Bacterial Pneumonia among HIV-Infected Patients: Decreased Risk After Tobacco Smoking Cessation. ANRS CO3 Aquitaine Cohort, 2000–2007

Antoine Bénard1,2,3, Patrick Mercie1,2,3, Ahmadou Alioum1,2, Fabrice Bonnet1,2,3, Estibaliz Lazaro3, Michel Dupon3, Didier Neau1,2,3, François Dabis1,2,3, Geneviève Chêne1,2,3, the Groupe d’Épidémiologie Clinique du Sida en Aquitaine (GECSA)1

1 INSERM, U897, CIC-EC7, Bordeaux, France, 2 Université Victor Segalen Bordeaux 2, ISPED, Bordeaux, France, 3 CHU de Bordeaux, COREVIH Aquitaine, Bordeaux, France

Abstract

Background: Bacterial pneumonia is still a substantial cause of morbidity and mortality in HIV-infected patients in the era of combination Antiretroviral Therapy. The benefit of tobacco withdrawal on the risk of bacterial pneumonia has not been quantified in such populations, exposed to other important risk factors such as HIV-related immunodeficiency. Our objective was to estimate the effect of tobacco smoking withdrawal on the risk of bacterial pneumonia among HIV-infected individuals.

Methodology/Principal Findings: Patients of the ANRS CO3 Aquitaine Cohort with ≥ two visits during 2000–2007 and without bacterial pneumonia at the first visit were included. Former smokers were patients who stopped smoking since ≥ one year. We used Cox proportional hazards models adjusted on CD4+ lymphocytes (CD4), gender, age, HIV transmission category, antiretroviral therapy, cotrimoxazole prophylaxis, statin treatment, viral load and previous AIDS diagnosis. 135 cases of bacterial pneumonia were reported in 3336 patients, yielding an incidence of 12 % patient-years. The adjusted hazard of bacterial pneumonia was lower in former smokers (Hazard Ratio (HR): 0.48; P = 0.02) and never smokers (HR: 0.50; P = 0.01) compared to current smokers. It was higher in patients with <200 CD4 cells/µL and in those with 200 to 349 CD4 cells/µL (HR: 2.98 and 1.98, respectively; both P<0.01), but not in those with 350 to 499 CD4 cells/µL (HR: 0.93; P = 0.79), compared to those with ≥500 CD4 cells/µL. The interaction between CD4 cell count and tobacco smoking status was not statistically significant.

Conclusions/Significance: Smoking cessation dramatically reduces the risk of bacterial pneumonia, whatever the level of immunodeficiency. Smoking cessation interventions should become a key element of the clinical management of HIV-infected individuals.

Introduction

Bacterial pneumonia is still a substantial cause of morbidity and mortality in HIV-infected patients in the era of combination Antiretroviral Therapy (cART). Indeed, around 10% of the causes of severe morbidity and 5% of the causes of death are related to a bacterial pneumonia in industrialized countries [1,2]. In the general population, tobacco smoking is well-known as a major modifiable risk factor of respiratory tract infections [3]. Pulmonary infections in tobacco smokers are mediated by local inflammatory modifications and a significant depression in the percentage and absolute numbers of CD4+ and CD8+ lymphocytes [4]. Among HIV-infected patients, the prevalence of tobacco smoking is very high, generally reported around 50% in European HIV cohorts [5,6], compared to 27% in the general population of comparable age and gender [7]. Recent studies have confirmed the effect of tobacco smoking on respiratory tract infections in HIV-infected patients [8,9,10], and promoted tobacco smoking withdrawal as a priority. However, the benefit of tobacco withdrawal on the risk of bacterial pneumonia has not been quantified in such populations, exposed to other important risk factors such as HIV-related immunodeficiency [11]. In addition, respiratory tract immunological modifications due to HIV infection might interact with the effect of tobacco smoking [12,13,14].

This study aimed at estimating the effect of tobacco smoking withdrawal on the risk of bacterial pneumonia among HIV-infected individuals in the era of cART, and its variation according to the level of immunodeficiency.
Methods

Ethics Statement
The ANRS CO3 Aquitaine cohort was granted ethics permission from the institutional review board of the ANRS (Agence nationale de recherche sur le sida et les hépatites virales). All patients included in the ANRS CO3 Aquitaine Cohort have signed an informed consent.

Study Sample
All patients of the ANRS CO3 Aquitaine Cohort with at least two visits during the 2000–2007 period, free of bacterial pneumonia at first visit and with available data on tobacco consumption were included in the study. The ANRS CO3 Aquitaine Cohort enrolls all HIV-infected in- or outpatients of the participating clinic wards of the Bordeaux University Hospital and five other public hospitals in Aquitaine, southwestern France, since 1985 [15]. Tobacco smoking status is recorded at inclusion (history of tobacco smoking and current status) and at each visit (current status only) with clinical, biological and therapeutic data.

Outcome Measures
Specific radiological signs including alveoli consolidation limited or not to a segment or lobe or diffuse bilateral reticulonodular pattern were a pre-requisite to define cases of bacterial pneumonia. Cases of pneumonia excluded tuberculosis and other mycobacteria. Bacterial pneumonia considered for this analysis were classified into two categories: (1) “confirmed” (clinical and specific radiological signs together with a bacteriological confirmation) or (2) “probable” (clinical and specific radiological signs together with a successful antibacterial treatment). Smoking status was defined as current smokers, never smokers and former smokers (i.e.: patients who had stopped smoking since at least one year) at each follow-up visit. Four categories of immunological status were defined based on CD4 count: <200 cells/µL, 200 to 349 cells/µL, 350 to 499 cells/µL and ≥500 cells/µL.

Statistical Analysis
Time to a first episode of bacterial pneumonia was calculated as the difference between the date of the first visit in the 2000–2007 period and the date of diagnosis of bacterial pneumonia or the date of the last visit within the study. Tobacco smoking status was taken into account as a time-dependent variable as cessation attempts and relapses are frequent in HIV-infected populations (Encrenaz G et al. Curr HIV Res. 2009. In press). Explanatory variables included gender, age at first visit, HIV infection through intravenous drug use [IDU] and, as time-dependent variables, CD4 cell count, previous diagnosis of AIDS, plasma HIV RNA, cART (defined as a combination of at least three antiretrovirals), cotrimoxazole prophylaxis and statin treatment. Indeed, this latter variable has been reported as a protective factor in recent studies in patients with chronic conditions [16,17]. Latest values were considered for time-dependent variables.

We estimated the incidence rate of bacterial pneumonia per 1000 patient-years with its corresponding 95% confidence interval (CI), assuming that time-dependent variables were constant between each measurement. Cox proportional hazards models including a term of interaction between CD4 cell count and tobacco smoking status were used. Statistical analyses were performed using SAS (version 9.1; SAS Institute, Cary, NC).

Results
Out of 4365 patients enrolled in the ANRS CO3 Aquitaine Cohort seen at least once in 2000–2007, 3336 (76%) were included in this study. Eight hundred and fifty five (20%) patients had no data available on tobacco smoking status, 30 (1%) presented a bacterial pneumonia at the first visit and 144 (3%) had only one visit during the 2000–2007 period. The 1029 excluded patients did not differ significantly from the 3336 patients available for the analysis in terms of age (40 years old on average in both groups), HIV transmission categories (injecting drug users: 20% versus 21%; heterosexual: 36% versus 30%), proportion of cART-treated patients (64% versus 67%), plasma HIV RNA (54% versus 50% with ≥1000 copies/ml), mean CD4 count (449 versus 450 cells/µL) and proportion of patients with a previous AIDS diagnosis (21% in both groups). Excluded patients were less frequently male (68% as compared to 74% in the study sample, P<0.001). Baseline characteristics of the 3336 included patients are shown in table 1.

Among the 1779 (53%) current smokers at inclusion, 277 (16%) stopped smoking for at least 1 year during follow-up and were considered former smokers. Among the 411 (12%) former smokers at inclusion, 164 (40%) relapsed during follow-up. Among the 1146 (34%) never smokers at inclusion, 41 (4%) did start smoking during follow-up. During a median follow-up of 3.3 years (inter-quartile range [IQR]: 1.6–5.1) and a median number of 12 visits (IQR: 6–21), 135 first episodes of bacterial pneumonia were reported. Of these, 51 (38%) were confirmed and 84 (62%) were classified as probable. Of the 31 (23%) with documented bacteria (confirmed cases), Streptococcus pneumoniae (22 cases) was the most common. Other identified organisms were Pseudomonas (6 cases), Staphylococcus aureus (2 cases) and Haemophilus influenzae (1 case). Seventy eight (58%) pneumonia episodes were diagnosed between November and April, i.e influenza virus epidemics period.

Overall, 104 (77%) of the bacterial pneumonia events were classified as probable. Of the 31 (23%) with documented bacteria (confirmed cases), Streptococcus pneumoniae (22 cases) was the most common. Other identified organisms were Pseudomonas (6 cases), Staphylococcus aureus (2 cases) and Haemophilus influenzae (1 case). Seventy eight (58%) pneumonia episodes were diagnosed between November and April, i.e influenza virus epidemics period.

The overall incidence of bacterial pneumonia was 12.0 per 1000 patient-years (CI: 9.9–14.0). It was 15.9 per 1000 patient-years (CI: 13.1–19.3) when patients were smoking, 7.9 per 1000 patient-years (CI: 4.2–14.9) in former smokers and 5.9 per 1000 patient-years (CI: 3.5–10.0) in never smokers. The incidence of pneumonia was significantly lower in former smokers compared to current smokers, respectively. The adjusted hazard of bacterial pneumonia was 0.48 (CI: 0.26–0.86; P=0.02) for former smokers and 0.50 (CI: 0.29–0.86; P=0.01) for never smokers.

In the multivariate analysis, the interaction between CD4 count and tobacco smoking status was not significant (P=0.44 and 0.34 for the relation between current smokers and former and never smokers, respectively). The adjusted hazard of bacterial pneumonia was significantly lower in former smokers compared to current smokers (Hazard Ratio [HR]: 0.48; CI: 0.26–0.90; P=0.02). It was also significantly lower in never smokers compared to current smokers (HR: 0.50; CI: 0.29–0.86; P=0.01). The hazard of bacterial pneumonia was higher when CD4 count was <200 cells/µL (HR: 2.98; CI: 1.80–4.94; P<0.001) or between 200 and 349/µm² (HR: 1.98; CI: 1.25–3.15; P<0.004) as compared when CD4 count ≥500 cells/µL. The hazard of bacterial pneumonia did not differ between patients with CD4 count between 350 and 499 cells/µL and those with CD4 count ≥500 cells/µL (HR: 0.93; CI: 0.54–1.60; P=0.79). In this final model, the hazard of bacterial pneumonia was also higher among...
patients with plasma HIV RNA $\geq 1000$ copies/ml (versus $<1000$ copies/ml) (HR: 1.75; CI: 1.19–2.56; $P$ = 0.004), among IDUs versus others (HR: 1.87; CI: 1.27–2.73; $P$ = 0.001), among women versus men (HR: 1.56; CI: 1.07–2.27; $P$ = 0.02) and among patients aged between 50 and 60 years (HR: 3.53; CI: 1.13–10.98; $P$ = 0.03) compared to those aged $\geq 60$ years (HR: 8.30; CI: 2.37–29.04; $P$ < 0.001) compared to those aged $<30$ years (table 2).

### Discussion

Bacterial pneumonia is a major cause of morbidity in HIV-infected patients in the cART era. In our study, the incidence of bacterial pneumonia was 12 per 1000 patient-years, comparing with results reported in previous studies (8 to 20 per 1000 patient-years) [1,9,10].
We identified three independent categories of risk factors for bacterial pneumonia: non modifiable risks factors such as age, infection through IDU and gender; HIV infection; and tobacco smoking.

As previously reported, patients infected through IDU had a higher risk of bacterial pneumonia than other patients [10,18]. This might be explained by a poorer antiretroviral therapy adherence in these patients [19]. Furthermore, daily cannabis consumption, a frequent practice in this category of patient [20,21], may contribute to this high rate of bacterial pneumonia [22].

HIV-infected women presented a higher risk of bacterial pneumonia than men. This result was observed after adjusting for the main risk factor for bacterial pneumonia. However, we did not adjust our analysis for precarious socio-economic conditions and delayed access to care which are more prevalent in women.

Table 2. Factors associated with the hazard of bacterial pneumonia among HIV-infected patients in the era of cART.

| Category | Sub-category | Univariate analysis (3336 patients/135 events) Incidence rates (% patients-years) | Multivariate analysis 3303 patients/135 events |
|----------|--------------|--------------------------------------------------------------------------------|---------------------------------------------|
|          |              | N events | Patients-years | Incidence | 95% CI | Hazard Ratio | 95% CI | P |
| Tobacco smoking status | Current smokers | 104 | 6546 | 15.9 | [13.1–19.3] | 1 |
|          | Former smokers | 12 | 1513 | 7.9 | [4.2–14.9] | 0.48 | [0.26–0.90] | 0.02 |
|          | Never smokers | 19 | 3228 | 5.9 | [3.5–10.0] | 0.50 | [0.29–0.86] | 0.01 |
| CD4+ lymphocyte count (cells/μL) | ≥500 | 39 | 4941 | 7.9 | [5.8–10.8] | 1 |
|          | [350–499] | 20 | 2717 | 7.4 | [3.9–13.7] | 0.93 | [0.54–1.60] | 0.79 |
|          | 200–349 | 38 | 2309 | 16.5 | [9.5–28.4] | 1.98 | [1.25–3.15] | 0.004 |
|          | <200 | 38 | 1317 | 28.8 | [16.7–49.8] | 2.98 | [1.80–4.94] | <0.001 |
| Gender | Men | 92 | 8433 | 10.9 | [6.8–17.4] | 1 |
|          | Women | 43 | 2853 | 15.1 | [11.2–20.3] | 1.56 | [1.07–2.27] | 0.02 |
| Age (years) | <30 | 4 | 1084 | 3.7 | [1.4–9.8] | 1 |
|          | [30–40] | 64 | 4697 | 13.6 | [3.3–55.7] | 2.38 | [0.86–6.62] | 0.10 |
|          | [40–50] | 47 | 3747 | 12.5 | [3.0–51.6] | 2.70 | [0.96–7.61] | 0.06 |
|          | [50–60] | 13 | 1294 | 10.0 | [2.3–44.5] | 3.53 | [1.13–10.98] | 0.03 |
|          | ≥60 | 7 | 464 | 15.1 | [3.1–72.7] | 8.30 | [2.37–29.04] | <0.001 |
| Plasma HIV RNA (copies/ml) * | <1000 | 72 | 7557 | 9.5 | [7.1–11.1] | 1 |
|          | ≥1000 | 63 | 3729 | 16.9 | [11.2–25.4] | 1.75 | [1.19–2.56] | 0.004 |
| HIV transmission categories | Other | 78 | 8778 | 8.9 | [7.2–11.2] | 1 |
|          | Injecting drug users | 57 | 2508 | 22.7 | [15.1–34.1] | 1.87 | [1.27–2.73] | 0.001 |
| Previous diagnosis of AIDS * | No | 90 | 8751 | 10.3 | [8.4–12.6] | 1 |
|          | Yes | 45 | 2535 | 17.8 | [11.7–26.8] | 1.20 | [0.82–1.77] | 0.35 |
| cART * | No | 30 | 2553 | 11.8 | [8.2–16.8] | 1 |
|          | Yes | 105 | 8733 | 12.0 | [7.0–20.7] | 0.89 | [0.57–1.39] | 0.60 |
| Cotrimoxazole prophylaxis * | No | 133 | 11141 | 11.9 | [10.1–14.1] | 1 |
|          | Yes | 2 | 145 | 13.8 | [3.4–56.2] | 0.76 | [0.18–3.18] | 0.71 |
| Statin treatment * | No | 131 | 10768 | 12.2 | [10.3–14.4] | 1 |
|          | Yes | 4 | 518 | 7.7 | [2.8–21.2] | 0.56 | [0.20–1.54] | 0.26 |

* Time-updated variables.

CART: combination Antiretroviral Therapy.

CI: Confidence Interval.

ANRS CO3 Aquitaine Cohort, 2000–2007.

doi:10.1371/journal.pone.0008896.t002
than men and may increase the risk of bacterial pneumonia [29,31]. We cannot exclude a higher susceptibility to bacterial pneumonia in HIV-infected women but further research is needed to explore this hypothesis.

We showed that HIV infection increases the risk of bacterial pneumonia through the virus itself and through the related immunodeficiency. As recognized in earlier cohort studies [9,10], we observed that the risk of bacterial pneumonia was higher in patients with plasma HIV RNA above 1000 copies/mL. One hypothesis is that HIV infection is associated with defects in humoral immunity in the lung, leading to an increase susceptibility to infections [25]. Immunodeficiency is generally reported as the major risk factor of bacterial pneumonia in HIV-infected individuals [1,18,25,27]. In our study, the incidence of bacterial pneumonia dramatically increased in patients with a CD4 count <350 cells/μL compared to those with a CD4 count above this threshold. This emphasizes the need for early HIV diagnosis and initiation of antiretroviral therapy at high levels of CD4 count, in order to preserve cellular immunity [29,29].

But there are other ways of preventing bacterial pneumonia in HIV-infected patients. Recent studies have reported that pneumococcal vaccination was effective in HIV-infected patients [30,31]. In our study, the most commonly reported bacterium was Streptococcus pneumoniae. We did not have any data on the proportion of patients who received a pneumococcal vaccine but these results should emphasize its use in HIV-infected patients. We also reported that 59% of bacterial pneumonia episodes were diagnosed during the period of influenza virus epidemics which is a risk factor for respiratory tract infections [32]. The use of influenza vaccination would also be an important option especially in the context of pandemic situation although data are lacking on its clinical efficacy and safety in HIV-infected patients [33].

Finally, our paper adds original information for the prevention of bacterial pneumonia in HIV-infected patients. Firstly, for the first time in such populations, we showed that after at least one year of tobacco smoking abstinence, the risk of bacterial pneumonia is significantly reduced and compares to the risk observed in never smokers. Secondly, as noted by the absence of interaction between tobacco smoking status and HIV induced immunodeficiency, tobacco smoking cessation is effective in preventing bacterial pneumonia whatever the level of immunodeficiency. These are evidences for promoting tobacco smoking cessation in HIV infected patients. However, we showed that a high proportion of former smokers relapsed during follow-up, even after at least one year of abstinence. This reinforces the need for specific tobacco cessation interventions in such populations to lower the burden of bacterial pneumonia and, more generally, all tobacco-related morbidities.

**Acknowledgments**

The Groupe d’Epidemiologie Clinique du Sida en Aquitaine (GECAS) steering the ANRS CO3 Aquitaine Cohort is organized as follows: Scientific Committee: F. Dabis (Chair and Principal Investigator), M. Dupon, M. Longy-Bourrier, P. Morlat, J.L. Pellegrin and J.M. Ragnaud; Epidemiology and Methodology: M. Bruyand, G. Chêne, F. Dabis, S. Lawson-Ayayi and R. Thiébaut; Infectious Diseases and Internal Medicine: M. Bonarek, F. Bonnal, F. Bonnet, N. Bernard, O. Caubet, L. Caunégre, C. Cazanave, J. Ceccaldi, F.A. Dauchy, C. De La Taille, S. De Witte, M. Dupon, P. Duffau, H. Dutrone, S. Farbos, M.C. Germain, C. Greß, D. Lacoste, S. Lafaerie-Castet, P. Laste, D. Malvy, P. Mercié, P. Morlat, D. Neau, A. Ochoa, J.L. Pellegrin, J.M. Ragnaud, S. Tehangnonou and J.F. Viaillard; Immunology: P. Blanco, J.F. Moreau and I. Pellegrin; Virology: H. Fleury, M.E. Lafon and B. Masquelier; Pharmacology: D. Breilh; Pharmacovigilance: G. Miremont-Salamé; Data Collection: M.J. Blaizeau, M. Decoin, S. Delveaux, S. Gillet, C. Hannapier, O. Leleux and B. Uwamahia-Nziyumvira; Data Management: S. Geffard, G. Palmer and D. Touchard.

**Author Contributions**

Conceived and designed the experiments: AB PM FB GC. Performed the experiments: AB. Analyzed the data: AB AA. Contributed reagents/materials/analysis tools: AB PM AA FB EL MD DN FD GC. Wrote the paper: AB PM FB EL MD DN FD GC.

**References**

1. Bonnet F, Chere G, Thiebaut R, Dupon M, Lawson-Ayayi S, et al. (2007) Trends and determinants of severe morbidity in HIV-infected patients: the ANRS CO3 Aquitaine Cohort, 2000–2004. HIV Med 8: 547–554.
2. Lewden C, May T, Rosenthal E, Burty C, Bonnet F, et al. (2000) Changes in Causes of Death Among Adults Infected by HIV Between 2000 and 2005: The “Mortalite 2000 and 2005” Surveys [ANRS EN19 and Mortavic]. J Acquir Immune Defic Syndr 40: 590–598.
3. Arcavi L, Benowitz NL (2004) Cigarette smoking and infection. Arch Intern Med 164: 2306–2316.
4. Nucetti JP, Butler JC, Farley MM, Harrison LH, McGeer A, et al. (2000) Cigarette smoking and invasive pneumococcal disease. Active Bacterial Core Surveillance Team. N Engl J Med 342: 681–689.
5. Benard A, Tessier JF, Rambelsourcie J, Bonnet F, Fossoux H, et al. (2006) HIV infection and tobacco smoking behaviour: prospects for prevention? ANRS CO3 Aquitaine Cohort, 2002. Int J Tuberc Lung Dis 10: 378–383.
6. Fris-Moller N, Weber R, Reiss P, Thiebaut R, Kirk O, et al. (2003) Cardiovascular disease risk factors in HIV patients: association with antiretroviral therapy. Results from the DAD study. AIDS 17: 1179–1193.
7. World Health Organization, regional office for Europe (available at URL: http://data.euro.who.int/tobacco//TabId=93302). Accessed date: July 15th, 2009.
8. Crotters K, Griffith TA, McGinnis KA, Rodriguez-Barradas MC, Leaf DA, et al. (2005) The impact of cigarette smoking on mortality, quality of life, and comorbid illness among HIV-positive veterans. J Gen Intern Med 20: 1142–1145.
9. Gordlin FM, Rodeniger MP, Girard PM, Lundgren JD, Miro JM, et al. (2000) Pneumonia in HIV-infected persons: increased risk with cigarette smoking and treatment interruption. Am J Respir Crit Care Med 167: 630–636.
10. Le Moing V, Rabaah C, Journet V, Duval X, Cuzin L, et al. (2006) Incidence and risk factors of bacterial pneumonia requiring hospitalization in HIV-infected patients started on a protease inhibitor-containing regimen. HIV Med 7: 261–267.
11. Bruyand M, Thiebaut R, Lawson-Ayayi S, Joly P, Sasco AJ, et al. (2009) Role of uncontrolled HIV RNA level and immunodeficiency in the occurrence of
21. Duval X, Baron G, Garelik D, Villes V, Dupre T, et al. (2008) Living with HIV, antiretroviral treatment experience and tobacco smoking: results from a multisite cross-sectional study. Antivir Ther 13: 389–397.
22. Tetrault JM, Crothers K, Moore BA, Mehra R, Concato J, et al. (2007) Effects of marijuana smoking on pulmonary function and respiratory complications: a systematic review. Arch Intern Med 167: 221–228.
23. Dray-Spira R, Lert F (2007) Living and working with HIV in France in 2003: results from the ANRS-EN12-VESPA Study. AIDS 21 Suppl 1: S29–36.
24. Joy R, Drayts EF, Branson EK, Lima VD, Rustad CA, et al. (2006) Impact of neighborhood-level socioeconomic status on HIV disease progression in a universal health care setting. J Acquir Immune Defic Syndr 47: 500–505.
25. Twigg HL 3rd, Spain BA, Soliman DM, Bowen LK, Heidler KM, et al. (1996) Impaired IgG production in the lungs of HIV-infected individuals. Cell Immunol 170: 127–133.
26. Burns DN, Hillman D, Neaton JD, Sherrer R, Mitchell T, et al. (1996) Cigarette smoking, bacterial pneumonia, and other clinical outcomes in HIV-1 infection. Terry Beirn Community Programs for Clinical Research on AIDS, J Acquir Immune Defic Syndr Hum Retrovirol 13: 374–383.
27. Sullivan JH, Moore RD, Keruly JC, Chaisson RE (2000) Effect of antiretroviral therapy on the incidence of bacterial pneumonia in patients with advanced HIV infection. Am J Respir Crit Care Med 162: 64–67.