight hill	62 (33)	1 (24)	
3. Walk slower than contemporaries on level ground or have to stop for breath when walking at your own pace	8 (4)	1 (1)	
4. Stop for breath after walking about 100 m or after a few minutes on level ground	13 (7)	1 (0)	
5. Too breathless to leave the house, or breathless when dressing/undressing	4 (2)	0 (0)	
St George's Respiratory Questionnaire [median (IQR)]			
Symptoms	25 (7–48)	18 (0–29)	< 0.01[#]
Activity	17 (6–36)	12 (0–19)	< 0.01[#]
Impacts	5 (0–15)	0 (0–6)	< 0.01[#]
Total	12 (6–25)	6 (2–14)	< 0.01[#]

IQR, interquartile range; SD, standard deviation.
*157 (80%) HIV-positive and 74 (80%) HIV-negative participants had acceptable spirometry results.
[A]t-test; comparison of FEV 1 % predicted.
[a]t-test; comparison of FVC % predicted.
[□]Fisher's exact test.
[#]Mann–Whitney U-test.

study sample (Table S1). Higher SGRQ scores were associated with impaired lung function, with a median total SGRQ score of 28.5 (IQR 7.2–41.9) in people with an $FEV_1 < 80\%$ predicted compared with 9.1 (IQR 4.4–17.4) in those with an FEV_1 in the normal range ($P < 0.01$). Symptoms of depression were associated with impaired self-reported respiratory health status: median SGRQ Total scores were 26.5 in the 52 participants with PHQ-9 scores of ≥ 10, and 7.7 in the 238 participants with PHQ-9 scores < 10.

Combining HIV-positive and HIV-negative groups, no significant associations were found between gender, ethnicity, smoking status or recreational drug use and impaired respiratory health status in univariable analyses. An association between body mass index (BMI) and SGRQ Total score was seen (with higher scores in those with BMI < 20 or > 25), which approached statistical significance ($P = 0.07$).

Associations between HIV-related parameters and respiratory health

In analyses restricted to HIV-positive participants, neither current nor nadir CD4 count was significantly associated with higher SGRQ Total score (although trends were seen for higher scores being related to lower current or nadir blood CD4 counts). No significant difference in median SGRQ Total score was identified in those with and without an HIV load < 40 copies/mL (Table 5). After adjustment for age in a log-scale linear regression model, there was a trend towards higher SGRQ Total scores in people with a longstanding HIV diagnosis. A significant association was present between higher SGRQ Total score and longer interval from HIV diagnosis to starting ART.

Multivariable analysis of factors associated with respiratory health status including all participants

To allow adjustment for potential confounding factors, we constructed multivariable (log scale) linear regression models including all participants, with log SGRQ as the dependent variable. In addition to those factors chosen *a priori* (smoking status, age and gender), PHQ-9 scores and BMI were also included as they reached statistical significance at the 5% level in univariable analysis.

After adjustment for these other factors, HIV infection remained independently associated with an increased SGRQ Total score, with a 54% higher SGRQ Total score compared with HIV-negative individuals [adjusted fold-change 1.54; 95% confidence interval (CI) 1.14–2.09; $P = 0.005$] (Table 6). Depression (PHQ-9 score ≥ 10) was also independently associated with a higher SGRQ Total score (adjusted fold-change 1.90; 95% CI 1.42–2.53; $P < 0.001$).

We found similar factors to be independently associated with an MRC dyspnoea score ≥ 2 in a multivariable logistic regression model (Table S2). Here, the adjusted odds ratio (aOR) for an MRC dyspnoea score of ≥ 2 was 2.84 (95% CI 1.35–6.00; $P = 0.006$) in HIV-positive compared with HIV-negative participants. Independent associations were found with female gender (aOR 4.69; 95% CI 1.85–11.45; $P = 0.001$) and depression (aOR 6.30; 95% CI 2.75–14.46; $P < 0.001$).

Comparing HIV-positive participants with an undetectable HIV viral load with HIV-negative participants

As other studies have suggested that untreated HIV infection is associated with chronic respiratory impairment [18, 19], a greater prevalence of respiratory symptoms

Table 5 Associations between HIV-related factors and St George's Respiratory Questionnaire (SGRQ) Total score

	HIV-positive participants [*n* (%)]	SGRQ Total [median (IQR)]	Unadjusted fold-change in SGRQ* (95% CI)	Age-adjusted fold-change in SGRQ (95% CI)*	*P*-value*
Current CD4 count (cells/μL)					
0–350	19 (11)	20 (6–35)	1.53 (0.88–2.18)	1.57 (0.94–2.62)	0.109
350–500	30 (18)	16 (9–34)	1.39 (0.91–2.54)	1.40 (0.90–2.19)	
≥ 500	119 (71)	11 (6–20)	Reference		
Viral load < 40 copies/mL at last clinic review					
No	19 (11)	15 (5–67)	1.31 (0.78–2.19)	1.52 (0.89–2.57)	0.12
Yes	149 (89)	13 (6–25)	Reference		
Nadir CD4 count (cells/μL)					
0–100	35 (21)	17 (7–43)	1.80 (0.98–3.32)	1.65 (0.88–1.74)	0.112
100–250	47 (28)	16 (8–33)	1.52 (0.85–2.73)	1.40 (0.76–2.55)	
250–500	67 (40)	12 (6–17)	1.04 (0.60–1.81)	1.0 (0.57–3.09)	
≥ 500	17 (10)	10.5 (4–14)	Reference		
Time since HIV diagnosis (years)					
> 20	47 (27)	16 (8–37)	1.72 (1.14–2.61)	1.56 (1.01–2.43)	0.067
10–20	62 (36)	11 (6–25)	1.24 (0.85–1.83)	1.19 (0.80–1.76)	
< 10	62 (36)	10 (4–20)	Reference		
Time between HIV diagnosis and ART (years)					
> 10	33 (20)	21 (11–48)	1.90 (1.23–2.91)	1.79 (1.17–2.75)	0.004
5–10	43 (25)	9 (5–26)	1.00 (0.68–1.45)	0.95 (0.64–1.39)	
< 5	93 (55)	12 (5–21)	Reference		
Duration of ART exposure					
> 10	68 (49)	17 (7–37)	1.73 (1.13–2.64)	1.58 (1.0–2.49)	0.148
5–10	26 (19)	13 (7–24)	1.54 (0.87–2.70)	1.41 (0.78–2.52)	
< 5	46 (33)	9 (4–21)	Reference		

ART, antiretroviral therapy; CI, confidence interval; IQR, interquartile range.
*Log-scale linear regression model.

Table 6 Associations with St George's Respiratory Questionnaire (SGRQ) Total score in multivariable log-scale linear regression analysis

	Adjusted fold-change in SGRQ* (95% CI)	*P*-value
HIV status		
HIV positive	1.58 (1.18–2.12)	0.002
HIV negative	Reference	
Age (per 1 year older)	1.01 (1.01–1.02)	0.335
Gender		
Female	1.37 (0.96–1.09)	0.083
Male	Reference	
Depression (PHQ-9)		
≥ 10	2.77 (1.98–3.88)	<0.001
< 10	Reference	
Body mass index (kg/m^2)		
< 20	1.18 (0.68–1.74)	0.681
≥ 20 < 25	Reference	
≥ 25 < 30	1.18 (0.85–1.37)	
≥ 30	1.23 (0.8–1.42)	
Smoking		
Current smoker	1.23 (0.89–1.24)	0.408
Ex-smoker	1.01 (0.72–2.57)	
Never smoker	Reference	

CI, confidence interval.
*Log-scale linear regression model.

within the PLWH population as a whole might result from increased symptoms only among HIV-positive individuals not yet taking ART, or on ART without virological suppression. We therefore undertook a subgroup analysis comparing HIV-negative participants with HIV-positive participants whose HIV viral load was measured as being undetectable (< 40 copies/mL) within 6 months of the study visit. Participants who declined consent to access clinical records were excluded from this analysis, leaving 157 HIV-positive participants with documented virological suppression, compared with the 93 HIV-negative participants. Those with virological suppression had similar demographic characteristics to the PLWH study population as a whole (Table S3) and had a median CD4 count of 684 (IQR 473–839) cells/μL. The differences in respiratory health scores between HIV-positive (HIV suppressed) and HIV-negative groups were similar to those present in the complete data set: median SGRQ Total scores were 12 (IQR 6–25) and 6 (IQR 2–14), respectively ($P < 0.001$); and seventy (47.3%) of the HIV-positive group had MRC dyspnoea scores of ≥ 2 compared with 20 (24.7%) of the HIV-negative group ($P = 0.001$). In a log-scale linear regression model (including the same predictive factors as the whole-group analysis), HIV status remained independently associated with a higher SGRQ Total score, with a similar effect size to that in the whole group (fold-change in SGRQ 1.53; 1.13–2.06; $P = 0.007$).

Sensitivity analysis

To further explore the possible effect of the difference in age distribution between the HIV-positive and HIV-negative groups, we undertook a sensitivity analysis where we examined only those aged ≤ 52 years (the 75th centile of the HIV-negative participants). Using this restricted analysis (including 127 HIV-positive and 69 HIV-negative participants), the age distributions were similar, with a median age of 42 years in both groups. The previously demonstrated differences remained, with median SGRQ Total scores of 11.2 (IQR 6–20) in the PLWH and 6.2 (IQR 3–15) in the HIV-negative group ($P = 0.01$). MRC dyspnoea scores were also higher, with 55 (46%) of the HIV-positive and 16 (27%) of the HIV-negative participants having a MRC dyspnoea score ≥ 2 ($P = 0.01$).

Discussion

We compared HIV-positive individuals to an HIV-negative group with similar exposures to risk factors such as tobacco smoking. Our results suggest that HIV infection remains associated with impaired respiratory health despite virological suppression on ART. Although some differences were present in the age and ethnicity compositions of our two groups, these could not explain the differences in respiratory health status seen after adjustment in multivariable analyses.

Breathlessness was common in HIV-positive participants, with 47% of PLWH reporting this to be present and of at least moderate severity, compared with 25% of the HIV-negative participants. Using the SGRQ respiratory health questionnaire (which assesses not only respiratory symptoms but also the impacts on activity and quality of life), we found a 6-point difference in the median total score between HIV-positive and HIV-negative groups (a minimum clinically important difference in SGRQ being around 4 points [20]), suggesting that there is a meaningful impairment of the respiratory health of PLWH. This difference was present despite the prevalence of airflow obstruction in our HIV-positive subjects (11%) being lower than that reported in other HIV-positive populations (for instance 23% in Italy and 27% in the USA [8, 21]). Of note, there was also no difference in the prevalence of airflow obstruction in our study between the HIV-positive and HIV-negative groups.

To our knowledge, no other study has used patient-reported outcomes to compare respiratory health status in HIV-positive and HIV-negative populations. Two previous reports used the SGRQ to evaluate respiratory health in HIV-positive adults: Hirani *et al.* evaluated 98 consecutive HIV-positive individuals (84% male) attending HIV care services in Philadelphia, USA, and found a mean SGRQ Total score of 7 [22]; in contrast, Leung *et al.* reported a mean SGRQ Total score of 32 in 199 HIV-positive men attending care services in Vancouver, Canada [9]. Our data therefore provide the first estimate of the difference in respiratory health (as experienced by individuals) between HIV-positive adults with optimized access to ART and HIV-negative adults.

What might be contributing to these findings? Impairment of lung function not measured by spirometry may be present, which could result from a higher frequency of respiratory infection prior to effective ART and lead to long-term lung damage [23]: this could also result from the direct effect of HIV in the lung [24]. Other possibilities include cardiovascular disease or other non-respiratory comorbidities which can lead to respiratory symptoms. Depression was strongly associated with impaired respiratory health in our population and this may contribute to the burden of physical symptoms (although it should be noted that the difference in respiratory health status persisted after adjustment for depression in our population). Persistent HIV-associated immune dysregulation despite ART may also contribute – an effect documented in the development of atherosclerosis [25]. This hypothesis is supported by Attia *et al.*'s finding that levels of circulating CD14 are associated with radiographic emphysema in HIV-positive individuals [26]. Prospective studies are needed to determine the relative importance of these possible causes.

The strengths of our study include the presence of an HIV-negative control group with similar exposures to tobacco smoking and recreational drugs and the use of well-validated measures of respiratory health status. Our high response rate (72%) suggests that participants were representative of the wider clinic population, who in turn are similar to the UK HIV-infected population as a whole [27].

Limitations include recruitment at a single study site and the cross-sectional nature of the data collected, meaning that temporality cannot be established. As people were not randomly selected to participate, recruitment bias is possible. Spirometry was our only objective measure of lung function, whereas several studies have suggested that impairment of gas transfer is more common than airflow obstruction in PLWH [28, 29]. The differences in age and ethnicity between groups could have influenced our results; however, our findings persisted after adjustment in multivariable analyses; and our sensitivity analysis is reassuring in that the difference in age distribution between HIV-positive and HIV-negative participants appeared to have little impact on the results.

Finally, we relied on self-reported HIV status in the HIV-negative participants, so we cannot exclude the possibility that HIV-positive or undiagnosed individuals were included in this group. However, we believe that this is unlikely to be a major issue as the prevalence of undiagnosed HIV infection in our sexual health clinics is low (data not shown). If this did occur, it would have acted to weaken the association between HIV infection and respiratory health status impairment.

Interventions that can preserve the respiratory health of people with chronic HIV infection are needed. The earlier use of ART may reduce non-AIDS-related comorbidities, including respiratory illness – although, notwithstanding a median duration of follow-up of only 2.8 years, this was not associated with differences in lung function decline in the recent Strategic Timing of Antiretroviral Treatment (START) trial [30]. However, the impaired respiratory health of PLWH despite effective virological suppression found in our study suggests that more than ART alone is required to maintain the health of this population. Reducing the effect of known risk factors such as tobacco is important, as many HIV-positive populations have high rates of smoking [31, 32]. Thus, the provision of appropriate smoking cessation services should be a priority. However, why HIV infection is associated with apparent worse respiratory health even in never-smokers remains uncertain and requires further investigation.

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Author contributions

J.B., S.C. and M.L. planned the study and obtained permissions; J.B., J.McG., H.C., D.L., M.J., L.S. and R.S. recruited participants for the study; J.B., J.McG., C.L., F.L., A.R. and M.L. analysed the data; J.B. created the first draft of the manuscript; all authors reviewed and approved the final manuscript.

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Supporting Information

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

Table S1. Univariable associations between participant characteristics and respiratory health status impairment.
Table S2. Multivariable logistic regression of associations between MRC score ≥2 and participant characteristics.
Table S3. Comparison of HIV positive participants with an undetectable HIV viral load and HIV negative participants.

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LETTER TO THE EDITOR

WILEY

Access to influenza immunisation services by HIV-positive patients in the UK

Influenza is an important cause of morbidity in HIV-positive adults, who may be more susceptible and more likely to develop severe disease.[1,2] Annual influenza immunisation is recommended for all HIV-positive adults in the UK, supported by British HIV Association (BHIVA) guidelines,[1] with evidence for reasonably good uptake.[3] HIV services do not receive specific funding to provide immunisation; and the National Flu Immunisation Programme offers this instead via primary care and pharmacies.[4] Whether this meets the needs of people living with HIV has not been evaluated.

To inform the design of services for influenza immunisation we undertook a survey of adults attending a metropolitan HIV service during autumn/winter 2015-2016. Participants were asked about their behaviour regarding influenza immunisation and preferences for services used to obtain this (Appendix). We obtained written consent to access clinical records and contact participants later in the season to establish whether immunisation had been received. We also documented whether participants consented to share details of their HIV status with their GP (General Practitioner), as we hypothesised that this might affect their ability to access influenza immunisation.

A total of 253 individuals participated: their median age was 48 years (IQR 41-54); 80% were male; 62% were White, 22% were Black and 4% were of Asian ethnicity. The median blood CD4 count was 627 cells/μL (IQR 434-873), 96% were using antiretroviral therapy, and 76% had an undetectable HIV load.

TABLE 1 Relationship between participant characteristics and uptake of immunisation

	Was participant immunised in 2015-2016 season?			
	Yes (n = 176)	No (n = 53)	Unknown (n = 24)	*P* value
Gender[a]				
Female n (row %)	37 (68)	12 (23)	5 (9)	.92
Male n (row %)	136 (71)	38 (20)	18 (9)	
Race/ethnic origin				
White n (row %)	109 (70)	34 (22)	13 (8)	.884
Black n (row %)	35 (64)	12 (22)	8 (14.5)	
Other n (row %)	16 (80)	3 (15)	1 (5)	
Mixed n (row %)	6 (86)	1 (14)	0	
Not stated n (row %)	10 (67)	3 (20)	2 (13)	
Age, years, median (IQR)	49 (43-55)	46 (39-50)	44 (39-51)	.005~
Blood CD4 count (cells/μL) median (IQR)[b]	625 (429-867)	627 (473-881)	636 (459-840)	.979~
Plasma HIV load <40 copies/mL[b] n (row %)				
Yes	143 (71.5)	40 (20)	17 (8)	.413#
No	25 (66)	9 (24)	4 (10)	
Consent to contact General practitioner (GP) n (row %)				
Yes	134 (74)	35 (20)	11 (6)	.05##
No	30 (61)	11 (22)	8 (16)	

#Chi-Squared test.
~Kruskal-Wallis test.
[a]3 participants did not state their gender.
[b]blood results available for 240 participants.
##calculation excludes 25 participants with no details regarding consent to GP contact.

Overall, 212 participants (84%) were aware of the recommendation for annual influenza immunisation. A total of 176 individuals (70%) were immunised during the 2015-2016 influenza season, 53 (21%) were not and in 24 (9%) we had no record of immunisation status as they did not respond to follow-up. Uptake did not differ significantly by gender, ethnicity, CD4 count or HIV load; those immunised were slightly older than those who were not (median 49 compared to 46 years, $P = .005$) (Table 1).

Of those immunised, 90 (51%) had this in their GP practice, 44 (25%) in an HIV service, 15 (9%) in a pharmacy and 20 (11%) elsewhere (7 individuals did not specify a location). Those immunised in their GP practice or pharmacy were older than those immunised in an HIV care service (mean age 50 and 49 years for those immunised in GP or pharmacies, respectively and 45 years for those immunised in HIV care services, $P = .004$). Consent to share information between HIV services and primary care appeared to influence uptake: overall, 180 participants (71%) consented to share details of their HIV status with their GP and 49 (19%) did not (for 24 participants this was not recorded). Uptake of influenza immunisation was higher in those consenting to data sharing (74% vs 61%, $P = .05$); and individuals immunised in primary care were more likely to consent to this than those immunised by HIV services (83% vs 67%, $P = .013$).

Our data suggest that HIV-positive adults have a reasonable uptake of influenza immunisation and around half of these immunisations are provided in primary care. Uptake was higher amongst people who consented to share details of their HIV status with their GP, and 25% of immunisations were provided by the HIV service. This suggests that the current (reasonably good) uptake of influenza immunisation relies on significant provision by HIV care providers.

The 2015 BHIVA guidelines on the immunisation of HIV-positive adults suggest that HIV services should work in partnership with primary care to ensure that patients receive annual immunisation.[1] Exploring the reasons why individuals do not want to share information between HIV services and primary care, and creating mechanisms for sharing clinical data (particularly in a way that is accessible to service users) could enable better targeting of immunisations for those who do not receive these in primary care.

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Presented conference abstracts:

British Thoracic Society conference 2015:

P226 **IMPAIRED RESPIRATORY HEALTH STATUS IN THE UK HIV INFECTED POPULATION DESPITE THE USE OF ANTIRETROVIRAL THERAPY**

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10.1136/thoraxjnl-2015-207770.362

Background The widespread use of antiretroviral therapy (ART) has led to a reduction in HIV related opportunistic infections. An increase in chronic non-HIV related co-morbidities has been observed in stable HIV positive individuals receiving ART. The extent to which HIV infection remains an independent risk factor for respiratory disease despite the use of antiretroviral therapy is uncertain and few studies have systematically evaluated respiratory disease in HIV-infected populations with access to antiretroviral therapy.

Aims We sought to evaluate the frequency of (a) smoking and (b) respiratory symptoms and (c) spirometric impairment in the ambulatory UK adult HIV infected population, compared to HIV uninfected controls.

Methods HIV-positive participants were recruited from a large HIV care service, HIV uninfected participants were recruited from Sexual Health services (where recruitment was stratified by age to approximate that of the HIV positive subjects). Participants completed a questionnaire which included questions on smoking history and respiratory health status using the St George's Respiratory Questionnaire (SGRQ), and undertook spirometry without bronchodilation.

Results 249 participants were recruited between April and July 2015 (Table 1). 28% of HIV positive and 33% of HIV negative participants were current smokers ($p = 0.22$). 9% of HIV positive and 7% of HIV negative participants had an FEV_1/FVC of <0.7 ($p = 0.38$). 92% of HIV positive participants were using antiretroviral therapy, 86% had an undetectable plasma HIV viral load and mean CD4 count was 684 cells/μL.

Significantly higher SGRQ scores were observed in HIV positive participants than HIV-negative participants with a median total SGRQ score of 12 for those with HIV infection and 8 for the HIV negative participants ($p = 0.03$). In a linear regression (log scale) model, HIV infection was associated with a 62% increase (95% CI 1.19–2.21, $p < 0.01$) in SGRQ in unadjusted analysis and 48% increase (1.08–2.02, $p = 0.01$) in a multivariable analysis adjusting for age, gender and smoking status.

Conclusions Despite widespread use of ART, HIV infection is independently associated with impaired respiratory health status. This does not appear to result from current smoking or obstructive lung disease.

S81 FEASIBILITY AND UPTAKE OF ENHANCED SMOKING CESSATION SERVICES WITHIN AMBULATORY HIV CARE

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10.1136/thoraxjnl-2015-207770.87

Background HIV infected individuals are at increased risk of smoking-related illness and smoking rates amongst populations with HIV are often significantly higher than the general population. Interventions that reduce the prevalence of smoking in this population are urgently required.

Aims We sought to establish the impact of initiating regular smoking screening and advice by healthcare assistants (HCAs) or nurses as part of routine care appointments in a HIV ambulatory care service.

Methods Individuals attending for ambulatory HIV care appointments were asked brief screening questions regarding cigarette smoking by Healthcare Assistants (HCAs) or nurses. This was completed whilst clinical observations were performed, allowing this intervention to be delivered as part of routine care. Those who were current smokers were given Very Brief Advice (VBA) regarding smoking cessation and offered referral to smoking cessation services. The number of referrals to smoking cessation services was compared to the six months prior to the introduction of the enhanced service.

Results 1,031 individuals were screened between October 2014 and March 2015: 262 (25%) reported that they were current smokers. 248 (93%) of these smokers were provided with VBA and the opportunity of referral to smoking cessation services. Of these, 103 (38%) accepted referral compared to 6 referrals from the HIV outpatient service in the preceding 6 months.

Conclusions An intervention to ask service users about smoking and provide smoking cessation advice can be undertaken as part of routine care in an ambulatory HIV care service and is effective in identifying smokers and increasing referrals to smoking cessation services. Further work will evaluate the impact of this intervention in HIV positive subjects.

European Respiratory Society congress 2016:

Attitudes to smoking and quitting in UK HIV positive adults: Would more education help?

James Brown, Jennifer McGowan, Hende Choual, Santino Capocci, Colette Smith, Daniel Ivens, Fiona Lampe, Margaret Johnson, Alison Rodger, Marc Lipman
European Respiratory Journal 2016 48: PA4603; **DOI:** 10.1183/13993003.congress-2016.PA4603

Abstract

Background: HIV infection is associated with increased risk of smoking-related illness, yet people living with HIV (PLWH) are more likely to smoke than the general population in the UK.

Aims: To explore attitudes towards smoking and readiness to quit in adult PLWH compared to HIV negative individuals.

Methods: Cross-sectional observational study: PLWH were recruited from a metropolitan HIV care service; HIV negative participants were recruited from Sexual Health clinics. Participants completed a questionnaire including details of tobacco smoking, attitudes towards smoking cessation and beliefs regarding HIV-related respiratory illness.

Results: 402 individuals were invited, of whom 197 PLWH and 93 HIV negative participants of similar demographic and socioeconomic backgrounds agreed. 30% and 33% respectively were current smokers and 28% vs 25% ex-smokers (p=0.94). 65% of PLWH and 59% of HIV negative smokers either intended to, or wished to, stop smoking (p=0.56). When asked to rate the importance of stopping smoking on a 1-10 scale, there was no significant difference in mean scores between HIV positive and negative participants. Only 33% of PLWH agreed with the statement that there is an increased risk of developing lung cancer and 21% agreed that HIV is associated with the early onset of smoking-related lung disease.

Conclusions: Our data suggest that PLWH should be priority group for smoking cessation services and HIV status does not adversely affect desire to quit smoking. Most PLWH were unaware of an increased risk of non-communicable respiratory illness associated with HIV, so provision of information regarding this might help to reduce tobacco use in this population.

Provision of influenza immunisation for UK HIV positive adults

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European Respiratory Journal 2016 48: PA2606; **DOI:** 10.1183/13993003.congress-2016.PA2606

Abstract

Background: Annual influenza immunisation (FI) is recommended for people living with HIV (PLWH) in the UK but HIV services do not receive specific funding for this, whereas GP practices and pharmacies do. High uptake of FI requires services that are easy to access. We explored behaviour and attitudes towards the provision of FI amongst PLWH in the UK.

Methods: Cross-sectional survey of uptake of influenza immunisation, services used and patient attitudes towards FI in a metropolitan HIV care service.

Results: 209 individuals responded to the questionnaire of whom 171 (81%) were aware of the recommendation for annual influenza immunisation for PLWH. 136 (65%) had received FI; 23 (11%) planned to do so and 40 (24%) did not want to be immunised. Of those immunised, 71 (54%) received this in their GP practice, 31(23%) in their HIV care service, 10 (8%) at a pharmacy and 24 (18%) elsewhere. Of the 33 individuals who did not plan to have FI this year, 28 (85%) reported that this was because they did not want it, 3 (9%) indicated that they did not know where to get it and 2 (6%) reported allergy to the products used. When asked if they would be able to receive the FI at their GP practice, 109/153 (73%) of respondents reported that they would be prepared to do this. Of the 45 who reported that they could not receive FI at their GP practice, 36 (80%) reported that they would prefer it to be given in their HIV service.

Conclusion: We found high levels of awareness of the need for FI, and reasonable uptake. Most immunisations were provided by GP practices, although one-quarter prefer to be immunised in their HIV care service. Resources for influenza immunisation of HIV positive individuals should facilitate patient choice.

High frequency of unexplained breathlessness among UK HIV positive adults

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European Respiratory Journal 2017 50: PA2601; DOI: 10.1183/1393003.congress-2017.PA2601

Abstract

Background: Antiretroviral therapy (ART) has transformed the lives of people living with HIV (PLWH). However, non-communicable non-AIDS conditions (including cardiovascular and respiratory disease) appear increasingly common.

Aims: To evaluate: (1) the proportion of PLWH who report breathlessness; (2) whether this is explained by known cardiac or respiratory diagnoses, or measured airflow obstruction.

Methods: Cross-sectional study of PLWH attending a HIV care service and age and gender-matched HIV negative individuals. Breathlessness was assessed using the MRC Dyspnoea scale and health-related quality of life with the St George's Respiratory Questionnaire (SGRQ) and EuroQoL 5D. Details of relevant exposures and comorbidities were collected and spirometry performed.

Results: We recruited 318 HIV positive and 166 HIV negative adults (median age 49 years in both groups, 81% and 74% male, 28% vs 30% current smokers). 89% of PLWH had an undetectable HIV load. PLWH were more likely to report breathlessness: 41% (131/318) of HIV positive compared to 19% of HIV negative participants (32/166) had an MRC breathlessness score >1 ($p<0.001$). Of the 131 PLWH with an MRC score >1, 80 (61%) had no known cardiac or respiratory diagnosis or evidence of airflow obstruction. Therefore 25% (80/318) of PLWH had unexplained breathlessness compared to 18/166 (11%) controls ($p<0.001$). Those with an MRC dyspnoea score >1 also had significantly worse SGRQ and EQ5D scores than those with an MRC dyspnoea score of 1.

Conclusions: unexplained breathlessness appears to be twice as common in an ART-treated HIV population compared to controls. Management strategies are needed to address an unmet need that is present in around 1 in 4 patients.

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Is there a difference in the frequency or severity of acute respiratory illness between HIV-positive and -negative individuals?

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Background: A growing prevalence of chronic non-communicable respiratory illness is recognised among people living with HIV (PLWH). Mechanisms underlying this are unclear: one possible cause could be a greater incidence and/or severity of acute respiratory illness despite antiretroviral therapy. We therefore sought to evaluate whether the frequency or severity of acute respiratory illness differed by HIV status now that most PLWH are using antiretroviral therapy (ART).

Methods: In this prospective observational cohort study, PLWH and age, gender and tobacco smoking matched HIV negative participants were followed for 12 months with weekly documentation of any acute respiratory illness using standardised illness definitions. Severity of illness was assessed using a scale asking about 9 different symptoms and impacts, each scored out of 6 points, where a higher score corresponded to more severe symptoms.

Results: 136 HIV positive and 73 HIV negative participants were recruited and followed-up for 12 months; the median number of weeks for which data were reported by each participant was 44/52 weeks (85%). Participants had a mean (SD) age of 50 (11) and 52 (8) years respectively; all but one HIV positive participant was using ART and 87% had an HIV viral load <40 copies/ml, HIV positive participants had a median (IQR) CD4 count of 686 (458-848) cells/μl. The frequency of acute respiratory illness did not differ with HIV status (incidence rate ratio in PLWH 0.87, (95% CI 0.65-1.12, p=0.28). However, when Acute Respiratory Illnesses occurred, HIV positive participants reported more severe symptoms, with a median total symptom score at the time of reporting illness of 14 points (IQR 8-22.5) in HIV positive and 9 (4.25-14) in HIV negative participants (p<0.001).

Conclusions: In an HIV positive population using ART with good levels of virological suppression, the frequency of acute respiratory illness did not differ compared to HIV negative individuals, but PLWH reported more severe symptoms when these illnesses occurred. Further work should evaluate whether this reported difference in severity reflects underlying changes in immune response despite antiretroviral therapy.

BHIVA Research Award Winner 2015, James Brown

P191

No difference in the detection of pathogenic respiratory pathogens between HIV-positive individuals using ART and matched negative individuals

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Background: Severe bacterial respiratory infections remain a significant cause of morbidity and mortality among people living with HIV (PLWH) despite the provision of antiretroviral therapy. A possible explanation is a greater frequency of bacterial colonisation by bacterial respiratory pathogens among PLWH.

Methods: As part of a cohort study evaluating acute respiratory illness among PLWH and a matched HIV negative population, we measured the prevalence of three common respiratory pathogens (*Streptococcus pneumoniae*, *Haemophilus influenzae* and *Moraxella catarrhalis*) in sputum samples obtained at baseline (when participants had no symptoms of acute respiratory illness). Culture-independent multiplex quantitative PCR assays were used to determine the prevalence and bacterial loads of these pathogens.

Results: 65 sputum samples from PLWH and 36 from HIV negative individuals were tested. Mean (SD) age of participants was 52 (9.5) years; PLWH had a median (IQR) CD4 count of 700 (471–848) cells/μl.

Overall, one or more of the three respiratory pathogens were detected in 71 (69%) of samples. There were no significant differences in the prevalence of detectable bacterial pathogens between HIV and negative participants with *Streptococcus pneumoniae* detected in 55% of samples from PLWH and 44% from HIV negative participants (p=0.29), *Haemophilus influenzae* from 41 and 33% respectively (p=0.31) and *Moraxella catarrhalis* from 8 and 14% respectively (p=0.89). The only significant difference in the quantitative bacterial load in those with bacteria detected was that the concentration of *Haemophilus influenzae* was lower in samples from HIV positive participants (median (IQR) count 8×10^2 (2×10^2–3×10^3) vs. 1×10^4 (2×10^3–7×10^4, p=0.01).

Conclusions: There was no evidence of a difference by HIV status in frequency of airway colonisation with the three commonest bacterial respiratory pathogens. Other causes of the higher incidence of bacterial pneumonia should be sought.

BHIVA Research Award Winner 2015, James Brown

A6096 / 810 - The Effect of HIV Status on the Frequency of Acute Respiratory Illness: A Longitudinal Cohort Study

Participant

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Abstract

Background: Acute respiratory illness is an important cause of morbidity and mortality among people living with HIV (PLWH). The provision of antiretroviral therapy (ART) has improved the outcome of many HIV-related conditions, but whether the use of ART means that PLWH have the same frequency of acute respiratory illness as HIV negative individuals is not known. Understanding this would guide clinical management and might reveal mechanisms leading to the development of chronic lung disease in this population. **Methods:** In this prospective observational cohort study, PLWH and HIV-negative participants matched by age, gender and tobacco smoking were followed for 12 months with weekly documentation of acute respiratory illnesses. Baseline data collected included respiratory health status measured using the St George's Respiratory Questionnaire (SGRQ). An acute respiratory tract illness was defined as the new occurrence of any of the following symptoms: cough, sore throat, blocked or runny nose with or without a sensation of facial pain or pressure, breathlessness or pain on breathing. **Results:** One hundred and forty three HIV positive and 73 HIV negative participants were recruited. Seventy-nine per cent of HIV positive and 75% of HIV negative participants were male (p=0.33), the median age in both groups was 51 years. HIV positive participants had a median (IQR) blood CD_4 count of 686 (458-848) cells/μL; 92% of HIV positive participants were using ART and 87% had an HIV viral load < 40 copies/ml. HIV positive participants reported greater impairment in respiratory health status at baseline with median (IQR) SGRQ Total scores of 13 (5-28) vs 6 (2-9), p<0.001. 418 acute respiratory illnesses were reported during 12 months of follow-up (median of 2, range between 0 and 9 events per participant). Overall, there was no significant difference in the incidence of acute respiratory illness between HIV positive and negative participants (IRR (95%CI) 0.86 (0.70-1.06)), p=0.15. **Conclusions:** In a population with a high uptake of antiretroviral therapy and good levels of virological suppression, the frequency of acute respiratory illness did not differ between HIV positive and negative individuals. Differences in the severity or patterns of acute respiratory illness in HIV positive and negative individuals require further investigation.