Title: Airway bacteria and respiratory symptoms are common in ambulatory HIV-positive UK adults

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Take-home message:

Airway bacterial colonisation and respiratory symptoms are common in ambulatory HIV-positive UK adults

The widespread use of antiretroviral therapy (ART) and Pneumocystis pneumonia (PCP) prophylaxis has led to significant declines in opportunistic infection rates in people with chronic HIV infection. Despite this, pulmonary disease is more common in these patients than HIV-negative individuals.[1] Observational studies suggest that while bacterial pneumonia is reduced by ART, it still occurs relatively frequently even after CD4 T cell reconstitution.[2,3] Non-recurrent HIV-associated bacterial pneumonia is 10 times more likely than in uninfected individuals,[3,4] and is now the most common infection and the leading cause of death after sepsis.[5]

HIV-positive individuals are particularly prone to Invasive Pneumococcal Diseases (IPD), mainly pneumococcal pneumonia,[6] which accounts for 40% of all bacterial pneumonia where the aetiological agent is identified.[3] *Haemophilus influenzae* is the next most common cause.[3] Proposed explanations for the increased susceptibility to bacterial pneumonia include dysfunctional CD4 and CD8 T cell responses, as well as the prevalence of lifestyle factors such as smoking and injecting drug use in HIV-positive individuals.[2]

In addition to the infectious complications of HIV, rates of obstructive airways disease are also higher. Studies in the USA have found that 16-20% of HIV-positive individuals have asthma or COPD, and poorly controlled HIV accelerates lung function decline.[1] In HIV-negative patients with COPD, one report identified lower airway bacterial colonisation (LABC) in 44% of stable-state COPD patients, and found it to be associated with increasing airway inflammation, frequency of exacerbations and lung function decline.[7] However work from Malawi, as well as the UK, using traditional culture methods with protected specimen brushing and bronchoalveolar lavage respectively found no difference in the frequency of LABC in HIV-positive and negative healthy volunteers.[8,9]

To our knowledge the extent of LABC in HIV has not been examined using modern molecular techniques. In this study we evaluate respiratory symptoms and determine levels of common airway bacterial pathogens by quantitative PCR (qPCR) in a cohort of HIV-positive individuals.

A representative sample of patients receiving ambulatory HIV care at the Royal Free Hospital, London, UK was recruited by stratified selection into a tuberculosis screening study between July 2013 and June 2014. A self-completed questionnaire was used to obtain medical and residential histories, lifestyle information (smoking, alcohol, injecting drug use), and respiratory symptom scores. Respiratory symptom questions were: “have you had a cough in the last 4 weeks?”, “do you ever wheeze?”, “do you get breathless walking up one flight of stairs?”, “do you get a cough (productive of sputum/phlegm) most winters?”

Spirometry was performed in accordance with ERS guidelines and percentage of predicted FEV_1 calculated. Current and nadir CD4 count, viral load and medication history were obtained from the hospital’s electronic medical records and HIV database. Patients had frontal chest radiographs performed followed by sputum induction with 3.5% hypertonic saline.

DNA extraction and multiplex qPCR processing for airway bacteria (*Streptococcus pneumoniae*, *Haemophilus influenzae*, *Moraxella catarrhalis*, *Legionella pneumophila*, *Chlamydia pneumoniae* and *Mycoplasma pneumoniae*) were performed as previously described.[7] Data were analysed using SPSS v22 (IBM Inc.). Pearson’s and Spearman’s rank correlation, chi-square, Fisher’s exact test, Mann Witney U and t tests were used as appropriate. A p-value <0.05 was taken to infer statistical

significance. The study was approved by the National Research Ethics Services Committee London – City and East (study number 12/LO/1516). All patients provided informed, written consent.

Demographic characteristics for the 218 originally enrolled patients and a subgroup of 53 who produced sufficient sputum for further analysis were similar (table). The median blood CD4 count was 609 cells/µL and 84% had an HIV plasma load less than 40 copies/mL. The only statistically significant differences identified were that patients able to produce sputum were more likely to report a cough in the previous week (p=0.04) and a history of winter cough (p=0.01). At least one respiratory symptom was given by 122/214 (57%) of patients.

Airway bacteria were present in 23/53 (43%) of samples. *S. pneumoniae* was the most commonly isolated species, in 16/53 (30%), followed by *H. influenzae* in 10/53 (19%) and *M. catarrhalis* in 1/53 (2%). None of the other bacterial species were detected. In samples where bacteria were isolated, mean (±1 SEM) total bacterial load was $10^{5.8(\pm0.2)}$ cfu/mL. Where *S. pneumoniae* or *H. influenzae* were found, mean bacterial loads of these bacteria were $10^{6.4(\pm0.2)}$ cfu/mL and $10^{4.8(\pm0.2)}$ cfu/mL respectively.

Patients with bacteria in their sputum were more likely to be smokers (table). This was also the case for *S. pneumoniae* in current smokers (detected in 6/37 [16%] smokers vs 8/15 [53%] non-smokers, p=0.01) but not ever smokers (10/16 [63%] vs 14/36 [39%], p=0.14). Patients reporting winter cough had a higher total bacterial load (p=0.04), while those reporting breathlessness had a trend to higher total bacterial load (p=0.05). Similarly, in patients where *S. pneumoniae* was identified, those reporting breathlessness had a higher *S. pneumoniae* bacterial load (p=0.01). There was an inverse relationship between *S. pneumoniae* bacterial load and nadir CD4 count (p=0.02). There were no other statistically significant associations with other measures of HIV control, respiratory symptoms, lifestyle factors (alcohol or injecting drug use) or lung function.

Our study shows that respiratory symptoms are common and, using nucleic acid amplification techniques, bacteria are often detected in induced sputum samples in an ambulatory HIV-positive cohort with generally good immune function. This is in contrast to previous studies that identified few bacteria in HIV patients using traditional culture techniques,[8,9] and suggests that the enhanced sensitivity afforded by tests for genetic material is an important consideration for future work.

The frequency of bacterial isolation in patients with HIV (43%) is similar to that in HIV-negative patients with stable-state COPD (44%),[7] where LABC is associated with increased exacerbation frequency[10] and long-term lung function decline.[11] Mean bacterial load in samples where bacteria were isolated from HIV patients was lower than in the COPD cohort ($10^{5.8(\pm0.2)}$ vs $10^{7.3(\pm0.2)}$ cfu/mL, p<0.01). However, comparison is difficult as sputum from HIV patients would have been diluted by saline used for induction whilst COPD patients produced sputum spontaneously.

Published descriptions of bacterial colonisation in healthy individuals generally come from comparisons of bronchoalveolar lavage or epithelial brushing culture in COPD patients and a control group. The reported frequency of LABC in healthy individuals varies from 10 to 27% and in COPD patients from 43 to 83%.[12-14] We found that smokers were more likely to have LABC, which is consistent with previous findings from a small study that showed airways colonisation levels were lower in healthy non-smokers (13%) than healthy smokers (40%) and patients with COPD (62%).[14]

In keeping with high rates of pneumococcal disease in HIV-positive individuals, *S. pneumoniae* was the most common colonising species (compared to *H. influenzae* in COPD patients). There was also an inverse correlation between *S. pneumoniae* bacterial load and nadir CD4 count, consistent with previous findings of more invasive pneumococcal disease in those with a nadir CD4 count under 200 even after access to ART.[15]

We found bacterial load to be associated with a history of breathlessness and winter cough, but not other respiratory symptoms or spirometry. However a properly powered study is needed to confirm this.

In this study we investigated the six most likely bacterial species to cause disease as surrogates for bacterial infection. We did not have matched nasal swabs, so were unable to assess nasopharyngeal bacterial colonisation or presence of viruses. It is possible that induced sputum may have been contaminated by upper airway bacteria. A study in Malawi comparing *S. pneumoniae* airway colonisation in nasal and oral swabs found it to be present in 1/27 (4%) HIV positive and 5/22 (23%) HIV negative individuals sampled. It was also cultured from right main bronchus brushings in one HIV positive person, but no distal airway brushings.[8]

Patients who could provide sputum (n=53) were more likely to report a cough. This may represent a source of bias, though both the complete cohort (n=218) and this subgroup had similar demographics, smoking history and spirometry; and reported a high overall frequency of respiratory symptoms.

The development of novel techniques such as bacterial 16S pyrosequencing now means it is possible to identify rapidly a wide range of bacteria from body sites, including the lung.[16] The Lung HIV Microbiome Project is currently the largest study of this type and may further our understanding of the interaction between the host and microbiome in HIV-related respiratory health.

Our study demonstrates that pathogenic airway bacteria, in particular *S. pneumoniae,* are common in HIV-positive individuals with apparently good systemic immunity using antiretroviral therapy. Quantitation indicates that there were higher levels of bacteria in smokers. Longitudinal studies could determine if LABC in the context of HIV leads to lung function decline in a similar manner to that found with COPD. We hypothesise that LABC contributes to the development of HIV-associated lung disease and provides a reservoir from which IPD may arise. Hence, testing for, and targeting, persistent airway bacteria, plus aggressive smoking cessation programmes may be important strategies to combat long-term HIV-related morbidity.

Table

	Entire HIV cohort, n=218	Subgroup with sputum analysis, n=53	Bacteria positive, n=23	Bacteria negative, n=30	p-value for interaction (bacteria positive v negative)
Male sex	160/218 (73.4%)	41/53 (77.4%)	18/23 (78.3%)	23/30 (76.7%)	>0.4
Age [years]	46.7 (±9.8)	47.2 (±9.4)	48.1 (±8.5)	46.5 (±10.1)	>0.4
Ethnicity					
White British	87/218 (39.9%)	21/53 (39.6%)	11/23 (47.8%)	10/30 (33.3%)	>0.4
Black African	60/218 (27.5%)	13/53 (24.5%)	5/23 (21.7%)	8/30 (26.7%)	>0.4
UK born	87/218 (39.9%)	21/52 (40.4%)	9/23 (39.1%)	12/29 (41.4%)	>0.4
Lifestyle					
Ever smoker	101/214 (47.2%)	24/52 (46.2%)	15/23 (65.2%)	9/30 (30.0%)	**0.01**
Current smoker	49/214 (22.9%)	14/52 (26.9%)	11/23 (47.8%)	3/30 (10.0%)	**<0.01**
Mean pack years of ever smokers	16.8 (±14.5)	17.9 (±15.3)	19.1 (±16.0)	15.9 (±14.7)	>0.4
Injecting drug use	21/192 (10.9%)	3/51 (5.9%)	3/23 (13.0%)	0/30 (0.0%)	0.08
Respiratory health					
Cough in last week	50/214 (23.4%)	20/52 (38.5%)**	7/23 (30.4%)	13/30 (43.3%)	>0.4
History of					
any respiratory symptom	122/214 (57.0%)	38/52 (73.1%)**	19/23 (82.6%)	19/30 (63.3%)	0.21
wheeze	59/211 (28.0%)	21/52 (40.4%)	11/23 (47.8%)	10/30 (33.3%)	>0.4
breathlessness	67/211 (31.8%)	17/52 (32.7%)	9/23 (39.1%)	8/30 (26.7%)	>0.4
winter cough	85/212 (40.1%)	32/52 (61.5%)**	15/23 (65.2%)	17/30 (56.7%)	>0.4
Previous PCP	25/198 (12.6%)	6/50 (12.0%)	4/23 (17.4%)	2/27 (7.4%)	0.39
Previous bacterial pneumonia	25/198 (12.6%)	8/49 (16.3%)	3/23 (13.0%)	5/30 (16.7%)	>0.4
FEV_1 (% of predicted)	93.5 (±25.0)	92.1 (±18.5)	91.4 (±19.0)	92.6 (±18.5)	>0.4
$FEV_1/FVC < 0.7$	14/205 (6.8%)	6/53 (11.3%)	2/23 (8.7%)	4/30 (13.3%)	>0.4
HIV management					
Median nadir CD4 T cell count	238 (109-330)	221 (81-307)	207 (59-301)	232 (95-327)	>0.4
Median latest CD4 T cell count	609 (447-784)	707 (428-808)	707 (427-814)	679 (418-807)	>0.4
ART					
Currently using	184/218 (84.4%)	42/53 (79.2%)	17/23 (73.9%)	25/30 (83.3%)	>0.4

Previously used	19/218 (8.7%)	7/53 (13.2%)	4/23 (17.4%)	3/30 (10.0%)	
Naïve	15/218 (6.9%)	4/53 (7.5%)	2/23 (8.7%)	2/30 (6.7%)	
HIV load undetectable	182/217 (83.9%)	42/53 (75.5%)	18/23 (78.3%)	24/30 (80.0%)	>0.4
Using PCP prophylaxis	10/211 (4.7%)	2/52 (2.8%)	0/23 (0.0%)	2/30 (6.7%)	>0.4

Table. Baseline patient characteristics for entire cohort of 218 and subgroup of 53 patients who produced sufficient sputum for bacterial investigations (with significant differences indicated by **). The subgroup that produced sputum are split into those with detectable bacteria (bacteria positive) and those without (bacteria negative), with p-value for difference between these two groups. Numbers in brackets indicate percentage, ±1 standard deviation or interquartile range as appropriate. ART = antiretroviral therapy; FEV_1 = forced expiratory volume in 1 second; FVC = forced vital capacity; PCP = Pneumocystis pneumonia.

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