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EFFECT OF SMOKING AND CESSATION IN HIV-INFECTED PEOPLE

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EFFECT OF SMOKING AND CESSATION IN HIV-INFECTED PEOPLE

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ABSTRACT

Cigarette smoking is prevalent in HIV-infected people, resulting in higher mortality rate and more premature heart and lung diseases in the highly active anti-retroviral therapy era. Smoking is a modifiable risk factor for these adverse outcomes and smoking cessation in HIV-positive smokers is feasible, although further efforts are needed to improve smoking cessation programs in HIV-positive persons.

In this thesis, I examined the role of smoking in mortality and morbidity in HIV-positive Ontarians, and piloted a smoking cessation program featuring a novel smoking cessation aid, varenicline, in HIV-infected smokers. In addition, I explored three different methods to resolve missing data, by excluding, grouping and multiply imputing missing data. I adopted three different study designs in my thesis studies: retrospective cohort, cross-sectional and open label study.

We found smoking prevalence in HIV-infected people was consistently higher than in the general population. Smoking was associated with a higher risk of death, of respiratory symptoms, hospitalization and chronic obstructive pulmonary disease, and with reduced lung function and less CD4-T-lymphocyte improvement over time. We found varenicline was as effective in HIV-positive smokers as in non-HIV smokers reported by previous studies.

ACKNOWLEDGEMENTS

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List of Abbreviations used in this Thesis

4W-CAR	Four-week continuous abstinence rate
7D-PP	Seven-day point prevalence of abstinence
%FEV$_1$	FEV$_1$ percent of age, gender, race and height predicted value
%FVC	FVC percent of age, gender, race and height predicted value
AE	Adverse event
AIDS	Acquired immunodeficiency syndrome
ALT	Alanine aminotransferase
ANOVA	Analysis of variance
ART	Antiretroviral therapy
AST	Aspartate aminotransferase
CAD	Coronary artery disease
CAR	Continuous abstinence rate
CE&B	Clinical Epidemiology & Biostatistics
CC	Complete case
CI	Confidence interval
CIHR	Canadian Institutes of Health Research
COPD	Chronic obstructive pulmonary disease
DAIDS	Division of AIDS
DAD study	Data Collection on Adverse Events of Anti-HIV Drugs study
ECG	Electrocardiogram
FEV$_1$	Forced expiratory volume in one second
FTND	Fagerström Test for Nicotine Dependence
FVC	Forced vital capacity
GOLD	Global initiative for chronic Obstructive Lung Disease
HAART	Highly active anti-retroviral therapy
HIV	Human immunodeficiency virus
HR	Hazard ratio
IDU	Intravenous drug use
IHD	Ischemic heart diseases
IR	Incidence rate
IU	International Units
LOCF	Last observation carried forward
log	Logarithm
MCAR	Missing completely at random
MNWS	Minnesota Nicotine Withdrawal Scale
MRC	Medical Research Council
OCS	Ontario HIV Treatment Network Cohort Study
OHTN	Ontario HIV Treatment Network
OR	Odds ratio
PI	Principal investigator
PJP	Pneumocystis jarovecii pneumonia

PVD	Peripheral vascular disease
RCT	Randomized controlled trial
REB	Research Ethics Board
RR	Relative risk
SD	Standard deviation
SE	Standard error
SIS	Special Immunology Services
STD	Sexually Transmitted Disease
TB	Tuberculosis
VACS	Veterans Aging Cohort Study
WBC	White blood cell

PREFACE

This Ph.D. thesis is a "sandwich thesis" consisting of three "core" individual chapters describing three separate clinical studies, together with introductory and concluding chapters. The three "core" chapters are written as manuscripts. I am first author on all submitted manuscripts, and played a lead or major supporting role in the study design, grant writing, data collection, data analysis, and manuscript writing for all three studies. Details for each study are outlined below. Publication or submission details are listed at the beginning of each chapter, where relevant.

Chapter 1 is introduction. In which I reviewed literature and proposed my thesis studies. It is entirely my own work.

Chapter 2 is a retrospective cohort study, with secondary data analysis. I examined smoking prevalence and the effect of smoking on mortality and morbidities in HIV-infected Ontarians in this study. I proposed the study, conducted data analysis and wrote manuscript. The Ontario HIV Treatment Network (OHTN) provided data. Dr. Lehana Thabane played a major role in helping me to make a detailed data analysis plan. My co-authors helped me to improve the design, analyses, and manuscript. I presented the preliminary results with two posters at annual research conference of Ontario HIV Treatment Network and annual Canadian Conference on HIV/AIDS Research, respectively. The manuscript was submitted and is currently undergoing peer-review for publication.

Chapter 3 is a cross-sectional study, in which I examined the impact of smoking in respiratory symptoms, lung function and respiratory diseases. I developed the study

from suggestions by my thesis supervisor, Dr. Marek Smieja, designed the questionnaire, carried out the medical chart review, executed statistical analysis and drafted manuscript. Dr. Andrew McIvor provided professional advice in interpreting data. Sue Carruthers consented subjects, performed the spirometry testing, and carried out the questionnaire survey. My co-authors helped me with study design, implementation and manuscript writing. I presented an oral and poster of the preliminary results at the annual research conference of the Ontario HIV Treatment Network. I also presented two posters at the Canadian Association for HIV Research (CAHR) annual conference. The manuscript was published in the journal "AIDS Research and Therapy", and is flagged as a highly accessed article soon after publication. To date, this paper has been cited in two publications.

Chapter 4 describes a multi-site open label study, using varenicline as a smoking cessation aid. As varenicline has not been tested in HIV-infected people prior to this study, we piloted 36 HIV-positive smokers to evaluate the effectiveness, safety and tolerability of this novel medication. As the principal investigator (PI), Dr. Marek Smieja conceived the study, and played a major role in grant application and research team building. Dr. Jeffrey Cohen was the co-PI of this smoking cessation study. I wrote the study protocol and applied for funding from Pfizer Canada. I designed or chose the questionnaires, enrolled subjects, and carried out the study at the Hamilton site. I performed all data cleaning, all statistical analyses, and drafted the manuscript. Dawn Elston helped with implementing the study. Linda Robinson and Nancy McFarland enrolled subjects at the Windsor site. Dr. Johannes Zeidler carried out serum cotinine test.

Chapter 5 is the conclusion of the thesis, in which I summarize the three core studies described in chapters 2 to 4, draw an overall picture of this thesis, discuss the findings of my thesis studies and conclude with a discussion of future studies. This chapter is entirely my own work.

Section 1:

Introduction

Chapter 1:

Background of the thesis studies and

methodological challenges

Structure of this thesis

This thesis consists of three sections and five chapters: an introduction, three "core" chapters describing three separate studies undertaken for this doctoral thesis, and a concluding chapter.

In this introductory chapter, I introduce the background of my thesis studies, research methodology challenges I encountered, and my solutions to these problems. I review smoking prevalence in human immunodeficiency virus (HIV)-infected subjects, the effect of smoking on health outcomes in HIV-infected subjects, and smoking cessation in HIV persons. I sketch a profile of HIV-positive people in Ontario, how smoking impacts their health, and how health providers can help with regard to smoking cessation.

I have divided my thesis studies into three "core" chapters, found in chapters two

to four, each written as a manuscript for publication. One was published in March 2010 [1], one was submitted to AIDS Patient Care and STDs (AIDS: Acquired immune deficiency syndrome; STD: Sexually Transmitted Diseases) in June 2011, and the third one will be submitted prior to thesis defense in September 2011.

In the concluding chapter, I summarize the findings of my thesis studies, discuss the role of smoking cessation in HIV care, methodological issues, and future studies.

Background of the thesis studies

HIV-infected population and smoking prevalence

According to the Public Health Agency of Canada, an estimated 65,000 (54,000 to 76,000) people were living with HIV in Canada at the end of 2008 [2], which is 0.2% of 33,316,000, the total Canadian population in 2008 [3]. While accounting for less than 39% of the Canadian general population [3], Ontario disproportionally accounted for 44% of HIV-positive population between 1985 and 2009 [2].

The HIV-positive Canadian population is dominated by men (82%). Whites account for 56% of cases, followed by Aboriginals (25%), Blacks (10%) and other ethnic groups. People aged 20-49 years account for 87% of the adult HIV-positive population. Eighteen percent of infections are due to intravenous drug use (IDU). In Ontario, there are slightly more male HIV-positive people compared to the national data (84% versus 82%, $p < 0.001$)[2].

Numerous studies have reported smoking prevalence of 50-70% or more in HIV-

infected subjects [4-12]. However, there are limited Canadian data, and little information on the change in smoking prevalence over time in this vulnerable population. I used the Ontario HIV Treatment Network (OHTN) cohort study (OCS) to provide an estimate of smoking in Ontario HIV-infected subjects between 1995 and 2007, as detailed in Chapter 2. However, as smoking information was collected inconsistently, particularly in the earlier years of the study, the data cut contained a large amount of missing data. I discuss the methodological challenges of dealing with missing data in this chapter.

How smoking impacts health in HIV-infected people

Mortality and life expectancy

With modern anti-retroviral therapies, there is a greatly increased chance of long term survival and a decreased chance of opportunistic infections. A recent study in British Columbia, Canada, estimated additional life expectancy of 23.6 years at the age of 20 years old for HIV-infected patients who initiated their antiretroviral therapy (ART) between 2002-2004 [13]. Today, prognosis is much better, with an estimated additional life expectancy of 49.4 years at the age of 20 years old in high-income countries for those who started on their first course of potent combination ART with at least three antiretrovirals between 2003-2005 [14]. With the decline in deaths from opportunistic infections, non-AIDS-related deaths are increasing in developed countries, including deaths from heart and lung diseases [15-18]. As a traditional risk factor for respiratory and cardiovascular diseases, smoking is associated with a 2-3 times higher risk of death in the

anti-retroviral therapy (ART) era [19,20]. A Canadian study showed that, in the ART era, smoking was associated with more deaths than in the pre-ART era [16]. However no Canadian study has examined the risk of death in HIV-positive smokers thoroughly.

Hospital admission

The incidence rate (IR) of hospitalization in HIV-positive patients is decreasing in the ART era as a result of the reduction in AIDS-related conditions. However, hospitalization rates are now fairly stable or modestly decreasing, with infection-related hospitalizations offset by increasing non-AIDS-related comorbidities [21,22]. A previous study found no association between smoking and hospitalization, with a non-significant odds ratio (OR) of 1.0-1.2 through each 3-year study period between 1994 and 2002 [21]. As there are very limited data in the literature on how smoking impacts hospitalization in HIV-positive patients, whether smokers are at a similar risk of being hospitalized as non-smokers is unclear. I examine the association of smoking with hospitalization amongst OCS participants in Chapter 2.

Respiratory diseases and lung function

Numerous studies have showed that HIV-positive smokers are more likely to have respiratory symptoms or illnesses, either compared to HIV-positive non-smokers [19,23-26], or compared to their HIV-negative counterparts [7,24,27,28]. However data in Canada are scarce. The incidence of respiratory diseases in HIV-positive Canadians, and how this is impacted by smoking, is unclear. Although some studies have examined the role of HIV infection in relation to lung function [29-32], few HIV studies focused on the association

between smoking and lung function. A previous HIV study showed increased pack-years of smoking was associated with reduced FEV_1/FVC ratio (FEV_1: Forced expiratory volume in one second; FVC: Forced vital capacity), an important measure of obstructive lung function[23]. However, no study has examined how pack-years of smoking affect FEV_1 percent predicted (%FEV_1), an important index to classify the phase of obstructive lung function or chronic obstructive pulmonary disease (COPD), in HIV-infected persons. In Chapter 3, I examine respiratory symptoms and lung function amongst 120 HIV-positive subjects attending scheduled clinic visits at McMaster University Medical Centre.

Cardiovascular diseases

With the use of ART, drug-induced metabolic complications, particularly cardiovascular events are increasingly of concern. Conflicting results are reported on the association between ART use and the incidence of cardiovascular diseases (CVD) [33-37]. In contrast, traditional risk factors of CVD consistently play an important role in the incidence of CVD in the ART era [38]. Specifically, smoking is undisputedly associated with a higher risk of myocardial infarction, ischemic heart diseases (IHD) and premature carotid lesions in HIV-positive people [5,33,36,39-42]. However, there are limited Canadian data in the literature. I examine the impact of smoking on cardiovascular events in the OCS in Chapter 2.

Immune function

CD4-T-lymphocyte recovery varies after initiation of HIV therapy. CD4 counts increase sharply within six months of ART initiation, followed by a slower increase for

the next 4-6 years, and a levelling-off afterwards [43-45]. However, no study has examined the impact of smoking on CD4 change. Conversely, if CD4 counts are adversely affected by continued smoking, smokers may need ART initiation sooner than non-smokers, but this has not been definitely studied. I examine the effects of smoking on CD4 improvement over time in OCS participants in Chapter 2, and the effect of smoking cessation and varenicline on CD4 counts in Chapter 4.

Smoking cessation in HIV-positive smokers

Given the disproportionately high smoking prevalence in the HIV-infected population, and the adverse impact of smoking on health consequences, smoking cessation programs in HIV-infected smokers are needed [46]. Quitting smoking can improve symptom burden and the quality of life in HIV-positive people [47]. Pharmacotherapy is an important medical aid to help with quitting smoking. Varenicline tartrate (Champix), a newly developed smoking cessation aid, has been shown to be effective and relatively safe in the general population [48-54], and was more effective than bupropion (Zyban), nicotine replacement therapy, or placebo[55]. However whether it works equally well, with similar safety, in HIV-positive smokers as in non-HIV smokers is unknown. I present data from my open-label study of varenicline for smoking cessation in HIV-positive subjects in Chapter 4.

Research methods used in thesis studies and methodological

challenges I encountered

In order to answer a series of questions with no or limited information in the literature, my co-authors and I designed three thesis studies as described in section 2. Chapter 2 is a retrospective cohort study, with secondary data analysis, in which I analyzed data from the OCS, examined the smoking prevalence over time and the effect of smoking in numerous health outcomes including mortality in HIV-infected Ontarians. Chapter 3 is a cross-sectional study, in which I examined the effect of smoking in lung function and lung diseases, particularly COPD, in HIV patients attending an Ontario HIV clinic. Chapter 4 is a multi-site open label study, in which I evaluated a promising smoking cessation aid, varenicline, in two Ontario HIV clinics. In each study, I identified methodological challenges and explored solutions.

Missing data and strategies to handle it

Missing data existed in all my three thesis studies. It appeared to be a serious problem in the OCS (Chapter 2). The OCS is an observational study prospectively collecting data from consenting HIV infected volunteers from HIV clinics across Ontario since 1994. Demographic, behavioural and medical information is collected by medical chart review and by in-person interview. Anonymous data cuts are available to the researchers upon request, after obtaining appropriate approval. By analyzing the OCS data, I expected to determine smoking prevalence by year, and how smoking impacted important health outcomes. Of 3,211 eligible HIV-infected adults who were included in

my analysis, 1,369 (43%) subjects had missing data including 1,330 (41%) subjects who had missing data on smoking. In the spirometry study (Chapter 3), I looked at the association between lung function, measured by spirometry, and self-reported smoking status and cumulative cigarette consumption. Data was missing on subjects who did not undergo post-salbutamol lung function test which was needed for the appropriate diagnosis of COPD. In the smoking cessation study (Chapter 4), I prospectively collected information on smoking, nicotine dependence and withdrawal, adverse events, lab test results and vital signs since the first dose of varenicline. Subjects had missing data during follow-up on several variables. In my thesis studies, all the missingness was item non-response, which means the subject had partial response on certain variables [56,57].

Generally speaking, there are three distinct methods to handle item non-response missing data. The most common and simple way is excluding them from the analysis, which is also the default set for many software programs, however this complete-case analysis method has two major drawbacks: 1) it reduces sample size, precision and power; 2) it biases results unless the data is missing completely at random (MCAR), which means the missing data is not associated with other known or unknown data, and that the missingness is simply an accident [56,58,59]. Moreover, even when the missing mechanism is MCAR, excluding missing data yields larger mean squared error of the estimate and hence is less efficient, compared to multiple imputation, despite similar negligible bias [60]. Another popular but inappropriate way for dealing with missing data is the missing-indicator method, consisting of creating an additional separate group for the missing data

in order to allow all of the subjects to be included in the analysis. Although there is no sample size reduction, the estimate would be biased even if the missingness mechanism is MCAR, because this missing group is indirectly related to the outcome, via the association between the known variable and outcome, and would distort the true association [56,61]. The third way to deal with missingness is imputation, using single or multiple imputation methods to replace missing data with one or more values [56,62]. In single imputation, one assumes the imputed value is the true value, and chooses one value to replace the missing data, either by series mean, median, last observation carried forward (LOCF), or expected value generated from linear regression or other methods. It is easy to conduct single imputation, however the major drawback with single imputation is that one would underestimate the variance, particularly by LOCF. In clinical studies researchers are usually interested in the change, while by using LOCF, the observations are assumed to not change over time, which is unrealistic and has a weak theoretical foundation [56,59]. In contrast to single imputation, multiple imputation means that missing data is replaced with a range of plausible values, instead of only one single value. By using multiple imputation, the mechanism of missingness is assumed to be MCAR, or missing at random (MAR), which means the missing data can be fully explained by observed measurements but has nothing to do with unobserved measurements. Unfortunately this assumption is not testable. However, when there are sufficient observed measurements, one can approximately ascertain if MAR is met and use multiple imputation technique to replace missing data [56-59].

One needs go through four steps to conduct a multiple imputation analysis [57,62,63]. The first step is to generate a probability model, using observed values to predict missing data. If observed information shows the effect modifier exists, the probability model needs to be generated by strata. Sufficient observed variables should be chosen to better predict the distribution of missing data, including: target variables, meaning variables which will be in the final analysis, such as outcome, predictors, confounders and effect modifiers; auxiliary variables, meaning variables which will not be in the final analysis but are highly related to target variables; and sample design variables, meaning variables used in the sample size calculation, such as the mean number of subjects per cluster. Sensitivity analysis can help to decide whether a variable is redundant, by comparing the results from models with and without the variable. However in practice one does not have to go through sensitivity analysis in order to identify redundant variables, because using redundant predictors will not cause bias, although it may reduce precision. On the contrary, if important predictors are omitted, the under-fitting may cause bias. By balancing the complex computation of sensitivity analysis and the influence of predictors on the results, the inclusive strategy as described above is practical[57,62]. At the second step, a certain number (m) of values are randomly chosen from a posterior distribution of the missing data, to replace the missing data. In this way, m imputed data sets are generated. The number of imputations, m, depends on the rate of missing data, and desired relative efficiency or tolerance for power falloff [57,64]. The third step is to analyze each imputed data set separately. If imputed data sets are generated by strata, one needs

to recombine each imputed data set before conducting the analysis. At the last step, final results are calculated by combining all the results obtained at the third step from m imputed data sets. The final estimate is the mean of m point estimates from m imputed data sets. The 95% confidence interval (CI) of the final estimate depends on within and between imputation variance, and m, rather than on total sample size. Compared to complete-case analysis, the missing-indicator method, or single imputation, multiple imputation is always the best choice because it yields robust results, gets less biased estimates and has more statistical efficiency.

Despite the advantages of multiple imputation and disadvantages of other methods, sometimes it can be difficult to choose multiple imputation over other options. For example, in the OCS (Chapter 2), one of the study objectives is to describe the smoking prevalence over time, but 41% of subjects never provided smoking information. In this circumstance, imputing for up to 13 correlated annual smoking status measures from 1995 to 2007 is methodologically challenging, because the imputed smoking status over time for a given subject may conflict with each other. For example, a subject is imputed as a former or current smoker in a certain year and is imputed as a non-smoker in several subsequent years within a same imputed dataset, despite defining all 13 annual smoking status as both predictors and dependants. Although we can correct the non-smoking as formerly smoking after the imputation process, the imputation itself is challenging. In this circumstance, I chose complete-case analysis to describe the smoking prevalence by year, by excluding those 41% subjects who never provided information on

smoking. I adopted a complete-case analysis in the spirometry study (Chapter 3) as well, because amongst 14 subjects with obstructive lung function by pre-salbutamol test, 9 (64%) did not undergo post-salbutamol lung function testing, which meant that if I chose multiple imputation to replace those missing post-salbutamol lung function, I would have used information obtained from 5 subjects to predict missing post-salbutamol lung function in 9 subjects. Due to very limit information, I simply used complete-case analysis to diagnose COPD and discuss the high probability of underdiagnosis of COPD.

I used different methods to handle missing data in my thesis studies, with awareness of the weaknesses and strengths for each method, as described earlier in this section, after balancing the ideal with feasibility. In OCS (Chapter 2), preliminary complete-case analysis showed that, amongst 1,881 subjects with smoking information, 1,677 (89%) did not change their smoking status (non-, former and current) during the study period, which means that LOCF would work for the majority of people with partial annual smoking data. Therefore, I imputed partially missing smoking data by carrying forward or backward known smoking data. The LOCF strategy was also adopted in the smoking cessation study (Chapter 4), in which subjects were intensively followed weekly during the first 12-week treatment period and then biweekly during the second 12-week non-treatment period. In this circumstance, LOCF is justified by the intensive follow up visits, despite the primary interest being change in the variables of interest. In OCS (Chapter 2), I replaced missing data on age and annual CD4 count by the series mean in each year, because the proportion of missingness was small, with 3 (0.1%) missing age

and 13 (0.4%) missing CD4 count, I concluded that the results would not be affected substantially. Therefore I chose single imputation over the more complex multiple imputation.

In the OCS, by using complete-case analysis I found subjects with missing smoking information differed from those with known smoking data in all aspects except race: they were older, male, and with a higher education level; were less likely to use intravenous drug, were diagnosed earlier, were more likely to have ART history before enrolment, had lower CD4 count, higher viral load and shorter duration of follow-up. No interaction between smoking and sex, age, HIV diagnosis era, baseline ART use or baseline CD4 count was found in the complete-case analysis versus death. I chose variables associated with smoking and with complete data, including sex, age, enrolment year, last follow up year, baseline and nadir CD4 count, IDU and ART use at baseline, together with the primary outcome, death, to predict the posterior distribution of missingness on baseline smoking status, race, education level, and baseline and peak viral load. I conducted multiple imputation without stratification. All the missing data were dependent. I chose 10 imputations in order to achieve relative efficiency greater than 0.95, given the fact that approximately 43% subjects had missing data [57]. In addition to reporting the results from my complete-case analysis, I also conducted missing-indicator and multiple imputation analysis in order to better understand to what extent missingness affects the results and contrast the results between methods.

Missing data is also a serious issue in other HIV cohort studies. In a large

combined cohort study involving 18,603 HIV-infected subjects from the USA (The HIV Insight database) and Netherlands (The Athena national cohort), 6,898 (37%) subjects had missing data in smoking, as well as other variables [41]. In the Data Collection on Adverse Events of Anti-HIV Drugs (DAD) study, an international collaboration involving 23,437 HIV-infected subjects from 21 countries in Europe, USA and Australia, 5,526 (24%) subjects had missing data on smoking, as well as other variables [36]. In both of these HIV cohort studies, missing-indicator methods were used to handle missing data, allowing all the subjects to be included in the analyses [36,41]. To the best of my knowledge, no HIV cohort study has used multiple imputation techniques to handle missing data, although the authors expressed clearly that the missingness needs to be addressed during ongoing follow-up [4]. In order to estimate how different methods dealing with missing data affect the results in my study, as well as to compare my results to the literature, I report results from complete-case analysis, missing-indicator method and multiple imputation respectively in the OCS.

Data quality control

In the OCS, the issue of data quality arose due to the large amount of missing smoking data. I was concerned that if missing smoking data reflects low quality of data collection in OCS, the results would inevitably be biased. In order to validate OCS smoking data, I reviewed 33 randomly selected medical charts at Special Immunology Services (SIS) clinic in Hamilton, accounting for 30% (33/104) of total subjects participating OCS at the Hamilton site. I calculated un-weighted and weighted kappa

with 95% CI, to estimate the strength of agreement between SIS and OCS [65] (Table 1-1). I treated non-, former, current smokers and unknown smoking status as ordinal categories and assumed the distance was evenly distributed through ordinal levels. Both linear and quadratic weights were used. The raw agreement was 85%, and unweighted kappa was 77% (95% CI: 59% to 96%). Quadratic weighted kappa was 86% (95% CI: 62% to 100%), and linear weighted kappa was 82% (95% CI: 66% to 98%). Thus, the strength of agreement was considered to be 'excellent' [65]. This small validation study showed that OCS data was quite reliable, despite a large amount of missing smoking data.

Correlated data

Correlated data existed in the OCS (Chapter 2) and in my smoking cessation study (Chapter 4) when a subject was followed up with repeated measurements. When a variable is measured repeatedly, the observations from a given subject are correlated rather than independent, and the joint distribution follows a marginal distribution rather than a normal distribution. Therefore the correlation amongst repeated measurements must be taken into account to increase efficiency [66-68]. I avoided using correlated data directly by converting the number of repeated annual CD4 counts into an annualized subject-specific change in CD4 counts, as a slope, obtained by fitting a linear regression line using calendar year as the independent variable. In the smoking cessation study (Chapter 4), I used general linear models for continuous variables and generalized estimating equations allowing for repeated measures [66-68].

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Summary

With the widespread use of ART in developed countries, HIV infection is becoming a manageable chronic condition, and HIV-infected people are living longer. Smoking may be a major contributor to long-term morbidity and mortality in the ART era. Smoking is associated with increased risks of death, heart and lung diseases, and reduced lung function in the ART era. However, information on smoking-related health consequences and utility of smoking cessation is limited, particularly in Canadian HIV-infected people. The effect of smoking on CD4 improvement and ART initiation remains unknown. Smoking is prevalent in the HIV-positive population, and smoking cessation is an urgent need in HIV care. As a proven effective pharmacotherapy for nicotine addiction in the general population, varenicline has not been tested in HIV-positive smokers.

In this chapter, I briefly reviewed the characteristics of the HIV-positive population in Canada, explained why smoking is a serious issue in this population, and what we can do to address it. I briefly summarized what we have learned from previous studies, identified topics with limited information and proposed a series of research questions. I discussed the key methodological challenges in HIV research cohort studies, focusing on missing data, data quality and correlated data, and discussed my solutions to each challenges. I also identified missing data as a methodological challenge in the HIV research literature.

In the remainder of this thesis, I present my thesis studies. I examined the effect of smoking on mortality, hospitalization, lung function, incidence of respiratory and

cardiovascular diseases and immune function change in HIV-infected subjects, by using the OCS data and conducting a cross-sectional study at a regional HIV clinic. I conducted a multi-site smoking cessation study in two Ontario HIV clinics. I addressed methodological challenges in each study. In the last chapter, I conclude my thesis work and suggest further areas for study.

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Tables

Table 1-1. Agreement on smoking in OCS and SIS clinic (n = 33)

OCS	SIS				Total
	Non-smoker	Former	Current	Unknown	
Non-smoker	9	0	0	0	9
Former	0	1	0	0	1
Current	0	0	14	3	17
Unknown	0	2	0	4	6
Total	9	3	14	7	33

For those 33 records I reviewed at SIS clinic, raw agreement was 85%. Unweighted kappa was 77% (95% CI: 59% to 96%). Quadratic weighted kappa was 86% (95% CI: 62% to 100%). Linear weighted kappa was 82% (95% CI: 66% to 98%). The strength of agreement was considered to be 'excellent' [65]. OCS = Ontario HIV Treatment Network cohort study. SIS = Special Immunology Services clinic in Hamilton.

Section 2:

Thesis studies

Chapter 2:

A retrospective cohort study of smoking prevalence

and the effect of smoking

In this chapter my co-authors and I describe the smoking prevalence over time in HIV-infected Ontarians participating in the OHTN Cohort Study (OCS), and examine the effect of smoking on mortality and morbidity. Our main finding was that smoking prevalence in HIV-positive people is very high, although decreasing gradually over time. Smokers were at higher risk of death, hospitalization and COPD. Smokers experienced less improvement in CD4-T-Lymphocyte count.

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I presented the preliminary results in part as a poster presentation at annual

research conference of Ontario HIV Treatment Network, Nov. 13-14, 2008, Toronto ON, and at the annual Canadian Conference on HIV/AIDS Research, May 13-16, 2010, Saskatoon SK.

Effect of smoking on mortality and morbidity

in the Ontario HIV Treatment Network Cohort Study, 1995-2007

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Abstract

Background

HIV-positive people are living longer with anti-retroviral therapy (ART) and smoking is becoming increasingly of concern. We conducted this study to better understand the impact of smoking on major health outcomes in the ART era.

Methods

We designed a retrospective cohort study using data from the Ontario HIV Treatment Network Cohort Study, from 1995 to 2007. We examined the hazard ratio (HR) by smoking for death, cardiac, and respiratory outcomes using Cox proportional hazards models. Annualized CD4 count changes were analyzed using multiple linear regression. Missing data was analyzed in three distinct methods: by excluding or grouping those with missing data, or by using multiple imputation.

Results

We included 3,211 subjects in the analysis. Mean baseline age was 38.1 years old, majority were male and white. Overall mortality rate was 26.6 deaths per 1,000 person-years. Missing smoking data affected 1,330 (41%) subjects. Baseline smoking prevalence was 60%, with an annual decrease of 0.6%. Compared to non-smokers, the hazard ratio (HR) for death in former smokers was 3.11 (95% confidence interval [CI]: 1.86 to 5.20, p

< 0.001), and 2.81 (95% CI: 1.60 to 4.93, p=0.001) for current smokers. Missing-indicator method showed subjects with missing smoking data had 2.1 times higher risk of death than former smokers and 2.3 for current smokers. The HRs for death were robust and more precise by using multiple imputation, compared to complete-case method. HR (95% CI) for hospital admission was 1.34 (1.13, 1.60) in current smokers (p = 0.001). Smokers had a 1.7-1.9 times higher risk of developing chronic obstructive pulmonary disease (COPD), and a non-significantly increased risk of lung cancer, pulmonary tuberculosis, PVD or stroke. Overall annualized CD4 count increased by 10 cells/µl/year, however smokers improved less by 8 cells/µl/year than non-smokers. Smoking had no impact on time to ART initiation.

Conclusions

Smoking prevalence in HIV-infected subjects was high. Missing smoking data affected the estimate of smoking on death. Smokers had approximately a 3-fold higher risk of death than non-smokers by complete-case, or multiple imputation method, with more precise results using multiple imputation. Smokers also had a higher risk of developing COPD, and of being hospitalized. Overall annualized CD4 counts increased less in smokers than in non-smokers. Smoking cessation is urgently needed in HIV-infected smokers.

Background

HIV infection has become a chronic manageable disease in the developed world since widespread access to anti-retroviral therapy (ART). The paradigm of mortality in HIV-infected population has shifted from opportunistic infectious diseases to non-AIDS deaths [1-3]. Chronic diseases are increasingly of concern for HIV infected subjects and their caregivers, particularly cardiovascular and respiratory diseases related to modifiable risk factors such as cigarette smoking [4-6]. As a traditional risk factor for respiratory and cardiovascular diseases in the general population, smoking is likely to have similar or greater deleterious effects in HIV-infected people. Smoking-related diseases are of increasing importance among HIV infected subjects and studies had found adverse effect of smoking on respiratory or cardiac outcomes [7-13]. However, there are no Canadian data on the effect of smoking on mortality, hospital admission, heart and lung diseases, and immune changes amongst HIV infected subjects in the ART era.

Missing data is common in HIV cohort studies [10,13,14]. Three different methods can be used to deal with missingness: complete-case analysis (excluding missing data), missing-indicator method (creating a separate group for missing data) and imputation (replacing the missing data with one value [single imputation], or with more than one values [multiple imputation]) [15]. Compared to other methods, multiple imputation is considered the best method of handling missing data and yields reliable and precise results [15-23]. In previous HIV cohort studies, missing-indicator methods were used to

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handle missing data [10,12]. To the best of our knowledge, multiple imputation techniques have not been used in any large published HIV cohort study. In our study, we examine the effect of these three methods of handling missing data (complete-case, missing-indicator, and multiple imputation) on the association between smoking and mortality and morbidity.

Methods

The OHTN Cohort Study (OCS)

The Ontario HIV Treatment Network (OHTN) Cohort Study (OCS) is an ongoing, community-governed, multi-site observational study collecting data from consenting HIV-infected subjects attending HIV clinics across Ontario. The OCS began recruitment in 1994, and has been funded by the Ontario Ministry of Health and Long-Term Care since 1998. Demographic, behaviour and medical information was collected by medical chart review.

This study was a secondary analysis of data from the OCS. The analysis was approved by the research ethics board at McMaster University / Hamilton Health Sciences, and by the OCS scientific steering committee. The data cut included ten study sites across Ontario, on HIV-infected subjects aged 18 or older after January 1st 1995 to December 31st 2007.

Measurements

Mortality and morbidity

Incidence rate (IR) was expressed as the number of events per 1,000 person-years with 95% confidence interval (CI). Target events which were documented before enrolment or within 6 months after enrolment were excluded from the calculated period at risk. Events missing the time of diagnosis were also excluded. Our primary outcome was all-cause mortality. We also determined all-cause hospitalization, time to initiation of antiretroviral therapy (ART), and smoking-associated respiratory and cardiovascular disease events. Respiratory diseases consisted of chronic obstructive pulmonary disease (COPD, including emphysema and chronic bronchitis), asthma, pulmonary tuberculosis (TB), lung cancer, bacterial pneumonia and *Pneumocystis jarovecii* pneumonia (PJP). Cardiovascular diseases consisted of myocardial infarction, ischemic heart disease (IHD, including myocardial infarction, angina pectoris, coronary heart disease, atherosclerotic heart disease and unspecified ischemic heart diseases), invasive coronary artery procedures (including coronary angioplasty or coronary artery bypass graft), peripheral vascular disease (PVD) and stroke. In addition, we examined clusters of diseases with presumed similar mechanisms (IHD or invasive procedures; and PVD or stroke), or with competing outcomes (lung cancer or death; IHD or invasive procedures or death; and stroke or death). All the classifications of disease and events were based on ICD-9 codes.

Annualized subject-specific changes in CD4 T-Lymphocyte count

For subjects who were followed up for more than one year and had at least two

recorded annual CD4 counts, we calculated annualized subject-specific changes in CD4 count, expressed as cells/μl/year, as the slope fitting a linear regression line using calendar year as the independent variable.

Statistical analysis

Continuous variables are reported as means (standard deviation [SD]). Categorical variables are reported as counts (percent). Baseline characteristics among different smoking groups were compared by analysis of variance (ANOVA) for continuous variables and by Chi-square test for categorical variables. The survivor function for death and incidence of other outcomes by smoking status was examined by the Kaplan-Meier method. Hazard ratios (HRs) for smoking and clinical events or death were assessed by Cox proportional hazards models, with adjustment for the following potential confounders: baseline age, gender, race, education level, intravenous drug use (IDU), year of HIV diagnosis, baseline ART use, baseline CD4 count and baseline viral load. A previously reported potential interaction between smoking and age was tested *a priori* [9]. In addition, we tested whether substitution of nadir CD4 counts and peak viral loads before enrolment, instead of study baseline values, would affect the results. HR with 95% CI and associated p-value were reported. Subjects were censored at the time of first development of target event or death, or at their last known visit. Multiple linear regression models were constructed to estimate the association between the annualized change in CD4 count (slope) and baseline smoking status, adjusting for the aforementioned potential confounders. The correlation coefficient β with 95% CI and

associated p-value were reported. R^2 was reported to estimate the goodness of fit of the model. Two-tailed significant level of 0.05 was adopted in all analyses. All p-values were reported to three digital places and those less than 0.001 were reported as $p < 0.001$. All analyses were performed using PASW 18 (Chicago, IL).

Additional details in the ways of handling missing data and conducting statistical analysis are described in Appendix 2-1 of this thesis.

Results

Demographic and baseline information

Of 3,237 study subjects, 26 were excluded: 6 had last follow-up before January 1st 1995 or were under 18 years old at initial visit, and 20 were followed for less than 6 months. The included 3,211 subjects entered the study between January 1995 and October 2006, and were censored between July 1995 and November 2007, with a total of 24,318 person-years of observation. Mean (SD) follow-up was 7.6 (3.8) years, and ranged from 0.5 to 12.8 years (Table 2-1). Study subjects were primarily male (87%) and White (81%), and 61% had a post-secondary education. Mean (SD) baseline age was 38.1 (9.1) years old; 74% were between 30-49 years old. There were 14% with a history of injection drug use (IDU). The majority of subjects (78%) were diagnosed as HIV-positive before 1996 in the pre-ART era, and half of the subjects had a history of ART use before or within 6 months of study enrolment. The mean (SD) baseline CD4 count was 311 (248) cells/µl, with 16% subjects having less than 50 cells/µl. Fewer than 2% subjects had

suppressed viral load at baseline, and the mean (SD) log viral load at baseline was 3.81 (1.00) if detectable.

Smoking prevalence at study baseline and changes over time

By excluding subjects with missing smoking information, at baseline 480 (26%) subjects were non-smokers, 277 (15%) were former smokers and 1,124 (60%) were current smokers. The smoking prevalence decreased gradually from 60% to 51% in the last year of follow up, for an annualized decrease in smoking of 0.6% (95% CI: -0.4% to -0.8%, $p < 0.001$, $R^2 = 0.836$) per year after enrolment (Appendix 2-2).

Baseline information by smoking status

There was no difference by smoking on duration of follow-up, year of HIV diagnosis, and mean baseline and peak viral load (Table 2-1). Former and current smokers were more likely than non-smokers to be male, White, and not on ART. The mean baseline age was similar in non- (38.5) and former (38.8) smokers but was younger in current smokers (37.0). Non- and former smokers had higher education than current smokers. Non-smokers had lower baseline and nadir CD4 count than current smokers, while former smokers had similar CD4 count as both non- and current smokers. The proportion of IDU increased in step-wise fashion from non-smokers to former and current smokers ($p < 0.001$).

Smoking information was completely missing for 1,330 (41%) subjects. They differed from those with known smoking data in all aspects except race (Table 2-1). In general, subjects with missing smoking data were older, male, and with a higher

education level; were less likely to use intravenous drug, were diagnosed earlier, were more likely to have started ART before study enrolment, had lower CD4 count, higher viral load and a shorter duration of follow-up.

Death

Six hundred and forty-six (20%) subjects died at a mean (SD) age of 44.5 (10.0) years after a mean (SD) follow-up of 4.6 (3.0) years. The mortality rate (95% CI) was 26.6 (24.6, 28.7) per 1,000 person-years during the over-all study (Table 2-2). The mortality rate was 15.3 in 1995, peaked in 1996 at 50.3, dropped to 31.3 in 1997, then decreased to 18.5 in 2007 (Figure 2-1). The mortality rate in non-smokers was consistently the lowest in each given year, whereas rates were similar for former and current smokers. Subjects with missing smoking information had the highest mortality rates before 2003, and had similar rates as non-smokers after 2004. Crude mortality rate significantly differed by baseline smoking status (p < 0.001, see Table 2-2), with the lowest rate of 5.3 (3.6, 7.9) in non-smokers, versus 19.2 (14.4, 25.4) in former and 16.5 (14.2, 19.2) in current smokers. Mortality rate was 57.5 (52.2, 63.4) per 1,000 person-years in subjects with missing smoking data and was 13.9 (12.3, 15.8) in subjects with known smoking data (p < 0.001).

Smoking was an independent predictor of death after controlling for potential confounders (Figure 2-2, Table 2-3 and Appendix 2-3). The HRs for former and current smokers were similar, regardless of whether missing data was excluded, grouped or imputed, or whether the association was unadjusted or fully-adjusted. After multiple

imputation and covariate-adjustment, compared with non-smokers, former smokers had an HR for death of 3.11 (95% CI: 1.86 to 5.20, p < 0.001), and current smokers had an HR of 2.81 (95% CI: 1.60 to 4.93, p = 0.001). No interaction was found between smoking and age. The HR (95% CI) was similar if we adjusted for nadir CD4 count and peak viral load before enrolment, instead of study baseline values (Table 2-3). Subjects with missing smoking data had an HR for death of 6.84 (95% CI: 4.49 to 10.43), which was 2.13 times higher than that in former smokers and 2.28 for current smokers (both p < 0.001).

In addition to smoking, older age (≥ 50 years old), White race, IDU, diagnosis before 1996, ART use before study enrolment and lower baseline CD4 counts were associated with a higher risk of mortality (Appendix 2-3).

Hospitalization

Of 2,853 (89%) study subjects with no history of hospital admission before or within 6 months of study enrolment, 1,223 (43%) were hospitalized during the follow-up period. The overall IR (95% CI) of hospitalization was 70.1 (66.3, 74.2) per 1,000 person-years between 1995 and 2007, and differed by smoking status (p = 0.001, see Table 2-2). Non-smokers had a lower IR of hospitalization than current smokers (52.0 versus 71.1, p < 0.001), and subjects with missing smoking data were more likely to be hospitalized compared to those with known data (84.4 versus 64.0, p < 0.001).

Estimates of HR for hospitalization for former and current smokers were similar. Crude and adjusted HRs were also similar, regardless of how the missing data was

handled (Table 2-4 and Appendix 2-4). After multiple imputation and multi-variable adjustment, HR (95% CI) for hospitalization was 1.21 (0.99, 1.49, p = 0.063) in former smokers and 1.34 (1.13, 1.60, p = 0.001) in current smokers. The missing-indicator method found no effect of missing smoking data on incidence of hospitalization, with similar estimates for former and current smokers.

Respiratory diseases

COPD was the most common smoking related respiratory disease measured, with an IR (95% CI) of 13.4 (12.0, 15.0) per 1,000 person-years, followed by PJP (13.1), bacterial pneumonia (3.4), asthma (3.4), pulmonary TB (1.0) and lung cancer (1.0) (Table 2-2). The crude IR differed by smoking status for COPD and for the composite outcome of lung cancer or death (both p < 0.001): IRs in non-smokers were the lowest, while IRs in former and current smokers were similar. On the other hand, compared to subjects with known smoking data, in subjects with missing smoking data the crude IR of PJP was higher (p < 0.001), while crude IRs were lower in those subjects for asthma (p = 0.006). In general, HR estimates for former and current smokers were very close for all the respiratory outcomes, and results were robust if we replaced baseline CD4 and viral load with nadir CD4 and peak viral load. HRs did not change appreciably after multiple imputation, except for rare events such as TB or lung cancer (Table 2-4 and Appendix 2-4). Generally the group with missing smoking data had similar estimates of HR as those in former and current smokers, except for the composite outcome of lung cancer or death. After multiple imputation and covariate adjustment, the incidence of COPD in former

smokers was 1.65 (95% CI: 1.05 to 2.60, p = 0.030) times higher than that in non-smokers and 1.86 (95% CI: 1.31 to 2.64, p = 0.001) times higher in current smokers than in non-smokers. The HR (95% CI) in ever smokers was elevated but not statistically significant for lung cancer at 3.76 (0.48, 29.68), and for TB at 2.13 (0.62, 7.34). Smoking was not conclusively associated with bacterial pneumonia, PJP, or asthma. Smoking was associated with the composite outcome of lung cancer or death, with an HR (95% CI) of 3.06 (1.83, 5.14) (p < 0.001) in former smokers and 2.78 (1.59, 4.86) (p = 0.001) in current smokers. No interaction between smoking and age was found.

Cardiovascular diseases

Myocardial infarction was the most common incident cardiac disease reported, with an IR (95% CI) of 1.7 (1.3, 2.4) per 1,000 person-years, followed by PVD 1.7 (1.2, 2.3), stroke 1.4 (1.0, 2.0) and invasive cardiac procedures 1.0 (0.7, 1.5). IR (95% CI) of IHD was 2.8 (2.2, 3.6) (Table 2-2). Crude IRs of cardiovascular diseases did not differ by smoking history, except for two composite outcomes in which death was considered as a competing outcome. Missingness of smoking data did not affect incidence of cardiovascular diseases either, except for analyses involving the two composite outcomes that included death. Overall, we found no association between smoking and incident cardiovascular disease, except for the association with the composite outcomes that included death as a competing outcome (Table 2-4 and Appendix 2-4): after multiple imputation and adjustment, both former and current smokers were at approximately 2.5 times higher risk of developing myocardial infarction, undergoing an invasive cardiac

procedure, or of dying, and missing-indicator method showed subjects with missing smoking data had an HR (95% CI) of 4.14 (2.98, 5.74), which was 2.1 times higher compared to former smokers and 1.9 for current smokers. Both former and current smokers were at 2.7-2.9 times higher risk for stroke or death, as compared to non-smokers after multiple imputation and adjustment, and subjects with missing smoking data had an HR (95% CI) of 5.75 (3.88, 8.51), which was 2.1 times higher compared to either former or current smokers. Crude and adjusted HR were similar for each cardiac outcome. Although not statistically significant, an HR of approximately 2 was observed for PVD or stroke, either as single or composite outcomes.

In addition, we found older age was associated with higher risk of cardiovascular diseases, except for PVD.

Immune outcomes

Annualized CD4 T-lymphocyte count change

Three thousand and twenty-eight (94%) subjects had at least two annual CD4 T-lymphocyte counts recorded. Overall, CD4 counts gradually increased during follow-up at a mean rate of 10.2 (95% CI: 8.1 to 12.3) cells/µl/year and at 13.8 (95% CI: 11.4 to 16.2) by excluding subjects with missing smoking data (Figure 2-3). The annual CD4 count increased faster in non-smokers than in former or current smokers (p < 0.001). Specifically, CD4 increased by 22.4 (95% CI: 18.6 to 26.2) cells/µl/year amongst non-smokers, compared with 12.3 (95% CI: 6.2 to 18.4) and 10.5 (95% CI: 7.2 to 13.8) in former and current smokers, respectively. Subjects with missing smoking information

had a smaller CD4 count improvement (4.6 versus 13.8, p < 0.001).

In uni-variable analysis, either former or current smokers had a significantly smaller annual CD4 change compared to non-smokers, by approximately -10 to -14 cells/µl/year depending on how we treated missing smoking data (Table 2-4 and Appendix 2-4). After multiple imputation and controlling for potential confounders, compared to non-smokers, the annualized increase in CD4 count was -9.7 (95% CI: -19.9 to 0.6) (p = 0.064) in former smokers, and -7.9 (95% CI: -14.1 to -1.8) in current smokers (p = 0.012). R^2 was 0.124 to 0.134 in 10 imputations respectively (0.146 and 0.138 by excluding and grouping missing data respectively). No interaction was found between smoking and age. The association was similar when we used nadir CD4 count and peak viral load instead. Missing smoking data affected the estimation: after multi-variable adjustment, subjects with missing smoking data had 13.3 cells/µl/year less improvement than non-smokers (p < 0.001), which was 7.2 less than former smokers (p = 0.041) and 7.6 less than current smokers (p = 0.001).

In addition, a slower adjusted increase in CD4 count was associated with higher baseline CD4 count, male gender IDU, lower education level, and earlier HIV diagnosis..

ART initiation

Of 1,580 (49%) subjects who had not initiated ART before or within 6 months of study enrolment, 1,324 (84%) subjects initiated ART during study follow-up. The overall IR (95% CI) of ART initiation was 307.9 (291.7, 324.9) per 1,000 person-years. Crude IR differed by smoking status (p = 0.036) (Table 2-2), with the lowest IR (95% CI) in non-

smokers at 255.1 (221.8, 293.3), intermediate in former smokers at 297.3 (251.5, 351.5), and highest in current smokers at 318.0 (292.4, 345.8). Missing smoking data did not affect crude IR.

All estimates of HR for time to ART initiation by smoking status ranged from 1.10-1.24, were very close regardless of whether the missing data was excluded, grouped or imputed, and regardless of covariable adjustment (Table 2-4 and Appendix 2-4). After multiple imputation and adjustment, smoking status was not associated with ART initiation, with non-significant point estimates of HR around 1 for both former and current smokers. No interaction with age was found. Estimates were almost identical if we used nadir CD4 and peak viral load instead of baseline values.

Discussion

Baseline smoking prevalence in HIV positive persons was 60% in our study, which is comparable to the literature [4]. Smoking prevalence in our study by calendar year varied from 54% to 60% between 1995 and 2007, with annualized decrease of 0.5% (95% CI: -0.7% to -0.4%, $p < 0.001$, $R^2 = 0.867$) per year. Available data in the province of Ontario general population shows smoking prevalence was from 16-23% between 1999 and 2007, with an annual decrease of 0.8% (95% CI: -0.4 to -1.2%) per year ($p = 0.002$, $R^2 = 0.766$) [24]. In this context, contemporary smoking prevalence of 54-60% in our study is consistently 2-3 times higher than in the general Ontario population, but with a similar annual decrease. In our study, the IR of death was 13.9 per 1,000 person-years in

all the subjects with known smoking information, and 5.3 in non-smokers, from which we can estimate an population attributable risk (PAR) percent of 62% ([13.9-5.3]/13.9). This suggests that, 62% of all deaths in this cohort occurred due to smoking, and might have been prevented if no one ever smoked [25]. The true PAR percent may be lower, if smoking is associated with other risky behaviours which are the true cause of death.

It is encouraging that fewer HIV-infected subjects smoke, although the lower prevalence reflects both successful cessation efforts, as well as lower survival amongst smokers. Regardless, the persisting very high smoking prevalence is likely to lead to appreciable future morbidity and mortality, and requires specially designed effective smoking cessation programs for this vulnerable population. Primary health care providers are in a unique position to reach smokers and initiate smoking cessation, and simply asking each patient at each clinical encounter, and offering cessation advice and medication, is known to improve quit rates [26]. In addition, motivating clinical staff and patients is a key to successful smoking cessation programs at HIV clinics [27].

Missing data was a serious issue in this study, as it is in many large HIV cohort studies. Smoking information was not available for 41% of our subjects. In addition, missing data also affected other variables, such as age, race, education level, CD4 count, viral load, ART use, death, hospitalization and all target diseases. Usually missing data is excluded from the analysis (complete-case analysis) because it is easier and is often the default set by software programmers. However, the major concerns for complete-case analysis are the reduced sample size and introduction of bias, unless the data is missing

completely at random [15-17]. The missing-indicator method is criticized for misuse because the additional missing group category is indirectly related to the outcome, via the association between the observed values and outcome, and therefore cannot provide true information on the relationship between exposure and outcome [15,19]. In our study, we handled missing data in three distinct ways to test the effect of missingness on our study results (Appendix 2-1). In addition to excluding or grouping missing data, we also applied multiple imputation technique to address this issue. Results from the missing-indicator method suggested the missing data significantly impacted the true association between smoking and death. Results showed the estimated risk of death was robust and had narrower 95% CI after multiple imputation, compared to the complete-case methods, suggesting that multiple imputation provides better estimates when there was a large number of missing data points for the predictor variable (Appendix 2-3).

Mortality in our study showed the same trends and comparable rates as those in the Southern Alberta Clinic programme between 1997 and 2003 [1]. Overall mortality rates in our study were also comparable to previous studies [10,28], but were lower than that in Veterans Aging Cohort 3 Site Study (VACS). In the latter study, HIV-positive subjects were more than ten years older than our study, which may explain the difference [4]. Nevertheless, we found smoking was an independent predictor of death, with an approximately 3-fold increased risk compared to non-smokers. This estimate is comparable to VACS and previous studies [4,28]. Some researchers speculated that the observed elevated risk of death in smokers in the ART era might be due to longer

survival and increasing smoking-related comorbidities [29].

The incidence rate of hospitalization in our study (Appendix 2-5) are consistent with previous reports [30,31]. We found a somewhat higher risk of hospitalization in current smokers (HR = 1.3) and former smokers (1.2), while in a previous study the risk was insignificant at 1.0-1.2 through each study period [31].

The incidence of respiratory diseases in our study was lower than in VACS, except for PJP [7], which was associated with younger age in our study. The difference in respiratory IRs between our study and previous work might be explained by younger age or by potentially missing data in our study. Subjects in our study were seven years younger than HIV-positive subjects in VACS, and the incidence of respiratory diseases increased with age, except with PJP and with asthma [7]. COPD underdiagnosis is common in the general population for various reasons [32,33], and was likely underdiagnosed in our study as well. Nevertheless, the HRs of smoking for respiratory diseases in our study are comparable to those reported in literature [7], with overlapping or wider 95% CI. We demonstrated a 1.7-1.9 times higher risk of COPD incidence in smokers. In addition, smokers had approximately 2-fold higher risk of TB and 4-fold higher risk of lung cancer than non-smokers, although these events were rare and the associations were not statistically significant. With the aging progress, we would expect higher IRs of respiratory diseases in our subjects, except for PJP. Moreover, those respiratory diseases are more likely to develop in former and current smokers.

A higher risk of myocardial infarction and IHD in former or current smokers has

been identified in previous HIV studies [8-13], however we could not verify this association in our study, presumably due to small number of events or to inaccuracy of coding. The overall IR of myocardial infarction in our study was comparable to some studies in which subjects' age are close to our subjects' [11,34], but was lower than that in the Data Collection on Adverse Events of Anti-HIV Drugs (DAD) study [12,13,35], which might be due to the younger mean age in our study. On the other hand, the IR of stroke in our study was comparable to that in DAD study [13,35], suggesting coding for cardiovascular diseases might be accurate but might not be for smoking. We found smokers had a non-significant 2-fold increased risk of PVD or stroke, consistent with previous studies, showing HIV-positive smokers had 2-fold higher risk of premature carotid lesions [36].

Overall the annualized CD4 count increased slightly by 10.2 cells/μl/year in our study, which is less than reported in the literature [37,38], and was comparable to that in HIV viral load suppressors four years after ART initiation. In our study, 1624 (51%) subjects were on ART already at study enrolment, and additionally 1324 (41%) subjects initiated ART after enrolment. By comparison, previous studies measured the change in CD4 counts beginning with ART initiation [37,38]. As CD4 recovery tends to level off 4-6 years after ART initiation [37], the different magnitude on annual CD4 change might also be due to longer follow up in our study: a mean 7.6 years in our study versus a median of less than 5 years in similar published studies [37,38]. Nevertheless, our study found that smokers had lower CD4 cell recovery by 8 cells/μl/year as compared with non-smokers, although we did not find any effect of smoking on time to ART initiation.

Our study has a number of limitations. Subjects may not have been representative of the contemporary HIV-positive population in Ontario, as study subjects were required to consent and do not represent the entire cohort of patients followed by participating clinics. Furthermore, 95% of the current studied cohort was enrolled between 1995 and 1998, and is not representative of the current clinic population which includes more women and immigrants from HIV endemic countries. For example, the study includes more subjects older than 40 years compared to all the tested HIV-positive Canadians between 1985 and 1998 (38% versus 27%, p < 0.001); and more men (87% versus 84%, p < 0.001), compared to all tested HIV-positive Ontarians between 1985 and 2007 [39]. As we had no HIV-negative control group, we are unable to assess the effect of HIV infection itself on target outcomes. Misclassification might have occurred in carrying known data forwards or backwards. We did not assess the effect of cigarette consumption in current study, and smoking intensity and duration were not available. Despite these limitations, our findings are consistent with previous HIV cohort studies, and provide important estimates of the effect of smoking on death, respiratory and cardiac disease. Unlike other cohort studies, we provide new insight into the effect of missing data on outcomes, by examining three different strategies for dealing with missing data. We recommend multiple imputation as the strategy of choice whenever the data are missing at random.

Conclusions

Smoking is very common in HIV-infected people, at 50-60%, with an approximately 3-fold higher prevalence than in non-HIV people. Our study found a 3-fold higher risk of death in smokers, regardless of the method used to handle missing data. Smokers also had higher risk of developing COPD and hospitalization, and slower gains in CD4 counts. Although smoking prevalence was steadily decreasing, given the continued high prevalence and high population attributable risk of death associated with smoking, smoking cessation in HIV-infected smokers is urgently needed and front-line health care providers can play an unique role in helping their patients quit.

Conflict of interests

No conflict of interest exists.

Acknowledgements

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Figures

Figure 2-1. Mortality rate by smoking over year (n = 3,211)
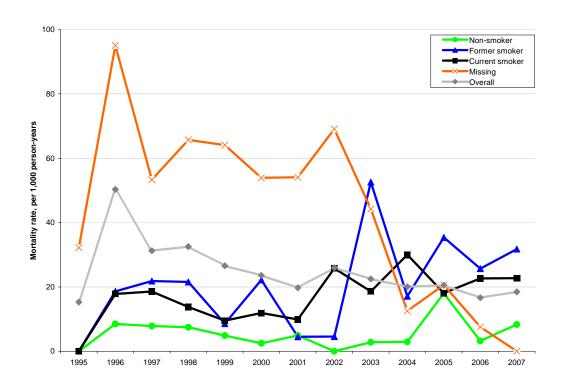
Figure 2-1 shows the mortality rate (per 1,000 person-years) by smoking status over time. The overall mortality rate (marked as diamond) was 26.6 during the overall study period: 15.3 deaths per 1,000 person-years in 1995, peaked in 1996 at 50.3, dropped to 31.3 in 1997, and then decreased to 18.5 in 2007. In general, the mortality rate in non-smokers (marked as circle) was consistently lower than in former (marked as triangle) and current (marked as square) smokers over year, while there was many crossovers between former and current smokers. Mortality rates in subjects with missing smoking information

(marked as cross) was the highest between 1995 and 2003, then decreased sharply to12.5

in 2004, and was close to non-smokers thereafter.
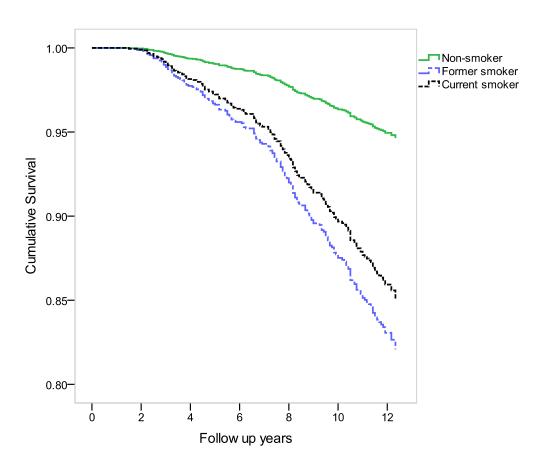
Figure 2-2. Adjusted survival by smoking status (n = 1,717)

Figure 2-2 shows cumulative adjusted survival by smoking status derived from Cox proportional hazard analysis. The model was adjusted for baseline age, gender, race, education level, intravenous drug use, year of HIV diagnosis, baseline antiretroviral therapy use, baseline CD4-T-lymphocyte count and baseline viral load. Subjects with missing data were excluded. Subjects were censored at the time of last known visit or death. Compared to non-smokers, HR (95%) of death was 3.58 (2.09, 6.13) for former smokers and 2.93 (1.83, 4.68) for current smokers (both p < 0.001).

Figure 2-3. Annual CD4 T-lymphocyte count by smoking status (n = 1,881)
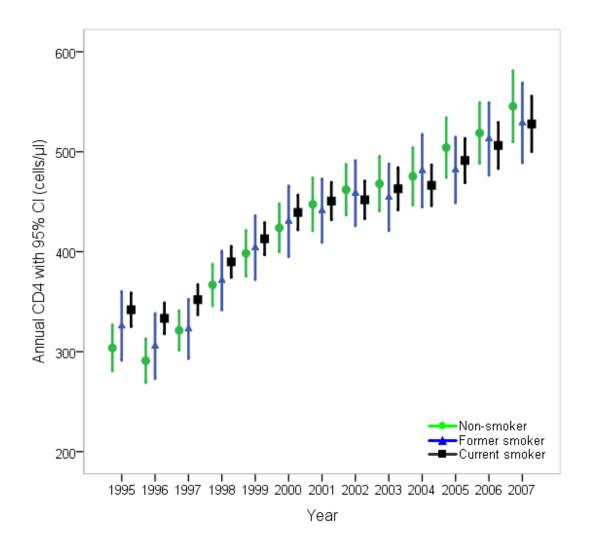
Figure 2-3 shows mean annual CD4-T-lymphocyte count with 95% confidence interval (CI) of mean, clustered by smoking status. Overall CD4 counts gradually increased during follow-up at a mean of 13.8 (95% CI: 11.4 to 16.2) cells/µl/year, excluding subjects with missing smoking data. The annual CD4 count increased faster in non-

smokers than in former or current smokers ($p < 0.001$). Specifically, CD4 increased by 22.4 (95% CI: 18.6 to 26.2) cells/μl/year amongst non-smokers, compared with 12.3 (95% CI: 6.2 to 18.4) and 10.5 (95% CI: 7.2 to 13.8) cells/μl/year in former and current smokers, respectively.

Tables

Table 2-1. Characteristics and baseline information by smoking

Parameters	Total, n = 3,211	Non-smoker, n = 480	Former, n = 277	Current, n = 1,124	p	Missing smoking, n = 1,330
Age, mean (SD) years	38.1 (9.1)	38.5 (10.0)	38.8 (9.7)	37.0 (8.2)	< 0.001	38.8 (9.1) ***
Male sex, n (%)	2791 (87)	381 (79)	244 (88)	970 (86)	< 0.001	1196 (90) ***
Race						
Non-White, n (%)	608 (19)	123 (26)	35 (13)	187 (17)	< 0.001	263 (20)
White, n (%)	2531 (79)	349 (73)	238 (86)	923 (82)		1021 (77)
Missing, n (%)	72 (2)	8 (2)	4 (1)	14 (1)		46 (3)
Highest education level						
Elementary, n (%)	144 (4)	9 (2)	14 (5)	75 (7)	< 0.001	46 (3) **
Secondary, n (%)	1069 (33)	129 (27)	80 (29)	455 (40)		405 (30)

65

Post-secondary, n (%)	842 (63)		585 (52)	179 (65)	338 (70)	1944 (61)
Missing, n (%)	37 (3)		9 (1)	4 (1)	4 (1)	54 (2)
Year of HIV diagnosis						
<1996, n (%)	1160 (87)***	0.202	798 (71)	207 (75)	343 (71)	2508 (78)
1996-1998, n (%)	163 (12)		238 (21)	51 (18)	87 (18)	539 (17)
> 1998, n (%)	7 (1)		88 (8)	19 (7)	50 (10)	164 (5)
IDU, n (%)	140 (11)***	< 0.001	258 (23)	27 (10)	11 (2)	436 (14)
Baseline ART use, n (%)	724 (55)***	0.034	522 (46)	124 (45)	254 (53)	1624 (51)
Baseline CD4, mean (SD) cells/µl	270 (249)***	0.015	351 (246)	333 (253)	313 (232)	311 (248)
Nadir CD4, mean (SD) cells/µl	253 (245)***	0.006	320 (242)	310 (249)	279 (223)	285 (243)
Baseline viral load, copies/ml						
≤ 50, n (%)	12 (1)**	0.219	23 (2)	4 (1)	16 (3)	55 (2)
51-9,999, n (%)	528 (40)		582 (52)	143 (52)	273 (57)	1526 (48)
10,000-99,999, n (%)	304 (23)		327 (29)	75 (27)	126 (26)	832 (26)
≥ 100,000, n (%)	152 (11)		115 (10)	36 (13)	45 (9)	348 (11)

Missing, n (%)	450 (14)	20 (4)	19 (7)	77 (7)		334 (25)
Log_{10} baseline viral load, mean (SD)	3.81 (1.00)	3.67 (1.03)	3.80 (1.02)	3.78 (0.99)	0.135	3.92 (0.98)[***]
Peak viral load, copies/ml						
≤50, n (%)	52 (2)	14 (3)	4 (1)	22 (2)	0.208	12 (1)
51-9,999, n (%)	1215 (38)	217 (45)	109 (39)	449 (40)		440 (33)
10,000-99,999, n (%)	918 (29)	153 (32)	84 (30)	360 (32)		321 (24)
≥100,000, n (%)	576 (18)	76 (16)	61 (22)	216 (19)		223 (17)
Missing, n (%)	450 (14)	20 (4)	19 (7)	77 (7)		334 (25)
Log_{10} peak viral load, mean (SD)	4.05 (1.07)	3.91 (1.10)	4.06 (1.08)	4.05 (1.07)	0.051	4.11 (1.04)[*]
Years of follow up, mean (SD)	7.6 (3.8)	9.4 (3.3)	9.0 (3.5)	9.1 (3.4)	0.189	5.3 (3.2)[***]

P-values for the overall comparison were made by one way ANOVA (analysis of variance) for continuous variables and by Chi-square tests for categorical variables, excluding missing data. Significance between missing and known smoking groups is marked next to the parameter value in the 'missing smoking' column. [*] $P < 0.05$, [**] $P < 0.01$, [***] $P < 0.001$. IDU = intravenous drug use. ART = anti-retroviral therapy.

Table 2-2. Incidence rate (IR) (95% CI) of target outcomes (per 1,000 person-years) by smoking status

Outcomes	Total	Non-smoker	Former	Current	p	Missing smoking
Death	26.6 (24.6, 28.7)	5.3 (3.6, 7.9)	19.2 (14.4, 25.4)	16.5 (14.2, 19.2)	< 0.001	57.5 (52.2, 63.4)[***]
Hospitalization	70.1 (66.3, 74.2)	52.0 (44.8, 60.3)	59.0 (48.9, 71.2)	71.1 (65.1, 77.7)	0.001	84.4 (76.8, 92.6)[***]
Respiratory diseases						
COPD	13.4 (12.0, 15.0)	7.6 (5.4, 10.7)	15.3 (11.0, 21.2)	16.9 (14.5, 19.8)	< 0.001	11.6 (9.3, 14.5)
Asthma	3.4 (2.7, 4.2)	2.5 (1.4, 4.5)	5.5 (3.2, 9.5)	4.4 (3.2, 5.9)	0.133	1.9 (1.1, 3.2)[**]
Lung cancer	1.0 (0.7, 1.5)	0.2 (0, 1.6)	0.8 (0.2, 3.2)	1.1 (0.6, 1.9)	0.248	1.4 (0.8, 2.6)
TB	1.0 (0.7, 1.5)	0.2 (0, 1.6)	0.4 (0.1, 2.9)	1.4 (0.8, 2.3)	0.070	1.3 (0.7, 2.5)
Bacterial pneumonia	3.4 (2.8, 4.3)	2.9 (1.7, 5.0)	1.2 (0.4, 3.7)	3.2 (2.3, 4.5)	0.243	4.9 (3.5, 6.9)
PJP	13.1 (11.6, 14.7)	7.6 (5.4, 10.9)	11.2 (7.5, 16.7)	11.3 (9.3,13.7)	0.176	20.4 (17.1, 24.5)[***]
Lung cancer /death	26.7 (24.7, 28.8)	5.3 (3.6, 7.9)	19.2 (14.4, 25.4)	16.7 (14.4, 19.4)	< 0.001	57.7 (52.3, 63.5)[***]
Cardiovascular diseases						

					p-value	
Myocardial infarction	1.7 (1.3, 2.4)	2.7 (1.5, 4.7)	1.6 (0.6, 4.3)	1.9 (1.2, 2.9)	0.552	1.0 (0.5, 2.1)
Invasive procedures	1.0 (0.7, 1.5)	1.6 (0.7, 3.3)	1.2 (0.4, 3.7)	1.2 (0.7, 2.1)	0.852	0.4 (0.1, 1.3)
PVD	1.7 (1.2, 2.3)	1.3 (0.6, 3.0)	2.8 (1.4, 6.0)	1.8 (1.1, 2.8)	0.367	1.3 (0.7, 2.5)
Stroke	1.4 (1.0, 2.0)	0.9 (0.3, 2.4)	0.8 (0.2, 3.2)	1.9 (1.2, 2.9)	0.236	1.3 (0.7, 2.5)
IHD	2.8 (2.2, 3.6)	3.8 (2.4, 6.1)	2.4 (1.1, 5.4)	3.1 (2.2, 4.4)	0.610	2.0 (1.2, 3.4)
IHD /invasive procedures	3.3 (2.6, 4.1)	4.0 (2.5, 6.4)	2.8 (1.3, 5.9)	3.8 (2.7, 5.2)	0.728	2.3 (1.4, 3.7)
IHD /invasive procedures /death	29.2 (27.1, 31.4)	9.4 (7.0, 12.7)	21.0 (16.0, 27.6)	19.5 (16.9, 22.4)	< 0.001	58.7 (53.2, 64.6)***
PVD /stroke	3.0 (2.4, 3.8)	2.0 (1.0, 3.9)	3.7 (1.9, 7.0)	3.7 (2.7, 5.0)	0.136	2.4 (1.5, 3.9)
Stroke /death	27.4 (25.4, 29.6)	6.2 (4.3, 9.0)	19.2 (14.4, 25.4)	17.9 (15.5, 20.7)	< 0.001	57.8 (52.4, 63.7)***
ART initiation	307.9 (291.7, 324.9)	255.1 (221.8, 293.3)	297.3 (251.5, 351.5)	318.0 (292.4, 345.8)	0.036	329.0 (299.8, 361.1)

Incidence rate (IR) as number of events per 1,000 person-years. P-values for the overall comparison of crude IR were

examined by the Log Rank test and excluded missing smoking data. Significance between missing and known smoking groups is marked next to the parameter value in the 'missing smoking' column. * p < 0.05, ** p < 0.01, *** p < 0.001. COPD = chronic obstructive pulmonary disease, including emphysema and chronic bronchitis. TB = pulmonary tuberculosis. PJP = *Pneumocystis jarovecii* pneumonia. IHD = ischemic heart diseases, either angina pectoris, myocardial infarction, coronary heart disease, atherosclerotic heart disease or unspecified ischemic heart diseases. Invasive procedures = invasive coronary artery procedures, including coronary angioplasty or coronary artery bypass graft. PVD = peripheral vascular disease. ART = antiretroviral therapy.

Table 2-3. Effect of smoking on death, estimated by hazard ratios (HRs) with 95% CI, by method handling missing data and adjustment

	Baseline smoking			
	Non-smoker	Former	Current	Missing
Uni-variable analysis				
Complete-case	1	3.64 (2.23, 5.95) ***	3.14 (2.05, 4.81) ***	
Missing-indictor	1	3.61 (2.21, 5.90) ***	3.11 (2.03, 4.77) ***	11.45 (7.57, 17.33) ***
Multiple imputation	1	3.44 (1.97, 6.02) ***	2.85 (1.58, 5.14) **	
Multi-variable analysis, model 1				
Complete-case	1	3.58 (2.09, 6.13) ***	2.93 (1.83, 4.68) ***	
Missing-indictor	1	3.22 (1.97, 5.27) ***	3.00 (1.95, 4.64) ***	6.84 (4.49, 10.43) ***
Multiple imputation	1	3.11 (1.86, 5.20) ***	2.81 (1.60, 4.93) **	
Multi-variable analysis, model 2				
Complete-case	1	3.56 (2.08, 6.09)***	2.85 (1.78, 4.56)***	

Missing-indictor	1	3.24 (1.98, 5.31)***	2.96 (1.92, 4.56)***	6.85 (4.49, 10.44)***
Multiple imputation	1	3.14 (1.90, 5.20)***	2.77 (1.60, 4.80)**

Hazard ratio (HR) is obtained by Cox proportional hazards analysis. Subjects were censored at the time of last visit or death.

Model 1 was adjusted for baseline age, gender, race, education level, intravenous drug use, year of HIV diagnosis, baseline

antiretroviral therapy use, baseline CD4-T-lymphocyte count and baseline viral load. Model 2 was adjusted for nadir CD4 and

peak viral load, instead of baseline values. Non-smokers were the referent. * p < 0.05, ** p < 0.01, *** p < 0.001.

Table 2-4. Effect of smoking on hospitalization, morbidity and immune function, estimated by hazard ratios (HRs) or correlation coefficient (β) with 95% CI, by adjustment after multiple imputation

Outcome	Uni-variable analysis		Multi-variable analysis	
	Former	Current	Former	Current
Hospitalization	1.26 (1.02, 1.57) *	1.39 (1.17, 1.66) ***	1.21 (0.99, 1.49)	1.34 (1.13, 1.60) **
COPD	1.80 (1.15, 2.80) *	1.99 (1.42, 2.80) ***	1.65 (1.05, 2.60) *	1.86 (1.31, 2.64) **
Asthma	1.90 (0.85, 4.24)	1.63 (0.85, 3.14)	1.87 (0.83, 4.20)	1.37 (0.68, 2.74)
PJP	1.52 (0.82, 2.78)	1.48 (0.93, 2.37)	1.34 (0.75, 2.37)	1.41 (0.88, 2.26)
Lung cancer /death	3.40 (1.94, 5.97) ***	2.82 (1.57, 5.06) **	3.06 (1.83, 5.14) ***	2.78 (1.59, 4.86) **
PVD	2.14 (0.74, 6.18)	1.45 (0.57, 3.69)	1.98 (0.66, 5.93)	1.59 (0.62, 4.06)
IHD	0.74 (0.29, 1.90)	0.95 (0.54, 1.68)	0.74 (0.29, 1.86)	1.16 (0.66, 2.04)
IHD /invasive procedures	0.80 (0.34, 1.86)	1.03 (0.60, 1.77)	0.79 (0.34, 1.84)	1.25 (0.73, 2.14)
IHD /invasive procedures /death	2.75 (1.73, 4.37) ***	2.35 (1.45, 3.82) **	2.49 (1.64, 3.79) ***	2.42 (1.55, 3.76) **
PVD /stroke	1.89 (0.80, 4.46)	1.81 (0.85, 3.88)	1.81 (0.74, 4.41)	2.07 (0.96, 4.47)

	Ever	Ever	Ever	
Stroke /death	3.23 (1.89, 5.52) ***	2.78 (1.59, 4.86) **	2.89 (1.76, 4.73) ***	2.71 (1.59, 4.60) **
ART initiation	1.13 (0.91, 1.40)	1.14 (0.97, 1.34)	1.10 (0.89, 1.37)	1.12 (0.95, 1.33)
CD4 slope, β	-13.8 (-24.8, -2.7) *	-13.6 (-20.0, -7.2) ***	-9.7 (-19.9, 0.6)	-7.9 (-14.1, -1.8) *
Lung cancer	3.18 (0.40, 25.14)		3.76 (0.48, 29.68)	
Tuberculosis	1.99 (0.59, 6.78)		2.13 (0.62, 7.34)	
Bacterial pneumonia	1.30 (0.71, 2.36)		1.08 (0.57, 2.04)	
Myocardial infarction	0.85 (0.42, 1.71)		0.99 (0.49, 2.01)	
Stroke	1.78 (0.58, 5.41)		1.93 (0.63, 5.95)	

Number of imputation = 10. Hazard ratio (HR) was by Cox proportional hazards analysis. All the parameters were HR unless specified. Correlation coefficient β was by multiple linear regression. Subjects were censored at the time of first event or last visit or death. All of the models are adjusted for baseline age, gender, race, education level, intravenous drug use, year of HIV diagnosis, baseline antiretroviral therapy use, baseline CD4-T-lymphocyte count and baseline viral load. Non-smokers were the referent in all the analysis. Former and current smokers were grouped together as ever smokers if the number of events in

any category was less than 5. * p < 0.05, ** p < 0.01, *** p < 0.001. COPD = chronic obstructive pulmonary disease, including emphysema and chronic bronchitis. PJP = *Pneumocystis jarovecii* pneumonia. IHD = ischemic heart diseases, either angina pectoris, myocardial infarction, coronary heart disease, atherosclerotic heart disease or unspecified ischemic heart diseases. PVD = peripheral vascular disease. Invasive procedures = invasive coronary artery procedures, including coronary artery bypass graft or coronary angioplasty. ART = anti-retroviral therapy. CD4 slope was annualized subject-specified annual CD4 count change for subjects who were followed up more than one year and had at least two recorded annual CD4 counts, expressed as cells/μl/year, calculated by fitting a linear regression line using calendar year as an independent variable.

Appendix

Appendix 2-1. Additional details in handling missing data and conducting statistical analysis

Missingness was found in variables of smoking status, age, race, education level, CD4 count, viral load, anti-retroviral therapy (ART) use, death, hospitalization and all target diseases. We handled missing data in different ways depending on the nature of the data. For death, hospitalization, all target diseases and ART use, missingness was treated as no disease or event. For the other variables, we replaced missing data in three steps. First, missing data was replaced by carrying last known data forward and backward for each subject; second, for the continuous variables age and CD4 count, with 3 (0.1%) missing age and 13 (0.4%) missing CD4 count, we replaced the missing data by the series mean in each year. Last, we used multiple imputation techniques to impute remaining missing data. The posterior distribution of missing data was estimated based on the known information. Preliminary complete-case analysis showed subjects with missing smoking information differed from those with known smoking data in all aspects except race and no interaction between smoking and age was found versus death. Variables associated with smoking and with complete data, including those replaced by series means, were used as predictors to conduct multiple imputation, including: sex, age, enrolment year, last follow up year, baseline CD4 count, nadir CD4 count before enrolment, nadir CD4 count during follow up, death, IDU and ART use at baseline.

Imputation was conducted without stratification. Ten imputations were randomly selected from the posterior distribution in order to achieve relative efficiency more than 0.95, giving the fact that approximately 43% subjects had missing data [21]. We used automatic imputation method, chose maximum 100 cases draws, maximum 10 parameter draws, and no maximum percentage missing. Analysis for ten datasets was conducted separately, and then the results from each dataset were combined to produce a pooled estimate, with 95% CI. We report results by excluding, grouping or multiply imputing missing data, respectively, in order to estimate how missing data affected the results.

By using ANOVA (analysis of variance) to compare baseline data by smoking, further pairwise comparison for continuous variables was tested by Dunnett's T3 method. For categorical variables, the observed upward or downward trend was further tested by z-test, and p-values were adjusted by the Bonferroni method. We classified all continuous variables into categorical variables in order to better make clinical sense. We combined the nearest levels if the number of events at a level was less than 5. Former and current smokers were grouped together as ever smokers when there were fewer than 5 events in either group. We used a pre-set criterion of $p \leq 0.2$ in uni-variable analysis to select the variables into multi-variable model. In Cox proportional hazards (PH) model, PH assumption was met if the p value of the Pearson correlation between ranked survival time and Schoenfeld partial residual was more than 0.05.

Appendix 2-2. Number of current smokers and smoking prevalence (%) over year

The figure shows the number of current smokers and smoking prevalence (%) during study period by year. The number of current smokers is shown in bar and the smoking prevalence is shown in line. By excluding subjects with missing smoking information, the smoking prevalence was 60% at baseline, steadily decreased to 51% in the last year of follow up, with annualized decrease of 0.6% (95% CI: -0.4% to -0.8%, p < 0.001, R^2 = 0.836) per year after enrolment.

Appendix 2-3. Hazard ratio (HR) (95% CI) for death by method handling missing data and adjustment

A. Uni-variable analysis

	Complete-case	Missing-indicator	Multiple imputation
Smoking status			
Non-smoker	1	1	1
Former	3.64 (2.23, 5.95)***	3.61 (2.21, 5.90)***	3.44 (1.97, 6.02)***
Current	3.14 (2.05, 4.81)***	3.11 (2.03, 4.77)***	2.85 (1.58, 5.14)**
Missing		11.45 (7.57, 17.33)***	
Age (year)			
< 30	1		
30-39	1.37 (1.04, 1.80)*		
40-49	1.61 (1.22, 2.14)**		
>= 50	2.36 (1.74, 3.22)***		
Female	0.52 (0.39, 0.69)***		
Race			
White	1	1	1
Non-white	0.63 (0.50, 0.79)***	0.63 (0.50, 0.79)***	0.63 (0.50, 0.79)***
Missing		1.44 (0.91, 2.27)	

HIV diagnosis year			
< 1996	4.74 (1.96, 11.44)[**]		
1996-1998	1.54 (0.61, 3.91)		
> 1998	1		
Baseline viral load (copies/ml)			
<= 50	1	1	1
51-9,999	0.62 (0.29, 1.33)	0.64 (0.30, 1.36)	0.87 (0.39, 1.96)
10,000-99,999	1.52 (0.71, 3.24)	1.51 (0.71, 3.22)	1.98 (0.90, 4.39)
>= 10,0000	2.78 (1.29, 5.97)[**]	2.66 (1.24, 5.71)[*]	3.62 (1.63, 8.03)[**]
Missing		10.93 (5.15, 23.21)[***]	
Baseline CD4 (cells/mm^3)			
< 50	5.71 (4.29, 7.60)[***]		
50-199	2.35 (1.74, 3.18)[***]		
200-349	1.25 (0.90, 1.74)		
350-499	1.03 (0.72, 1.48)		
>= 500	1		
IDU	1.19 (0.96, 1.48)		

No baseline	0.45 (0.38, 0.53)***		
ART use			
Highest			
education			
Elementary	1.12 (0.78, 1.59)	1.12 (0.78, 1.59)	1.11 (0.78, 1.59)
Secondary	1.04 (0.88, 1.23)	1.04 (0.88, 1.23)	1.04 (0.88, 1.23)
Post-secondary	1	1	1
Missing		1.66 (0.99, 2.79)	

B. Multi-variable analysis

	Complete-case	Missing-indicator	Multiple imputation
Smoking status			
Non-smoker	1	1	1
Former	3.58 (2.09, 6.13)***	3.22 (1.97, 5.27)***	3.11 (1.86, 5.20)***
Current	2.93 (1.83, 4.68)***	3.00 (1.95, 4.64)***	2.81 (1.60, 4.93)**
Missing		6.84 (4.49, 10.43)***	
Age (year)			
< 30	1	1	1
30-39	0.89 (0.56, 1.43)	0.87 (0.66, 1.15)	0.96 (0.73, 1.27)
40-49	0.90 (0.54, 1.49)	1.10 (0.82, 1.47)	1.18 (0.88, 1.58)
>= 50	2.22 (1.28, 3.87)**	1.37 (1.00, 1.88)	1.78 (1.29, 2.46)***

Female	0.65 (0.38, 1.09)	0.76 (0.56, 1.02)	0.79 (0.59, 1.07)
Race			
White	1	1	1
Non-white	0.82 (0.54, 1.25)	0.75 (0.59, 0.96)[*]	0.71 (0.56, 0.90)[**]
Missing		0.98 (0.62, 1.56)	
HIV diagnosis year			
< 1996	0.92 (0.37, 2.30)	2.79 (1.14, 6.83)[*]	4.22 (1.74, 10.25)[**]
1996-1998	0.54 (0.20, 1.46)	1.07 (0.42, 2.72)	1.61 (0.63, 4.09)
> 1998	1	1	1
Baseline viral load (copies/ml)			
<= 50	1	1	1
51-9,999	0.60 (0.22, 1.67)	0.48 (0.22, 1.02)	0.65 (0.27, 1.57)
10,000-99,999	1.17 (0.42, 3.24)	0.93 (0.43, 2.00)	1.23 (0.52, 2.88)
>= 10,0000	1.49 (0.52, 4.25)	1.42 (0.66, 3.08)	1.88 (0.80, 4.43)
Missing		5.59 (2.60, 11.98)[***]	
Baseline CD4 (cells/mm^3)			
< 50	2.51 (1.49, 4.23)[**]	3.10 (2.30, 4.17)[***]	4.31 (3.18, 5.83)[***]
50-199	1.93 (1.21, 3.09)[**]	1.87 (1.39, 2.52)[***]	2.13 (1.58, 2.89)[***]

200-349	1.37 (0.84, 2.22)	1.21 (0.88, 1.66)	1.38 (1.01, 1.90)[*]
350-499	1.03 (0.61, 1.73)	0.93 (0.65, 1.32)	0.94 (0.66, 1.34)
>= 500	1	1	1
IDU	1.86 (1.29, 2.68)[**]	1.73 (1.37, 2.18)[***]	1.39 (1.09, 1.78)[**]
No baseline ART use	0.58 (0.43, 0.79)[**]	0.56 (0.47, 0.67)[***]	0.58 (0.48, 0.69)[***]

Number of imputation = 10. Hazard ratio (HR) is obtained by Cox proportional hazards analysis, adjusting for all the co-variables listed in the table. Education level was not selected into multi-variable analysis because p values for all the levels were more than 0.2 in uni-variable analysis. Subjects were censored at the time of last visit or death. Non-smokers were the referent. [*] $p < 0.05$, [**] $p < 0.01$, [***] $p < 0.001$. IDU = intravenous drug use. ART = anti-retroviral therapy.

Appendix 2-4. Effect of smoking on hospitalization, morbidity and immune function, estimated by hazard ratios (HRs) or correlation coefficient (β) with 95% CI, by complete-case analysis, missing-indicator method and adjustment

Outcomes	Smoking status	Uni-variable analysis		Multi-variable analysis	
		Complete-case	Missing-indicator	Complete-case	Missing-indicator
Hospitalization	Former	1.14 (0.89, 1.44)	1.14 (0.89, 1.44)	1.08 (0.84, 1.39)	1.09 (0.86, 1.39)
	Current	1.38 (1.16, 1.63) ***	1.37 (1.16, 1.63) ***	1.26 (1.05, 1.52) *	1.32 (1.10, 1.57) **
	Missing		1.66 (1.39, 1.99) ***		1.44 (1.20, 1.73) ***
COPD	Former	2.01 (1.25, 3.25) **	2.01 (1.25, 3.25) **	1.73 (1.05, 2.83) *	1.83 (1.13, 2.97) *
	Current	2.23 (1.53, 3.27) ***	2.23 (1.53, 3.26) ***	2.15 (1.44, 3.19) ***	2.07 (1.40, 3.07) ***
	Missing		1.49 (0.98, 2.25)		1.38 (0.90, 2.09)
Asthma	Former	2.19 (0.98, 4.89)	2.20 (0.98, 4.90)	2.43 (1.06, 5.59) *	2.14 (0.95, 4.81)
	Current	1.74 (0.90, 3.37)	1.74 (0.90, 3.37)	1.78 (0.87, 3.64)	1.45 (0.72, 2.90)

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Variable	Category				
	Missing		0.71 (0.32, 1.59)		0.69 (0.30, 1.55)
Lung cancer	Ever	4.58 (0.60, 35.00)	4.63 (0.61, 35.37)	5.80 (0.75, 44.70)	5.69 (0.74, 43.58)
	Missing		7.22 (0.91, 57.06)		6.93 (0.88, 54.83)
TB	Ever	5.28 (0.70, 40.01)	5.28 (0.70, 40.01)	5.59 (0.74, 42.56)	5.74 (0.76, 43.61)
	Missing		4.67 (0.59, 37.02)		4.86 (0.61, 38.50)
Bacterial pneumonia	Ever	0.96 (0.51, 1.81)	0.96 (0.51, 1.81)	1.05 (0.50, 2.20)	0.77 (0.40, 1.50)
	Missing		1.38 (0.72, 2.63)		0.89 (0.46, 1.74)
PJP	Former	1.45 (0.85, 2.47)	1.45 (0.85, 2.47)	1.22 (0.70, 2.15)	1.30 (0.75, 2.23)
	Current	1.45 (0.97, 2.17)	1.45 (0.97, 2.16)	1.35 (0.88, 2.09)	1.42 (0.94, 2.15)
	Missing		2.15 (1.44, 3.20)***		1.58 (1.05, 2.37)*
Lung cancer /death	Former	3.65 (2.23, 5.95)***	3.62 (2.22, 5.90)***	3.56 (2.08, 6.09)***	3.22 (1.97, 5.28)***
	Current	3.18 (2.07, 4.87)***	3.16 (2.06, 4.84)***	2.99 (1.87, 4.78)***	3.05 (1.98, 4.71)***
	Missing		11.47 (7.58, 17.35)***		6.88 (4.51, 10.49)***
Myocardial infarction	Ever	0.69 (0.34, 1.38)	0.69 (0.34, 1.38)	0.91 (0.44, 1.92)	0.83 (0.40, 1.69)
	Missing		0.51 (0.20, 1.31)		0.52 (0.20, 1.35)

PVD	Former	2.13 (0.71, 6.33)	2.13 (0.72, 6.34)	2.00 (0.64, 6.23)	1.97 (0.63, 6.15)
	Current	1.33 (0.53, 3.35)	1.33 (0.53, 3.35)	1.55 (0.61, 3.92)	1.50 (0.59, 3.80)
	Missing		1.11 (0.39, 3.18)		0.99 (0.34, 2.85)
Stroke	Ever	1.86 (0.64, 5.40)	1.86 (0.64, 5.40)	2.06 (0.69, 6.20)	2.10 (0.72, 6.16)
	Missing		1.52 (0.46, 5.01)		1.21 (0.36, 4.06)
IHD	Former	0.64 (0.25, 1.63)	0.64 (0.25, 1.62)	0.73 (0.28, 1.87)	0.66 (0.26, 1.67)
	Current	0.82 (0.45, 1.47)	0.81 (0.45, 1.47)	1.10 (0.60, 2.04)	1.04 (0.57, 1.89)
	Missing		0.67 (0.33, 1.38)		0.63 (0.30, 1.30)
IHD /invasive	Former	0.71 (0.30, 1.69)	0.71 (0.30, 1.69)	0.76 (0.31, 1.84)	0.73 (0.30, 1.75)
procedures	Current	0.95 (0.54, 1.66)	0.94 (0.54, 1.65)	1.19 (0.66, 2.15)	1.21 (0.68, 2.13)
	Missing		0.73 (0.37, 1.45)		0.70 (0.35, 1.40)
IHD /invasive	Former	2.25 (1.50, 3.37)***	2.23 (1.49, 3.35)***	2.12 (1.36, 3.30)**	2.01 (1.33, 3.02)**
procedures	Current	2.09 (1.50, 2.91)***	2.07 (1.49, 2.89)***	2.08 (1.46, 2.98)***	2.22 (1.59, 3.10)***
/death	Missing		6.70 (4.86, 9.23)***		4.14 (2.98, 5.74)***
PVD /stroke	Former	1.82 (0.72, 4.57)	1.82 (0.72, 4.58)	1.77 (0.68, 4.60)	1.76 (0.68, 4.56)
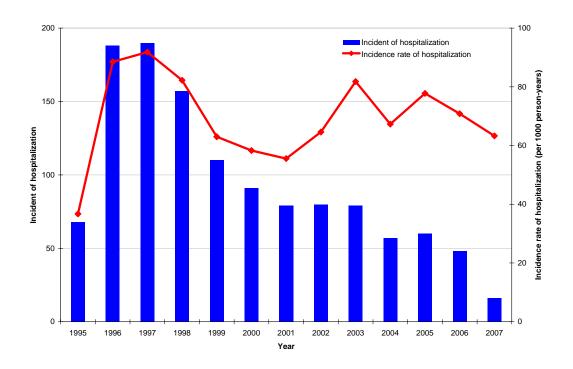
	Current	1.82 (0.88, 3.77)	1.82 (0.88, 3.78)	2.13 (1.01, 4.49)*	2.15 (1.03, 4.50)*
	Missing		1.36 (0.60, 3.09)		1.18 (0.52, 2.70)
Stroke /death	Former	3.11 (1.95, 4.95)***	3.08 (1.94, 4.92)***	2.93 (1.76, 4.87)***	2.69 (1.68, 4.30)***
	Current	2.90 (1.95, 4.32)***	2.88 (1.94, 4.29)***	2.69 (1.74, 4.15)***	2.74 (1.83, 4.10)***
	Missing		9.81 (6.67, 14.43)***		5.75 (3.88, 8.51)***
ART initiation	Former	1.16 (0.94, 1.45)	1.16 (0.93, 1.44)	1.15 (0.91, 1.44)	1.14 (0.91, 1.42)
	Current	1.24 (1.05, 1.45)*	1.23 (1.04, 1.45)*	1.21 (1.02, 1.44)*	1.20 (1.01, 1.41)*
	Missing		1.26 (1.06, 1.49)**		1.16 (0.97, 1.38)
CD4 slope, β	Former	-10.2 (-17.9, -2.5)*	-10.2 (-19.0, -1.4)*	-7.4 (-14.3, -0.6)*	-4.5 (-12.8, 3.8)
	Current	-11.9 (-17.5, -6.4)***	-11.9 (-18.3, -5.6)***	-6.9 (-12.1, -1.8)**	-4.4 (-10.5, 1.8)
	Missing		-17.8 (-24.1, -11.5)***		-13.3 (-19.4, -7.2)***

Number of imputation = 10. Hazard ratio (HR) was by Cox proportional hazards analysis. All the parameters were HR unless specified. Correlation coefficient β was calculated by multiple linear regression. Subjects were censored at the time of first event or last visit or death. All of the models are adjusted for baseline age, gender, race, education level, intravenous drug use,

year of HIV diagnosis, baseline antiretroviral therapy use, baseline CD4-T-lymphocyte count and baseline viral load. Non-smokers were the referent in all the analysis. Former and current smokers were grouped together as ever smokers if the number of events in any category was less than 5. * p < 0.05, ** p < 0.01, *** p < 0.001. COPD = chronic obstructive pulmonary disease, including emphysema and chronic bronchitis. TB = pulmonary tuberculosis. PJP = pneumocystis jarovecii pneumonia. IHD = ischemic heart diseases, either angina pectoris, myocardial infarction, coronary heart disease, atherosclerotic heart disease or unspecified ischemic heart diseases. PVD = peripheral vascular disease. Invasive procedures = invasive coronary artery procedures, including coronary artery bypass graft or coronary angioplasty. ART = anti-retroviral therapy. CD4 slope was annualized subject-specified annual CD4 count change for subjects who were followed up more than one year and had at least two recorded annual CD4 counts, expressed as cells/µl/year, calculated by fitting a linear regression line using calendar year as an independent variable.

Appendix 2-5. Number and incidence rate of hospital admission (per 1,000 person-years) over year

The figure showed number and incidence rate (IR, per 1,000 person-years) of hospital admission by year. Subjects who were hospitalized before or within 6 months after study enrolment were excluded. Subjects were censored at the time of first hospitalization or last visit or death. The number of patients being hospitalized was shown in bar and IR was shown in line. The overall IR (95% CI) of hospital admission was 70.1 (66.3, 74.2) per 1,000 person-years. The IR was 36.7 in 1995, increased sharply to 88.5 in 1996, peaked at 91.8 in 1997, then decreased to 55.5 in 2001. IR hit the second peak at 81.8 in 2003, and third peak at 77.8 in 2005, and then finally decreased to 63.3 per 1,000 person-

years in 2007.

Section 2:

Thesis studies

Chapter 3:

A cross-sectional study of lung function

In this chapter my co-authors and I examine the effect of smoking on lung function, respiratory symptoms and diseases amongst HIV-infected subjects. Our main study finding was that %FEV_1 decreased with the number of pack-years of smoking, after multi-variable adjustment, and this reduction was comparable to that previously seen in the general population. I also found current smokers had more respiratory symptoms compared to non- and former smokers.

This chapter consists of a published manuscript. I have made no changes from the published manuscript, except I added academic degrees for each co-author and added post-publication correspondence for consistency throughout the thesis. The full citation is:

Cui Q, Carruthers S, McIvor A, Smaill F, Thabane L, Smieja M. Effect of smoking on lung function, respiratory symptoms and respiratory diseases amongst HIV-positive subjects: a cross-sectional study. *AIDS Res Ther*. 2010;7(6).

I presented the preliminary results at annual research conference of Ontario HIV

Treatment Network orally, Nov. 27-28, 2006, Toronto ON, and as a poster presentation,

Nov. 19-20, 2007, Toronto ON, and at the annual conference of the Canadian Association

for HIV/AIDS Research (CAHR) as a poster presentation, Apr. 26-29, 2007, Toronto ON,

and Apr. 23-26, 2009, Vancouver BC, respectively.

Effect of smoking on lung function, respiratory symptoms and respiratory diseases

amongst HIV-positive subjects: a cross-sectional study

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Word Count: Abstract: 346; Text: 4499.

Abstract

Background

Smoking prevalence in human immunodeficiency virus (HIV) positive subjects is about three times of that in the general population. However, whether the extremely high smoking prevalence in HIV-positive subjects affects their lung function is unclear, particularly whether smoking decreases lung function more in HIV-positive subjects, compared to the general population. We conducted this study to determine the association between smoking and lung function, respiratory symptoms and diseases amongst HIV-positive subjects.

Results

Of 120 enrolled HIV-positive subjects, 119 had an acceptable spirogram. Ninety-four (79%) subjects were men, and 96 (81%) were white. Mean (standard deviation [SD]) age was 43.4 (8.4) years. Mean (SD) of forced expiratory volume in one second (FEV_1) percent of age, gender, race and height predicted value (%FEV_1) was 93.1% (15.7%). Seventy-five (63%) subjects had smoked 24.0 (18.0) pack-years. For every ten pack-years of smoking increment, %FEV_1 decreased by 2.1% (95% confidence interval [CI]: -3.6%, -0.6%), after controlling for gender, race and restrictive lung function ($R^2 = 0.210$). The loss of %FEV_1 in our subjects was comparable to the general population. Compared to non-smokers, current smokers had higher odds of cough, sputum or breathlessness,

after adjusting for highly active anti-retroviral therapy (HAART) use, odds ratio OR = 4.9 (95% CI: 2.0, 11.8). However respiratory symptom presence was similar between non-smokers and former smokers, OR = 1.0 (95% CI: 0.3, 2.8). All four cases of COPD (chronic obstructive pulmonary disease) had smoked. Four of ten cases of restrictive lung disease had smoked (p = 0.170), and three of five asthmatic subjects had smoked (p = 1.000).

Conclusions

Cumulative cigarette consumption was associated with worse lung function; however the loss of %FEV$_1$ did not accelerate in HIV-positive population compared to the general population. Current smokers had higher odds of respiratory symptoms than non-smokers, while former smokers had the same odds of respiratory symptoms as non-smokers. Cigarette consumption was likely associated with more COPD cases in HIV-positive population; however more participants and longer follow up would be needed to estimate the effect of smoking on COPD development. Effective smoking cessation strategies are required for HIV-positive subjects.

Background

In the developed world, mortality from HIV/AIDS has decreased significantly since the introduction of highly active anti-retroviral therapy (HAART) in 1996 [1]. Consequently, people are living with HIV/AIDS longer than ever. In this context, chronic diseases, whether HIV/AIDS related or not, are increasingly of concern amongst the HIV-positive population, and for clinicians caring for them.

Prior to 2001, annual smoking prevalence in the Ontario Cohort Study (OCS) of HIV-positive adult subjects was more than 70%, and steadily decreased to 58% in 2007 (data unpublished), which was constantly about three times higher than that in the Ontario general population from 1999 to 2007 [2]. A smoking prevalence of 60% or more in HIV-positive subjects has been reported in other studies [3-7]. Therefore, smoking-related outcomes, such as lung function problems, respiratory symptoms and lung diseases, are likely to increase in this population.

We hypothesized that HIV infection would accelerate smoking-related respiratory symptoms and diseases. Studies have shown that HIV-positive subjects were more likely to have respiratory symptoms and diseases compared to their HIV negative counterparts [3,8,9]. Moreover, some studies showed among HIV-positive subjects, smokers were more likely to have respiratory problems [8,10]. Other studies found that HIV-positive subjects had similar lung function compared to their HIV negative counterparts [11,12], and they had similar changes of lung function over time [13,14]. Although in the general population, the

effects of cigarette smoking on lung function has been well demonstrated in the general population, using different measurements of smoking and lung function [15-21], no study has been done to address whether cigarette smoking affects lung function in a similar way in HIV-positive population.

Hence, the literature is unclear on the effects of smoking on lung function in HIV-positive subjects, particularly whether lung function decline would be greater in HIV-positive subjects compared to the general population. The primary objective of this study was to determine the association between smoking and lung function amongst HIV-positive subjects. The secondary objective was to examine the association between smoking and respiratory symptoms and diseases amongst HIV-positive subjects.

Methods

Study design, setting and participants

This was a cross-sectional study. The study protocol was approved by the Research Ethics Board (REB) at Hamilton Health Sciences / McMaster University. Consecutive consenting HIV-positive subjects attending the regional HIV clinic (Special Immunology Services [SIS] clinic) at McMaster University, aged 18 years or more were eligible to take part in the study.

Study description

Our study respiratory technologist approached potentially eligible subjects

attending regularly scheduled clinical visits at the SIS clinic. Participants provided signed informed consent, filled out a questionnaire, and underwent spirometry testing. The questionnaire contained information on demography, respiratory symptoms, and history of respiratory diseases, cigarette smoking and other drug uses. Spirometry testing followed the standardization of spirometry testing recommended by the American Thoracic Society (ATS) and European Respiratory Society (ERS) [22-24]. We used a VIASYS JAEGER FlowScreen V2.1.1 (Hoechberg, Germany). All subjects who had forced expiratory volume in one second (FEV_1) percent of age, gender, race and height predicted value (%FEV_1) less than 90% were given two puffs of salbutamol (Ventolin, a short acting β-agonist) and repeated spirometry to assess the change in FEV_1. Medical information such as CD4 T-lymphocyte count, HIV viral load, date of HIV diagnosis and antiretroviral medication was abstracted from the medical chart. Information on history of respiratory diseases was abstracted from the medical chart if information was absent in the questionnaire.

Measurements

Each subject was self-classified as a non-smoker, ex-smoker or current smoker. Cumulative exposure to cigarette smoking was measured by pack-years, which was calculated by multiplying the number of packs of cigarette smoked per day and the number of years of smoking. Marijuana use was similarly measured as never, former and current use. Marijuana consumption was measured by the number of times of use per day and the number of years of use. The primary outcome of lung function was measured as

FEV_1, %FEV_1, forced vital capacity (FVC) and FVC percent of age, gender, race and height predicted value (%FVC). All measurements were automatically printed by the VIASYS JAEGER FlowScreen. Per cent FEV_1 and %FVC were calculated by dividing the measured FEV_1 and FVC by their age, gender, race and height predicted values, which were also automatically printed by FlowScreen. Respiratory symptoms included cough, sputum and breathlessness. Cough was described as current cough, productive cough and nocturnal cough. Sputum was measured at 5 levels: no sputum, 1 tea spoon, 1 table spoon, 2 table spoon and 1/2 cup in 24 hours. We used the Medical Research Council (MRC) dyspnea scale to measure breathlessness. Grade 3 or more (breathlessness walking on the level) was considered having breathlessness [25].

The diagnoses of obstructive and restrictive lung diseases could be interpreted by the spirogram, however we diagnosed these diseases based on our calculation and the GOLD (Global initiative for chronic Obstructive Lung Disease) guidelines. The diagnosis of obstructive lung function was defined as pre-salbutamol FEV_1/FVC < 70% without post-salbutamol values. The diagnosis of COPD was defined by post-bronchodilator FEV_1/FVC < 70%, and COPD level was classified based on %FEV_1. A COPD case with %$FEV_1 \geq$ 80% was classified as mild, 30% $\leq$ %FEV_1 < 80% as moderate and %FEV_1 < 30% as severe [26]. Subjects who did not undergo post-salbutamol testing could not be diagnosed as COPD by definition. For subjects whose post-salbutamol FEV_1/FVC was between 66.5% and 73.5%, the diagnosis was made by the committee's judgment, taking account of the pre-salbutamol FEV_1/FVC value and clinical symptoms of cough, sputum

and breathlessness. The diagnosis of restrictive lung function was defined as FEV_1/FVC $\geq$ 70% and %FVC < 80%, either before or after salbutamol inhalation [27,28]. Asthma was defined as reversible FEV_1, which improved more than 12% and 200 ml after salbutamol inhalation [26]. A history of asthma was defined as previous diagnosed or treated asthma. Normal lung function was defined as FEV_1/FVC $\geq$ 70%, %FEV_1 $\geq$ 80% and %FVC $\geq$ 80% accordingly, by both pre- and post-salbutamol tests. Pre-salbutamol values were used to classify normal lung function if post-salbutamol test was not done.

Statistical analysis

Continuous variables were reported as mean (standard deviation [SD]) if they were normal distributed or reported as median (first quartile [Q_1], third quartile [Q_3]) if they were not normal distributed. Normality was visually tested by P-P plots. Categorical variables were reported as count (percent). Analysis of variance (ANOVA) was used to compare continuous variables among different groups and χ^2 test was used for categorical variables. Fisher's exact test was adopted if the number of cases in any cell is less than 10. Multiple regression method was used to adjust for possible confounders further. Multiple linear regression was used to model %FEV_1. Multiple logistic regression model was used for lung function, respiratory symptoms, respiratory diseases and subject classification. We used the criterion of $\alpha = 0.20$ in uni-variate regression analysis to decide whether or not to select appropriate variables into a multivariable regression model. Possible interaction between independent variables was tested. The criterion for statistical significance for multivariable analysis was set at $\alpha = 0.05$. All p-values were reported to

three digital places with those less than 0.001 were reported as p < 0.001. All analyses were performed using SPSS 15 (Chicago, IL).

In the multiple linear regression model, the dependent variable was %FEV$_1$ before salbutamol. The selected predictor was pack-years of smoking or smoking status, depending on which variable had the smaller p value in uni-variate analysis. Restrictive lung function was co-variable related to %FEV$_1$. Productive cough and age were potential confounders based on the previous literature [17,19,20]. We examined gender and race as they were common confounders, although previous studies were inconclusive [18,19]. In addition, current CD4 T-lymphocytes count, current viral load, current antiretroviral treatment and marijuana use were examined a priori as potential confounders as well. Potential interactions between independents were tested. The results were reported as estimates of model coefficients (95% confidence interval [CI]) and associated p-values. Results for all subjects and for smokers (including former and current smokers) were presented when pack-years of smoking was the predictor variable. We examined the residuals to assess model assumptions and goodness-of-fit (reported using R^2).

In the multiple logistic regression model, the dependent variable was lung function (normal / abnormal), respiratory symptom (yes / no), respiratory disease (yes / no) and subject classification (normal lung function and no symptom / abnormal lung function or respiratory symptom) in each analysis respectively, and the predictor was smoking status. We considered the same potential confounders as we did in multiple linear regression analysis. We selected no more than one independent variable for each

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ten cases, which was considered to be the lesser number of the outcome group. If the lesser number of the outcome group was less than 10, logistic regression analysis was not conducted. The results were reported as estimates of odds ratio (OR) (95% CI) and their p-values. Nagelkerke R square was reported to assess the goodness-of-fit of logistic regression model.

Results

Demographic and baseline information

We recruited 120 consecutive consenting HIV positive subjects, of whom 119 had an acceptable spirogram. Demographic and baseline information are listed in Table 2-1. Ninety-four (79%) subjects were men (one trans-gendered individual was classified as a woman). Ninety-six (81%) subjects were white, including 83 (88%) men and 13 (52%) women ($p < 0.001$). Mean (standard deviation [SD]) age was 43.4 (8.4) years. Men were 5.4 years older than women ($p = 0.004$). Mean (SD) number of years of living with HIV was 9.0 (6.6) years. One hundred (84%) HIV-positive subjects were on antiretroviral treatment at the time of study. Mean (SD) current CD4 T-lymphocytes count was 484 (274) cells/mm^3, and 102 (86%) of subjects had current CD4 count of 200 cells/mm^3 or more. Seventy-three (61%) subjects had current undetectable viral load. Amongst those with detectable viral load, median (Q_1, Q_3) viral load was 907 (193, 28630), and mean (SD) of log viral load was 3.38 (1.26). No gender or race difference was found in terms of current CD4 T-lymphocytes count or HIV viral load.

Smoking status and marijuana use

Forty-four (37%) subjects never smoked cigarettes, of whom 3 subjects currently used marijuana at the time of survey. Twenty-three (19%) subjects had formerly smoked, of whom 6 subjects currently used marijuana. Fifty-two (44%) subjects currently smoked, of whom 21 subjects currently used marijuana. Males accounted for 68% in non-smokers and 85% in smokers respectively (p = 0.036). On average smokers had smoked 24.0 (18.0) pack-years. Mean (SD) pack-years of smoking was 16.8 (13.9) for former smokers and 27.2 (18.7) for current smokers respectively (p = 0.020).

Sixty (50%) subjects never used marijuana. Twenty-nine (24%) subjects formerly used marijuana, of whom only 7 (24%) subjects used once or more per day, with mean (SD) year of use of 8.7 (4.5) years. Thirty (25%) subjects were currently using marijuana at the time of survey, of whom 26 (87%) subjects used once or more per day, with mean (SD) year of use of 18.3 (9.5) years. Current users used marijuana more frequently (p < 0.001) and for a longer time (p < 0.001) than former users.

Association between lung function and smoking

Lung function by smoking status and gender was summarized in Table 2-1. Mean (SD) of FEV_1 before salbutamol was 3.5 (0.8) litres. Mean (SD) of $\%FEV_1$ before salbutamol was 93.1% (15.7%). Mean FVC before salbutamol was 4.5 (1.0) litres. Forty-six (39%) HIV-positive subjects had $\%FEV_1 < 90\%$ and 27 (59%) of them underwent post salbutamol spirometry test. Mean improvement was 143 (193) ml for FEV_1 and 79

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(263) ml for FVC respectively.

According to our preset criterion of α = 0.2, four variables from uni-variate regression were selected to build the multiple linear regression model: pack-years, gender, race and restrictive lung function. For every ten pack-years of smoking increment, %FEV$_1$ significantly decreased by 2.1% (95% CI: -3.6%, -0.6%), after controlling for gender, race and restrictive lung diseases (p = 0.006). Moreover white subjects had 8.8% (95% CI: 1.2%, 16.3%) higher %FEV$_1$ than non-white, after controlling for pack-years, gender and restrictive lung diseases (p = 0.023). Gender did not affect %FEV$_1$ significantly (p = 0.640). No interaction between independents was found. The point estimate of β coefficient of -2.0% (95% CI: -4.2%, 0.2%) was similar when non-smokers were excluded, with wider 95% CI (p = 0.077). The point estimate of pack-years did not change when the association was not adjusted for gender. Coefficients and 95% CIs of each variable versus %FEV$_1$ in different populations were summarized in Table 2-2.

Among 24 (20%) subjects who had abnormal lung function, there were 8 non-smokers, 4 former smokers and 12 current smokers respectively (p = 0.782) (Table 2-1). According to preset criterion of α = 0.2, smoking status was not selected into multiple logistic regression model for abnormal lung function.

Association between respiratory symptoms and smoking

Cough: Sixty-one (51%) subjects coughed, including 16 (36%) non-smokers, 8 (35%) former smokers and 37 (71%) current smokers (p = 0.001) (Table 2-1). Compared to non-smokers, current smokers had higher odds of cough, OR = 4.3 (95% CI: 1.5, 12.0)

after controlling for marijuana use, race, current HAART status and current viral load (p = 0.005). For former smokers the OR of 0.8 (95% CI: 0.3, 2.6) was not statistically significant compared to non-smokers (p = 0.753). No interaction was found. Moreover, subjects who were on HAART had higher odds of cough (OR = 5.5, 95% CI: 1.4, 21.5, p = 0.014), and subjects who had suppressed viral load had lower odds of cough (OR = 0.3, 95% CI: 0.1, 0.9, p = 0.025). The results are presented in Table 2-3.

Sputum: Fifty-one (43%) subjects produced sputum, including 11 (25%) non-smokers, 8 (35%) former smokers and 32 (63%) current smokers (p = 0.001) (Table 2-1). Compared to non-smokers, current smokers had higher odds of sputum, OR = 5.0 (95% CI: 1.9, 13.3) after controlling for marijuana use (p = 0.001). For former smokers the OR of 1.7 (95% CI: 0.5, 5.3) was not significant compared to non-smokers (p = 0.382). No interaction existed. The results are presented in Table 2-3.

Breathlessness: Eight subjects (7%) had breathlessness (Table 2-1). All of them were smokers including 1 former smoker and 7 current smokers (p = 0.027), however we could not compute the OR because of 0 cases in the reference (non-smoker) group, nor could we use logistic regression to estimate the risk factors further.

Any symptom: In terms of three respiratory symptoms (cough, sputum and breathlessness), 63 (53%) subjects had at least one respiratory symptom, including 16 (36%) non-smokers, 9 (39%) former smokers and 38 (73%) current smokers (p = 0.001) (Table 2-1). Compared to non-smokers, current smokers were 4.9 (95% CI of OR: 2.0, 11.8) times more likely to have at least one symptom, after controlling for current

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HAART status ($p < 0.001$). For former smokers the OR was 1.0 (95% CI: 0.3, 2.8) compared to non-smokers ($p = 0.969$). No interaction existed. The results are presented in Table 2-3 and Figure 2-1.

Association between respiratory diseases and smoking

COPD: Three (3%) subjects were diagnosed with COPD originally. In addition, one subject with borderline lung function was diagnosed with COPD by the committee. All these 4 cases of COPD were moderate in severity according to GOLD guidelines, and asthma co-existed in 2 COPD cases. All of them were white men, mean (SD) age was 49.8 (7.3), ranging from 43 to 57 years old. All were smokers with mean (SD) pack-years of 29.5 (13.8), however COPD was not associated with ever smoking status ($p = 0.295$), probably due to the small number of cases. The OR could not be calculated because of zero case in non-smokers. Notably 14 (12%) subjects had obstructive lung function by pre-salbutamol spirometry testing, however only 5 (38%) of them underwent post salbutamol testing. Therefore we could not confirm the other 9 potential COPD subjects. Among 14 subjects with pre-salbutamol obstructive lung function, there were 2 non-smokers, 3 former smokers and 9 current smokers. Although smokers had 4.0 times the odds of pre-salbutamol obstructive lung function, this crude OR was not significant ($p = 0.079$) and its 95% CI (0.9, 18.8) was very wide. COPD results are presented in Table 2-1 and Figure 2-1.

Restrictive lung function: Ten (8%) subjects had restrictive lung function. Mean (SD) age was 42.5 (7.0), ranged from 34 to 56 years old. Seven (70%) of them were

women, including 6 black women. Amongst 10 subjects with restrictive lung function, there were 4 smokers including 1 former smoker and 3 current smokers (p = 0.170) (Table 2-1). Multiple logistic regression analysis was not conducted because there were ten cases of restrictive lung function only. Results are also presented in Figure 2-1.

Asthma: Thirteen (11%) subjects had a history of asthma, which was not associated with smoking status (p = 0.553) (Table 2-1). Only 5 (38%) of them underwent post-salbutamol testing and 2 subjects were asthmatic at the time of study. In addition, among 106 subjects without an asthma history, 3 (3%) were asthmatic. In total 5 subjects were asthmatic at the time of study, which was not associated with smoking status (p = 0.985). In total 16 (13%) subjects were asthmatic or had an asthma history, which was not associated with smoking status (p = 0.551). A history of asthma and spirometry diagnosed asthma are also presented in Figure 2-1.

Respiratory diseases history: A history of bronchitis was present in 38 (32%) subjects, pneumonia in 46 (39%) subjects, tuberculosis in 5 (4%) subjects, emphysema in 1 (1%) subject and asthma in 13 (11%) subjects. Former smokers had 3.7 times the odds of a history of bronchitis than non-smokers (95% CI: 1.1, 12.5), after controlling for race and HAART use (p = 0.038), while the OR of 1.5 (95% CI: 0.6, 4.1) for current smokers was insignificant (p = 0.421). Moreover, white subjects had 12.4 (95% CI: 1.5, 101.7) times the odds of a history of bronchitis compared to non-white subjects (p = 0.019). A history of other respiratory diseases was not associated with smoking status.

Subject classifications and their association with smoking

The exclusive subject categories based on lung function, respiratory symptoms, COPD and restrictive lung disease are shown in the study flow chart (Figure 2-1). The category of 'normal lung function without symptom' represented subjects who had normal lung function and did not have any respiratory symptoms. Forty-eight (40%) subjects were in this category, including 2 subjects with a history of asthma. In this category there were 24 non-smokers, 12 ex-smokers and 12 current smokers, accounting for 55%, 52% and 23% in each smoking category respectively (p = 0.004) (Table 2-1). All potential confounders had p > 0.2 in uni-variate analysis except smoking status. Comparing to non-smokers, current smokers were 0.3 times less likely to be classified as 'normal lung function without symptom', OR = 0.3 (95% CI: 0.1, 0.6) (p = 0.002). The OR of 0.9 (95% CI: 0.3, 2.5) was not significant for former smokers (p = 0.853). Results are listed in Table 2-3 and Figure 2-1.

Discussion

We did not find excessive decline of %FEV$_1$ in HIV-positive subjects compared with published reference ranges for the general population. In our study ten pack-years %FEV$_1$ change was -2.1% (95% CI: -3.6%, -0.6%). In a population-based cross-sectional study, the %FEV$_1$ before salbutamol in 2050 white people decreased by 0.29% (95% CI: -0.33%, -0.25%) for every one pack-years increment [17], which would equal an %FEV$_1$ change of -2.9% (95% CI -3.3%, -2.5%) per ten pack-years. The findings in our study are

comparable to that in the general population. Similar results were reported in previous studies, where HIV-positive subjects had similar loss of lung function as their HIV-negative counterparts [13,14], which did not support the hypothesis that lung function decline is greater in the HIV-positive population.

We should keep in mind that our study was cross-sectional and the effect of smoking we found might not apply to a cohort study [16,29]. In a cross-sectional study, for every one pack-years of smoking increment FEV_1 decreased by 7.4 ml (95% CI: 6.4, 8.4) in a typical male (173 cm tall) and by 4.4 ml (95% CI: 3.2, 5.6) in a typical female (161 cm tall) respectively [16]. While in this same population after 6-year follow up, the longitudinal analysis showed that among smokers, for every one pack/day of cigarette smoking, the rate of FEV_1 decrease was 12.6 ml/year (95% CI: 9.7, 15.5) for men and 7.2 ml/year (95% CI: 4.8, 9.6) for women [29]. Therefore we should not extrapolate the same coefficient of pack-years of smoking found in a cross-sectional study to a prospective cohort study. In other words, we could not predict an HIV-positive smoker would decrease %FEV_1 by 2.1% if s/he continued smoking for another 10 pack-years.

In the multiple regression model for %FEV_1, the coefficient of gender was not significant (p = 0.640), and the point estimate of coefficient of pack-years did not change regardless of adjustment for gender, suggesting that gender did not affect %FEV_1 in this HIV positive population. Similar results were reported in a meta-regression analysis where eight large population-based cross-sectional studies were synthesized: neither gender nor race affected the association of cigarette smoking with lung function

measured by residual FEV_1 (observed - expected value) [18]. However, other population-based studies showed that smoking affected the annual decrease of FEV_1 significantly more in males than in females [16,19,29]. Further study is needed to compare the result in our study to the general population.

Our study found that current smokers had significantly higher odds of cough and sputum than either non-smokers or former smokers, while the difference between non-smokers and former smokers was not significant, after controlling for possible confounders. The findings were consistent with other studies [8,30,31]. Therefore, effective smoking cessation projects would help HIV-positive smokers to have less cough and sputum. Moreover, the prevalence of smoking in our study was 2.4 (95% CI: 2.0, 3.0) times higher than that in Ontario general population in 2007 [2], which reinforced the need for smoking cessation programmes in the HIV-positive population. Fortunately 20 (38%) of current smokers were trying to quit smoking at the time of study. Fifteen (65%) former smokers quit smoking successfully without medication or counselling, implying insufficient involvement of health care providers in terms of helping smokers quit. Further, our study showed an association of smoking with childhood household smoking environment (p = 0.023): current smokers accounted for 7 (24%) of those subjects whose parents did not smoke, 12 (33%) if the father smoked, 6 (60%) if the mother smoked and 27 (63%) if both parents smoked. Therefore, an effective smoking cessation program should target not only current smokers, but also health professionals and families.

Marijuana use was evaluated in our study when the effect of smoking was

estimated. Marijuana use might be associated with respiratory symptoms such as cough and sputum production. We found current marijuana users tended to use more frequently and for longer time than former users, however we did not know how many joints a subject consumed each time. More measurement of cumulative marijuana consumption might be more helpful to further examine the effect of marijuana use more deeply.

Subjects in our study represented the source population at the SIS clinic fairly well. Only five patients refused participating. In a clinical database of our study population (data unpublished), mean (SD) age among 726 active patients was 43.0 (10.5) years old in 2007, males accounted for 68% (95% CI: 65%, 72%), and smoking prevalence was 48% (95% CI: 44%, 53%). Compared to this clinical database, the subjects in our study was comparable in terms of age ($p = 0.718$) and smoking prevalence ($p = 0.365$), however we recruited a slightly greater proportion of males in our study (rate ratio RR = 1.1, 95% CI: 1.0, 1.3). Notably the smoking prevalence of 44% (95% CI: 35%, 53%) in our study was significantly lower than 58% (95% CI: 55%, 61%), the lowest smoking prevalence in OCS over time in 2007 (data unpublished). As OCS was a province-wide study, we considered it the best resource to assess smoking prevalence in Ontario HIV-positive population, although the subjects in OCS might not represent the whole HIV positive population in Ontario due to voluntary participation. Nevertheless the representativeness of our study subjects was limited to our clinic only.

Since we only detected four cases of COPD, we had low power to examine the effect of smoking on COPD. Nevertheless all four COPD cases were smokers, and

smokers had a crude OR of 4.0 (95% CI: 0.9, 18.8) of pre-salbutamol obstructive lung function compared to non-smokers in our study. In a prospective observational study with 867 HIV-positive veterans, either former or current smokers were 5.3 times more likely to develop COPD than non-smokers (95% CI was 1.5 to 18.0 for former smokers and 1.6 to 17.0 for current smokers) [10]. Our study was comparable with these results, albeit underpowered to detect statistically significant difference due to the small number of COPD cases.

We likely would have captured more cases of COPD, if all the subjects with pre-salbutamol obstructive lung function had undergone post-salbutamol testing. According to our preset criteria, all subjects with $\%FEV_1 < 90\%$ should undergo post salbutamol test, which should have included 46 subjects. However only 27 (59%) of these subjects agreed to salbutamol inhalation followed by repeat spirometry, primarily due to time limitations. As a result, amongst 14 subjects whose spirogram suggested COPD by pre-salbutamol test, 9 (64%) subjects did not undergo post-salbutamol test and could not be confirmed. We might expect 5 to 6 more cases of COPD in our study. In a prospective observational study the prevalence of COPD in 1014 HIV positive veterans was 10% by ICD-9 codes and 15% by self report respectively [9]. Comparing this HIV-positive veteran population to our study population, the median age of study population was 50 versus 44.0 years old, the median age of COPD cases was 52 (by ICD-9 codes, 51 by self report) versus 49.5 years old. COPD usually is often diagnosed in patients 50 years or older, and longer follow up will be needed to observe development of additional COPD cases.

Conclusions

In conclusion, we found cigarette smoking affected HIV infected subjects similarly to estimates of its effect in the general population. Cumulative cigarette consumption was associated with worse lung function and higher odds of respiratory symptoms. However the loss of %FEV$_1$ did not accelerate in HIV-positive population compared to the general population. Current smokers were at significant higher odds to present respiratory symptoms compared to non-smokers, but former smokers were at the similar risk compared to non-smokers. Although all four COPD cases had smoked, we could not evaluate the effect of smoking on COPD due to small number of cases. More participants and longer follow up would be needed to estimate the effect of smoking on COPD development. Our study highlighted the importance of smoking cessation in the HIV-positive population in terms of improving lung function and reducing respiratory symptoms, and may prevent the development of COPD.

Competing interests

Qu Cui and Marek Smieja are currently leading an open label study sponsored by the Pfizer company, where we offer Champix to HIV-positive smokers to help them quit smoking and we evaluate the effectiveness, safety and tolerability of Champix in this HIV-positive population.

Authors' contributions

QC wrote study protocol, designed the questionnaire, carried out medical chart review, performed the statistical analysis and drafted the manuscript. SC performed the spirometry test, carried out the questionnaire survey and coordinated the study. AM made substantial contributions to interpretation of data, and was involved in revising the draft critically for important intellectual content. FS made substantial contributions to acquisition of data, and was involved in revising the draft critically for important intellectual content. LT made substantial contributions to analyze data, and was involved in revising the draft critically for important intellectual content. MS conceived of the study, participated in its design, made substantial contributions to acquisition of data, and helped to draft the manuscript. All authors read and approved the final manuscript.

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Acknowledgements

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Tables

Table 3-1. Demographic and baseline information by gender and smoking status (n = 119)

	Gender		Smoking status				Total
	Male	Female	None	Former	Current		
	(n = 94)	(n = 25)	(n = 44)	(n = 23)	(n = 52)		(n = 119)
Male, n (%)	—	—	30 (68)	21 (91)	43 (83)		94 (79)
White, n (%)	83 (88)	13 (52)	28 (64)	19 (83)	49 (94)		96 (81) ***##
Age (years), mean (SD)	44.5 (8.3)	39.2 (7.7)	42.7 (7.7)	46.0 (8.8)	42.8 (8.8)		43.4 (8.4) **
Years of living with HIV, mean (SD)	9.6 (6.7)	6.9 (6.0)	7.3 (5.8)	10.6 (6.9)	9.7 (7.0)		9.0 (6.6)
On HAART, n (%)	86 (92)	14 (56)	35 (80)	22 (96)	43 (83)		100 (84) ***
CD4 (cells/mm^3), mean (SD)	478 (264)	505 (312)	510 (261)	402 (252)	498 (291)		484 (274)
Undetectable viral load, n (%)	62 (66)	11 (44)	25 (57)	17 (74)	31 (60)		73 (61)
Pack-years in smokers [1], mean (SD)	24.0 (17.6)	24.0 (20.7)	—	16.8 (13.9)	27.2 (18.7)		24.0 (18.0) #
Marijuana use							###

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None, n (%)	60 (50)	17 (33)	9 (39)	34 (77)	18 (72)	42 (45)
Former, n (%)	29 (24)	14 (27)	8 (35)	7 (16)	5 (20)	24 (26)
Current, n (%)	30 (25)	21 (40)	6 (26)	3 (7)	2 (8)	28 (30)
FEV_1 before salbutamol (litres), mean (SD)	3.5 (0.8) ***	3.62 (0.79)	3.46 (0.81)	3.48 (0.87)	2.7 (0.5)	3.8 (0.7)
$\%FEV_1$ before salbutamol (%), mean (SD)	93.1 (15.7) *	93.5 (16.1)	91.1 (18.3)	93.6 (14.0)	86.9 (14.1)	94.7 (15.8)
FVC before salbutamol (litres), mean (SD)	4.5 (1.0) ***	4.76 (0.95)	4.58 (0.89)	4.26 (1.09)	3.3 (0.7)	4.9 (0.8)
%FVC before salbutamol (%), mean (SD)	94.5 (13.8) **	97.1 (14.4)	94.6 (11.7)	91.4 (13.7)	88.2 (16.7)	96.2 (12.5)
Abnormal lung function [2], n (%)	24 (20)	12 (23)	4 (17)	8 (18)	8 (32)	16 (17)
Asthma history, n (%)	13 (11) **	7 (14)	3 (13)	3 (7)	7 (29)	6 (7)
Asthmatic by spirometry [3], n (%)	5 (4)	2 (4)	1 (4)	2 (5)	2 (8)	3 (3)
Cough, n (%)	61 (51) ##	37 (71)	8 (35)	16 (36)	12 (48)	49 (52)
Sputum, n (%)	51 (43) ##	32 (63)	8 (35)	11 (25)	10 (40)	41 (44)
Breathlessness, n (%)	8 (7) #	7 (14)	1 (4)	0	2 (8)	6 (6)
Any respiratory symptom [4], n (%)	63 (53) ##	38 (73)	9 (39)	16 (36)	12 (48)	51 (54)
COPD [5], n (%)	4 (3)	2 (4)	2 (9)	0	0	4 (4)

Restrictive lung diseases [6], n (%)	3 (3)	7 (28)	6 (14)	1 (4)	3 (6)	10 (8) **
Abnormal lung function [2] or symptomatic [4], n (%)	17 (68)	54 (57)	20 (45)	11 (48)	40 (77)	71 (60) ##

P value was obtained by Analysis of variance (ANOVA) for continuous variables listed as mean (SD) and was obtained by χ^2 test for categorical variables listed as n (%). Fisher's exact test was adopted if the number of cases in any cell was less than 10. — Not applicable. * $p < 0.05$ by gender. ** $p < 0.01$ by gender. *** $p < 0.001$ by gender. # $p < 0.05$ by smoking status. ## $p < 0.01$ by smoking status. ### $p < 0.001$ by smoking status. [1] Pack-year of smoking was calculated by multiplying the number of packs of cigarette smoked per day and the number of years of smoking. [2] Abnormal lung function was defined as either $FEV_1/FVC <$ 70% or %$FEV_1<$ 80% or %FVC < 80%, either pre- or post-salbutamol test. [3] Asthmatic by spirometry was defined as reversible FEV_1, which improved more than 12% and 200 ml after salbutamol inhalation. [4] Any respiratory symptom was defined as having cough, sputum or breathlessness. [5] COPD referred to chronic obstructive pulmonary disease, was defined as post-salbutamol $FEV_1/FVC <$ 70%. [6] Restrictive lung function was defined as $FEV_1/FVC \geq$ 70% and %FVC < 80%, either before or after salbutamol inhalation.

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Table 3-2. β Coefficients and 95% CIs of each variable versus %FEV$_1$ by model and population

	In all the subjects		In smokers	
	Model 1 [#]	Model 2 [##]	Model 1 [#]	Model 2 [##]
Per 10 pack-years	-0.021 (-0.036, -0.006) [**]	-0.021 (-0.036, -0.006) [**]	-0.020 (-0.042, 0.002)	-0.020 (-0.042, 0.002)
Male	0.017 (-0.054, 0.088)	Not assessed	-0.034 (-0.144, 0.077)	Not assessed
White	0.088 (0.012, 0.163) [*]	0.093 (0.020, 0.165) [*]	0.050 (-0.086, 0.187)	0.052 (-0.083, 0.188)
Restrictive lung diseases	-0.167 (-0.270, -0.065) [**]	-0.174 (-0.272, -0.077) [**]	-0.133 (-0.309, 0.042)	-0.121 (-0.290, 0.049)

All the results were from the multiple linear regression analysis, in which the dependent variable was %FEV$_1$. Pack-year of smoking was calculated by multiplying the number of packs of cigarette smoked per day and the number of years of smoking. Restrictive lung function was defined as FEV$_1$/FVC ≥ 70% and %FVC < 80%, either before or after salbutamol inhalation.

[#] In model 1, the association between %FEV$_1$ and pack-years of smoking was adjusted for by gender, race and restrictive lung diseases. R^2 = 0.210 in all the subjects and R^2 = 0.084 in smokers. [##] In model 2, the association between %FEV$_1$ and pack-years of smoking was adjusted for by race and restrictive lung diseases. R^2 = 0.209 in all the subjects and R^2 = 0.080 in smokers. [*] p<0.05 for β coefficient. [**] p<0.01 for β coefficient.

Table 3-3. ORs and 95% CIs of each variable versus respiratory symptoms, respiratory diseases and subject classification
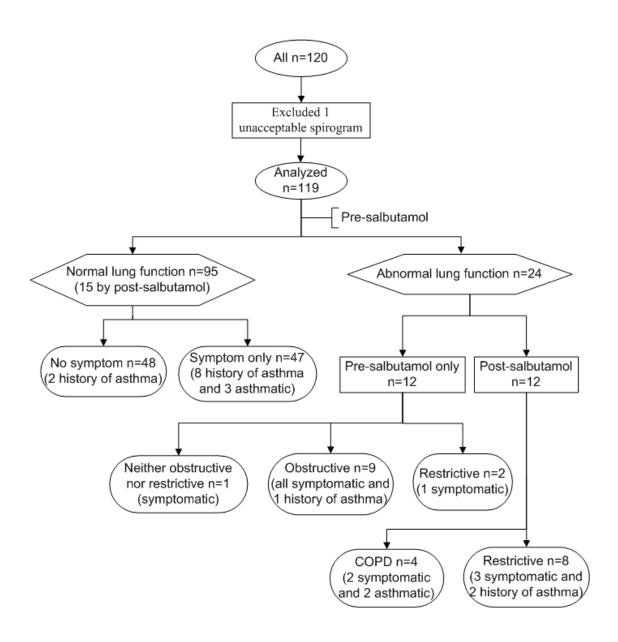
	Cough [a]	Sputum [b]	Any respiratory symptom [c]	Normal lung function without symptom [d]
Former smoker	0.8 (0.3, 2.6)	1.7 (0.5, 5.3)	1.0 (0.3, 2.8)	0.9 (0.3, 2.5)
Current smoker	4.3 (1.5, 12.0) **	5.0 (1.9, 13.3) **	4.9 (2.0, 11.8) ***	0.3 (0.1, 0.6) **
Former marijuana user	0.7 (0.3, 2.1)	0.6 (0.2, 1.7)	—	—
Current marijuana user	1.5 (0.5, 4.6)	1.3 (0.5, 3.5)	—	—
White	1.1 (0.4, 3.5)	—	—	—
On HAART	5.5 (1.4, 21.5) *	—	2.7 (0.9, 8.1)	—
Undetectable viral load	0.3 (0.1, 0.9) *	—	—	—

All the results were from multiple logistic regression analysis. Non-smoker group was the reference group in all the analysis. Reference group for other variables was non-marijuana user, non-white subject, subject who was not on HAART and subject who had detectable viral load respectively.

— The variable had $p > 0.2$ in uni-variate analysis and was not selected into multiple logistic regression analysis.

* $p < 0.05$ for the OR. ** $p < 0.01$ for the OR. *** $p < 0.001$ for the OR.

[a] Subject having no cough was reference group. Nagelkerke $R^2 = 0.252$.

[b] Subject having no sputum was reference group. Nagelkerke $R^2 = 0.177$.

[c] Subject having no respiratory symptom at all was reference group. Respiratory symptom was defined as cough, sputum or breathlessness. Nagelkerke $R^2 = 0.194$.

[d] Subject having either abnormal lung function or any respiratory symptom was reference group. Abnormal lung function was defined as either $FEV_1/FVC < 70\%$ or $\%FEV_1 < 80\%$ or $\%FVC < 80\%$, either pre- or post-salbutamol test. Nagelkerke $R^2 = 0.128$.

Figures

Figure 3-1. Study flow chart and subject classifications

Normal lung function was defined by $FEV_1/FVC \geq 70\%$ and $\%FEV_1 \geq 80\%$ and $\%FVC$ $\geq 80\%$, by both pre- and post-salbutamol tests. Pre-salbutamol values were used to classify normal lung function if post-salbutamol test was not done. Abnormal lung function was defined by either $FEV_1/FVC < 70\%$ or $\%FEV_1 < 80\%$ or $\%FVC < 80\%$, either pre- or post-salbutamol test. Symptomatic was defined as having cough, sputum or breathlessness. Obstructive lung function was classified as pre-salbutamol $FEV_1/FVC <$ 70% without post-salbutamol values. COPD was defined as post-salbutamol FEV_1/FVC $< 70\%$. Restrictive lung function was defined by $FEV_1/FVC \geq 70\%$ and $\%FVC < 80\%$, either before or after salbutamol inhalation. Asthma was defined as reversible FEV_1, which improved more than 12% and 200 ml after salbutamol inhalation.

Section 2:

Thesis studies

Chapter 4:

A multi-centre open label study of smoking cessation

In chapter 3, my co-authors and I evaluated the effectiveness, safety and tolerability of varenicline tartrate (Champix) in HIV-positive smokers. I found that varenicline was as effective and safe as in non-HIV smokers, and the most frequently reported adverse event was nausea.

This manuscript was submitted to AIDS Patient Care and STDs in June 2011 and is currently undergoing peer-review. I have made no changes from the submitted manuscript. The full citation is:

Cui Q, Robinson L, Elston D, Smaill F, Cohen J, Quan C, McFarland N, Thabane L, McIvor A, Zeidler J, Smieja M. Effectiveness, Safety and Tolerability of Varenicline Tartrate (Champix/ Chantix) for Smoking Cessation in HIV-Infected Subjects: An Open-Label Study. Submitted to AIDS Patient Care and STDs, June 2011.

I presented an oral abstract of the preliminary results at the annual conference of the Canadian Association of HIV/AIDS Research (CAHR), Nov. 15-16, 2010, Toronto ON, and at the Department of Clinical Epidemiology & Biostatistics 8th annual research day conference on Mar. 25th 2011, Hamilton ON.

Effectiveness, Safety and Tolerability of Varenicline Tartrate (Champix/ Chantix)

for Smoking Cessation in HIV-Infected Subjects: An Open-Label Study

Running title: Varenicline for smoking cessation in HIV patients

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Abstract

Background

The smoking prevalence in HIV-infected subjects is 2-3 times higher than in the general population. Varenicline (Champix/Chantix) was approved as a smoking cessation aid in Canada in 2007, but has not been previously evaluated in HIV-infected smokers.

Methods

In this multi-centre open label study, varenicline 1.0 mg was used twice daily for 12 weeks with dose titration in the first week. Self-reported abstinence was validated by serum cotinine at week 12. Adverse events (AEs) during the treatment period were recorded. Changes from baseline in daily cigarette consumption, nicotine dependence and withdrawal, laboratory test and vital signs were measured through week 24.

Results

We enrolled 36 subjects with a mean of 29 pack-years of smoking. Serum cotinine-verified four-week continuous abstinence rate (95% CI) through weeks 9-12 was 42% (26%, 58%). Subjects who never quit smoked 5-11 fewer cigarettes/day and had lower Fagerström Test for Nicotine Dependence scores. The most frequently reported AEs were nausea (33%), abnormal dreams (31%), affect lability (19%), and

insomnia (19%). Six (17%) subjects discontinued varenicline due to AEs. No grade 3/4 laboratory abnormalities or serious adverse event occurred during the study. There was no significant change in HIV viral load. CD4 count significantly increased by 69 cells/mm^3 at week 24. Abstinence rates and AEs were generally comparable to those in published randomized controlled trials conducted in generally healthy HIV-negative subjects.

Conclusions

Varenicline was effective and safe amongst HIV-infected smokers, although AEs were common. The most common AE was nausea, with no adverse effect on HIV treatment outcome.

Key words:

HIV, varenicline, smoking cessation, safety, effectiveness, open-label study

Background

With modern anti-retroviral therapies (ART), there is a greatly increased chance of long term survival and a decreased chance of opportunistic infections in HIV-infected persons [1,2]. The majority of HIV deaths in developed countries are now caused by cancer and by heart, lung and liver disease [3,4]. Smoking is the most common and important modifiable risk factor for these diseases. Smoking prevalence in HIV-infected Canadians is 30-70% [3,5], compared with less than 20% in the general Canadian adult population [6]. A recent study showed that the incidence rate ratios of cardiovascular diseases decreased in HIV-infected former smokers who quit smoking during study, compared to HIV-infected non-smokers [7]. After appropriate initiation of ART, smoking cessation may be the single most important intervention for improving long-term survival and quality of life in HIV-infected people. However, there is limited data regarding the efficacy of smoking cessation in HIV-infected people.

In February 2007, Health Canada approved varenicline tartrate (Champix in Canada and many countries, or Chantix in the United States) as a new smoking cessation aid in adults, to be used in conjunction with smoking cessation counselling. Varenicline acts as a partial agonist at $\alpha 4 \beta 2$ nicotinic acetylcholine receptors in the brain, reducing nicotine craving. A previous review showed that varenicline is more effective than bupropion (Zyban), nicotine replacement therapy, or placebo [8]. The most frequently reported adverse event (AE) of varenicline was nausea, which was generally mild or

moderate. Dose-titration over the first week helps minimize nausea. As a new promising smoking cessation aid, varenicline had not yet been tested in HIV-infected subjects prior to our study.

The study protocol was approved by Hospital Research Ethics Boards (REBs) at the Hamilton Health Sciences, Hamilton, Ontario, Canada and at Windsor Regional Hospital, Windsor, Ontario, Canada. Health Canada issued 'No objection letter'. All participants gave written consent.

Methods

Study design, setting and participants

We designed a multi-centre open-label study, conducted at two regional HIV clinics in Hamilton and Windsor, Ontario, Canada between October 2008 and July 2010. Subjects satisfied all of the following five inclusion criteria: 1) Age 19-64 years; 2) Smokes one or more cigarettes per day; 3) Has attempted to quit at least once previously; 4) No abstinence period of greater than 3 months during the past year; and 5) Weight 45-125 kg. Subjects who had any one of the following seven conditions were excluded: 1) Pregnant or nursing women; 2) Allergy to varenicline; 3) Cancer or transplant; 4) Epilepsy, on anti-epileptic drugs, on anti-depressants, or anti-psychotic drugs; 5) Significant cardiovascular disease, uncontrolled hypertension, or cataract; 6) Nausea, irritable bowel or other significant gastrointestinal symptoms; or 7) Currently on nicotine replacement therapy. In addition, if the subject was not on ART, a CD4 T-lymphocyte

count of at least 350 cells/mm^3 was required, and subjects in whom the physician was planning to start ART within the next 6 months were excluded. If the subject was on ART, a CD4 T-lymphocyte count of over 200 cells/mm^3 was required, with undetectable viral load (less than 50 copies/ml) for the past 6 months, and he/ she should be judged by the physician to not require a change in anti-retroviral therapy in the next 6 months. Potential eligible subjects were referred to research staff by their physician or were approached by our trained research staff during routine clinical visits. After consenting, all the subjects underwent an electrocardiography to rule out signs of past cardiovascular disease.

Study intervention and follow up

We titrated the dosage of varenicline (Saint-Laurent, QC, Canada) in the first week: 0.5 mg once daily for days 1-3, then 0.5 mg twice daily for days 4-7, followed by 1.0 mg twice daily from day 8 until week 12. We offered each subject a package of reading materials, including educational materials from the Canadian Cancer Society (Ontario), Hamilton Public Health Services and the Ontario Lung Association [9-16]. Counselling was delivered to the subject by a physician or a trained counsellor, in accordance with a U.S. clinical practice guideline on treating tobacco use and dependence [17]. A target quit date was set 8-14 days after starting varenicline.

We followed subjects weekly during the 12-week treatment period and biweekly during the 12-week non-treatment period in Hamilton. In Windsor, we followed subjects weekly during the first 4 weeks, then at weeks 8, 12, 16 and 24. At both sites, four

clinical visits were scheduled at baseline, weeks 4, 12 and 24 when self-administered questionnaire, physical exam and blood work were obtained. Fifteen (in Hamilton) or five (in Windsor) follow up visits were scheduled over the 24 week study by telephone, e-mail or personal interview, to collect information on smoking and AEs and to deliver brief counselling. Subjects who discontinued varenicline before week 12 were still followed according to the original schedule, or information was collected by medical chart review if they declined further participation.

Measurement of effectiveness, nicotine dependence and withdrawal

Effectiveness was measured by the serum cotinine-verified four-week continuous abstinence rate (4W-CAR) from weeks 9-12, which was defined as the proportion of subjects who remained abstinent from smoking for the consecutive 4-week period from weeks 9 through 12. Abstinence from smoking was defined as no smoking, not even a puff, for at least seven consecutive days prior to each follow up. Self-reported abstinence at week 12 was validated by serum cotinine concentrations below 3 ng/ml [18]. Cotinine was quantified by liquid chromatography-mass spectrometry [19].

Secondary effectiveness estimates included: serum cotinine-verified continuous abstinence rate (CAR) through weeks 9-24; self-reported seven-day point prevalence of abstinence (7D-PP) at each follow up; changes in daily cigarette consumption; changes in Fagerström Test for Nicotine Dependence (FTND) score [20-22] and in Minnesota Nicotine Withdrawal Scale (MNWS) score [23-25]. The FTND questionnaire was self-administrated at baseline for all subjects and at subsequent clinical visits for subjects

who still smoked. FTND scores of 0-2 indicate very low nicotine dependence, 3-4 low, 5 medium, 6-7 high, and 8-10 very high [22]. The MNWS questionnaire was self-administered at each clinical visit for all the subjects, measuring five sub-domains of nicotine withdrawal symptoms: negative affect (including depressed mood, irritability /frustration /anger, anxiety /nervousness and difficulty concentrating), with sub-score ranging 0-16; insomnia 0-8; craving 0-4; restlessness 0-4 and increased appetite 0-4. In each sub-domain, higher scores indicated more severe nicotine withdrawal symptoms [25].

Safety assessment

Adverse events were collected through regular follow up and patient report. We asked about AEs starting with a defined open-ended question: "Since the last follow up, have you had any new symptoms?" We then assessed AE severity, duration, date of onset, action taken, and the suspected relationship to varenicline [26-28]. All the AEs were self-reported. An SAE was judged by the subject's physician and by the principal investigator.

Blood work was measured at each clinical visit, including: complete blood count, creatinine, aspartate aminotransferase (AST), alanine aminotransferase (ALT), CD4-T-lymphocyte count, and plasma HIV viral load (Chiron 3.0 bDNA assay). Abnormalities in laboratory results were graded according to the Division of AIDS (DAIDS) adverse events scale [29]. In addition, for subjects with elevated AST or ALT at baseline, grading was based on changes relative to baseline rather than upper limit of normal. Grade numbers 1-4 represent mild, moderate, severe, and potentially life-threatening

abnormality respectively [29,30].

Weight and blood pressure were measured at baseline, weeks 12 and 24.

Statistical analysis

This study was an exploratory investigation using a pre-post design. The sample size of 36 was determined based on feasibility considerations. Demographic and baseline characteristics were summarized using descriptive statistics reported as mean (standard deviation [SD]) for continuous variables and count (percent) for categorical variables. Proportions were calculated as the number of events divided by the total number of subjects, and expressed with 95% confidence intervals (CIs). The change from baseline to follow-up visit was reported as mean (standard error [SE]) and analyzed by general linear models allowing for repeated measures. Repeated measured proportions were analyzed using generalized estimating equations. Missing data was replaced by the data from last follow-up visit. All statistical tests were 2-sided with alpha of 0.05. We did not adjust the overall level of significance for multiple testing as the analyses were primarily exploratory. Effect size was calculated by the change divided by its standard deviation. We considered an effect size of 0.2 to 0.3 to be small, 0.5 to be medium, and 0.8 to be large [31]. We used PASW Statistics 18 (Chicago, IL) to analyze data.

In addition, we found randomized controlled trials (RCTs) conducted in HIV-negative generally healthy smokers from the published literature, identified RCTs with at least one treatment arm using varenicline at the same dosage and duration as in our study, and compared our results to the pooled published results. Pooled estimation from

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external comparison studies was weighted by the variance in each study. We used one sample *t*-tests to compare the results in our study to pooled estimates.

Results

Demographic and baseline information

We recruited 36 subjects, including 20 subjects from Hamilton and 16 from Windsor (Table 4-1). Subjects were similar between the two sites, with mean (SD) age of 46 (8) years old. The majorities were male, white, on ART and had viral load less than 50 copies/ml. Mean (SD) CD4 T-lymphocyte count was 601 (291) cells/mm^3. Subjects had smoked an average of 29 (22) pack-years, with 23 (64%) having medium, high or very high levels of nicotine dependence by FTND score. Thirty (83%) subjects had previously quit smoking for more than seven days with a mean (SD) of 2.3 (2.5) previous quit attempts.

Effectiveness

Continuous Abstinence Rates (CARs) and seven-day point prevalence (7D-PP) of abstinence

Serum cotinine-confirmed 4W-CAR (95% CI) through weeks 9-12 was 42% (26%, 58%), and CAR through weeks 9-24 was 28% (13%, 42%) (Table 4-2). We found seven published RCTs conducted in HIV-negative generally healthy smokers, serving as our external comparison studies [32-38]. Both abstinence rates were comparable to those in

external studies (Table 4-2).

The self-reported 7D-PP increased markedly to 39% in the first three weeks, was 50% at week 12 and 42% at the end of study at week 24 (Figure 4-1). Twenty (56%) subjects quit for at least 7 days at least once during the 12-week treatment period and 23 (64%) subjects did so during the entire 24-week study.

Effect on daily cigarette consumption, FTND and MNWS score

Seventeen (47%) subjects never quit, although they cut down significantly, with mean (SE) decrease of -11 (2), -7 (2) and -5 (2) cigarettes/day at weeks 4, 12 and 24 respectively (Table 4-3). Effect size of treatment on daily cigarette consumption was large at weeks 4 and 12, and was medium at week 24 [31]. Those continuous smokers also had significantly decreased FTND scores with a large effect at each visit respectively. On the other hand, 10 (28%) continuous smokers had medium, high or very high FTND scores at baseline, while the proportion significantly decreased to 2 (6%) at week 4 (p < 0.001) and to 4 (11%) at week 24 (p = 0.002).

The changes in MNWS sub-scores of negative affect, restlessness and increased appetite were not significantly different (Table 4-3). For insomnia, the change was significant by week 12 only, with a small effect size. For craving, the sub-score significantly decreased with large effect size at each clinical visit.

Safety and tolerability

Incidence and severity of AEs, subjects' responses to AEs, and discontinuation of varenicline

Twenty-eight (78%) subjects reported AEs during the treatment period (Table 4-4). The most frequently reported AE was nausea, reported by one-third of subjects including 2 (6%) who reported it as severe; followed by abnormal dreams (31%); affect lability (irritability, mood swing, agitation or anger) (19%) and insomnia (19%). Other AEs were generally reported as trivial, mild, moderate or marked. The incidence rate of abnormal dreams was 29% (5/17) in those on efavirenz versus 32% (6/19) in those not on efavirenz (p = 1.000). No SAEs occurred during the drug treatment period. A 61 year old white man, who had smoked for 45 years at 10 cigarettes a day, was diagnosed with lung cancer at week 22. Because of the long latency of lung cancer, we did not consider this event to be related to varenicline.

Four (11.1%) subjects decreased their dose of varenicline because of AEs during the treatment period, including 3 (8%) subjects with nausea. Only one subject completed the entire 12-week treatment at the decreased dose, whereas the remaining three subjects discontinued varenicline before week 12. Nine (25%) subjects took medications for AEs during the treatment period, and one (3%) visited his doctor for pneumonia as well as for a sleeping disorder. No subject was hospitalized because of AEs.

In total, ten (28%) subjects discontinued varenicline before week 12, and 6 (17%) of these discontinuations were due to AEs, including 4 (11%) cases of nausea, 1 (3%) of

anger and 1 (3%) of insomnia. Of these ten subjects, 3 (8%) withdrew from the study.

Incidence rates in overall AE and nausea in our study were comparable to those in published RCTs conducted in HIV-negative smokers (Table 4-2), however we had a significantly higher incidence rate of abnormal dreams (31% vs 9%, p < 0.001) and discontinuation rate (28% vs 13%, p = 0.011).

CD4-positive T-lymphocyte count and plasma HIV viral load

CD4-positive T-lymphocyte counts increased significantly at week 24 by a mean (SE) of 69 (20) cells/mm^3 (p = 0.001), with a medium effect size of 0.58 (Table 4-3). The change was neither associated with study site, age, race, HIV infection category, smoking status or discontinuation of varenicline before week 12, nor with suppressed viral load or ART use.

All twenty-nine (80.6%) subjects with suppressed viral load at baseline were still undetectable at each clinical visit. In addition, one subject started ART after completing 12-week varenicline treatment and had undetectable viral load at week 24. Log$_{10}$ HIV viral load did not vary for six subjects who always had an detectable HIV viral load.

Other laboratory tests

The changes in white blood cell (WBC) count, platelet count and AST were not significantly different during the study. For haemoglobin and creatinine, although the changes were significantly different at some visits, the changes were considered within normal range at all visits. Mean (SD) baseline ALT was 32 (20) IU/L, the mean (SE) change was significantly different at week 12 by 10 (4) (p = 0.011) and at week 24 by 8

(3) IU/L (p = 0.004) respectively. All the ALT elevations were grade 1 at all follow up visits: mild elevation in 2 (6%) subjects at week 4, in 6 (17%) subjects at week 12 and in 7 (19%) subjects at week 24 respectively, versus at baseline when 4 (11%) subjects had mild and 1 (3%) had moderate ALT elevations.

Blood pressure and weight changes

Baseline mean (SD) blood pressure was 123 (15) / 76 (9) mmHg. Systolic blood pressure did not change during the study, whereas diastolic blood pressure increased significantly by a mean (SE) of 6 (1) mmHg (p < 0.001) at week 12 and 4 (2) (p = 0.049) at week 24 respectively. Eight (22%) subjects had elevated diastolic blood pressure resulting in hypertension, including 6 (17%) at week 12 and 4 (11%) at week 24. No grade 3/4 abnormality in diastolic blood pressure occurred.

Subjects gained a significant amount of weight by a mean (SE) of 1.7 (0.6) kg at week 12 (p = 0.007) and 2.8 (0.7) kg at week 24 (p < 0.001). This represented a medium effect size (0.48) at week 12 and a large effect size (0.72) at week 24. Weight changes were not associated with study site, or smoking status.

Discussion

In this study, we found that stable HIV-positive subjects were able to take varenicline for 12 weeks, with temporary smoking cessation in over 50% of subjects and 12-week laboratory-verified smoking cessation in 40%. Side effects were common but generally tolerable, and varenicline had no adverse effects on HIV control. Indeed,

CD4-T-lymphocyte counts improved during the study.

Our study has important limitations. We had a small sample size in this open label, non-randomized study. Thus, we can provide estimates of efficacy and of side effects, but with fairly wide confidence intervals. We studied only stable subjects, who were not on antidepressant or antipsychotic medication, with a high mean (SD) baseline CD4 count of 601 (291) cells/mm^3, and 81% of our subjects had undetectable viral load. Thus, we cannot generalize to acutely ill or to more severely immunocompromised HIV patients, or those on treatment for mental illness, in whom side effects or efficacy could be different. We only verified self-reported smoking status at week 12, and could have slightly over-estimated the effectiveness of varenicline. However, we classified all invalidated subjects as smokers when we calculated 4W-CAR through weeks 9-12 and CAR through weeks 9-24. Therefore this possible over-estimation was minimized.

We had no direct comparison in our study; however, we compared our results with that in HIV-negative smokers from the published RCTs and found our rates comparable for efficacy, but perhaps increased for certain side-effects. The serum cotinine-verified 4W-CAR through weeks 9-12 in our study was similar to the pooled estimate in the literature, as well as the verified CAR through weeks 9-24. The comparable efficacy of varenicline in our study is also supported by the significantly decreased daily cigarette consumption, FTND scores and MNWS craving sub-scores in our study subjects and in the literature [37,38].

In addition to estimating efficacy, we designed our study to examine the safety

and tolerability of varenicline in the context of HIV infection. Our results are comparable to the seven non-HIV RCTs in overall incidence rates of any adverse events, and of nausea. However, the incidence rate of abnormal dreams was significantly higher in our study, and our discontinuation rate was also somewhat higher.

While side effects were common, they were not necessarily bothersome. AEs such as weight gain or abnormal dreams were desirable to some subjects. Conversely, HIV-positive subjects are often questioned regarding abnormal dreams as part of their routine HIV care since certain commonly-used HIV medications such as efavirenz frequently cause abnormal dreams. Our study subjects might be more aware of this side effect compared to HIV-negative subjects, and HIV-infected smokers as a whole might report more abnormal dreams than their HIV-negative counterparts, reflecting potential ascertainment bias. However, in HIV-infected smokers, we found no association between self-reported abnormal dreams and the use of efavirenz, indicating that varenicline itself is likely the cause of abnormal dreams in HIV-positive subjects. The higher incidence of reported abnormal dreams and the higher discontinuation rate in our study suggest precautions for using varenicline in HIV-infected smokers, and questioning about these side effects at follow-up visits. In addition, a most recent published meta-analysis including 14 RCTs conducted in non-HIV smokers showed varenicline was associated with an elevated OR of 1.72 (95% CI: 1.09 to 2.71) for any ischemic or arrhythmic cardiovascular event [39]. Given the existing concerns of anti-retroviral therapy related cardiovascular events [40-42], precautions and close monitor of

serious cardiovascular AEs are needed in HIV-infected smokers using varenicline.

Changes on laboratory tests and blood pressure verified that varenicline generally was safe, without significant changes or grade 3/4 abnormalities during the study in haemoglobin, WBC, platelet, creatinine, AST and systolic blood pressure. However we found mild ALT elevations during the study. We also detected small but statistically significant changes in diastolic blood pressure, which were not associated with weight change. On the other hand, in three external comparison studies we used, only one case of ALT elevation was reported respectively [32,34,35], and no significant change in blood pressure was reported in any external comparison study. Although inconclusive, due to limitations in our study, our results suggest that HIV-positive patients taking varenicline may benefit from monitoring of their liver enzymes and blood pressure.

We detected no adverse effect of varenicline on HIV control. Viral load did not change. We found a statistically significant and clinically important improvement in CD4 T-lymphocyte counts by week 24 of follow up. By comparison, a previous study showed that CD4 count significantly increased by 93-151 cells/mm^3 within 6 months after highly active antiretroviral therapy initiation, then slowed down to 22-36 cells/mm^3/year through the first four years, and then levelled off [43]. In this context, the CD4 increase in our study (69 cells/mm^3 in 24 weeks) is potentially important, and was not associated with either smoking status or with use of ART. However, as this was not an anticipated finding, it could be due to chance and must be interpreted with caution. Nevertheless, larger cohort studies should examine the long-term effects of varenicline

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and of smoking cessation on CD4 lymphocyte counts, as an immunological benefit would argue for an even greater emphasis on smoking cessation in HIV care.

In summary, varenicline efficacy in HIV-infected smokers was comparable to the rates amongst HIV-negative smokers from previously published trials, with verified 12-week quit rates of 42%. Furthermore, even amongst subjects who never quit smoking, nicotine dependence and craving scores decreased significantly, as did their daily cigarette consumption. Varenicline was as safe in HIV-infected smokers as in HIV-negative smokers, although AEs were common and occasionally resulted in drug cessation. Monitoring of side effects such as nausea, abnormal dreams and cardiovascular AEs, clinical parameters such as blood pressure and weight, are recommended until there is a greater experience with varenicline in HIV-infected patients.

We conclude that varenicline is an effective adjunct for smoking cessation amongst HIV-infected patients, together with appropriate counselling, and encourage further studies to improve smoking cessation in this vulnerable population.

Acknowledgements

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Tables

Table 4-1. Demographic and Baseline Information

	Total n = 36	Hamilton n = 20	Windsor n = 16
Age, mean (SD) years	46 (8)	46 (7)	46.4 (9)
Male, n (%)	35 (97)	20 (100)	15 (94)
White, n (%)	33 (92)	19 (95)	14 (88)
Years living with HIV, mean (SD)	11 (7)	11 (7)	12 (7)
ART use, n (%)	31 (86)	15 (75)	16 (100)
CD4, mean (SD) cells/mm^3	601 (291)	605 (295)	595 (297)
Suppressed viral load, n (%)	29 (81)	14 (70)	15 (94)
Log$_{10}$ viral load if detectable, mean (SD)	3.92 (0.75)	3.85 (0.80)	4.31 (-)
Cigarettes per day, mean (SD)	19 (10)	17.5 (8)	20 (13)
Number of years smoked, mean (SD)	29 (8)	28 (7)	31 (10)
Pack-years of smoking, mean (SD)	29 (22)	25 (14)	34 (29)
FTND score, mean (SD)	5.4 (2.2)	5.7 (1.8)	5.1 (2.6)
Medium/ high/ very high FTND score, n (%)	23 (64)	14 (70)	9 (56)

Previous quit attempts of more than 7 days, mean (SD)	2.3 (2.5)	2.9 (2.5)	1.7 (2.3)

Proportions were calculated within each column. P values were obtained by one way ANOVA for continuous variables and by chi-square test for categorical variables. Fisher's exact test was used if the number in any cell was less than 10. All p values were more than 0.05.

ART, anti-retroviral therapy. Suppressed viral load, viral load less than 50 copies/ml. FTND, Fagerström Test for Nicotine Dependence.

Table 4-2. Comparison of Varenicline Efficacy and Adverse Events % (95% CI) Between the Current and Previously Published Studies

Study	Total n	4W-CAR weeks 9-12	CAR weeks 9-24	Any AE	Nausea	Abnormal dreams	Discontinuation
Current study	36	42 (26, 58)	28 (13, 42)	78 (64, 91)	33 (18, 49)	31 (16, 46)	28 (13, 42)
Wang et al, 2009 [32]	165	50 (42, 58)	38 (31, 46)	77 (71, 83)	29 (22, 36)	4 (1, 7)	3 (0, 6)
Aubin et al, 2008 [33]	376	56 (51, 61)	32 (28, 37)	85 (81, 88)	37 (32, 42)	12 (8, 15)	17 (13, 21)
Nakamura et al, 2007 [34]	156	65 (57, 74)	38 (29, 46)	80 (74, 86)	24 (18, 31)	-	8 (4, 13)
Tsai et al, 2007 [35]	126	60 (51, 68)	47 (38, 56)	87 (81, 92)	44 (35, 52)	6 (2, 10)	-
Oncken et al, 2006 [36]	130	55 (46, 63)	-	79 (72, 86)	35 (27, 43)	19 (13, 26)	23 (16, 31)
Jorenby et al, 2006 [37]	344	44 (39, 49)	30 (25, 34)	-	29 (25, 34)	13 (10, 17)	24 (20, 29)
Gonzales et al, 2006 [38]	352	44 (39, 49)	30 (25, 34)	79 (75, 83)	28 (23, 33)	10 (7, 14)	26 (21, 30)
Pooled study [†]		51 (49, 54)	33 (31, 36)	82 (80, 84)	32 (29, 34)	9 (8, 11)	13 (11, 14)
p-value		0.248	0.495	0.545	0.819	< 0.001	0.011

†Pooled study: studies 31-37, all of them were conducted in non-HIV smokers, and only the arm using varenicline 1.0 mg

twice daily for 12 weeks was included. Estimations in pooled study were weighted by the variance in each study. p-values

were calculated by one sample *t*-test, where the proportions in our study were compared to that in pooled study.

4W-CAR, four-week continuous abstinence rate. CAR, continuous abstinence rate. AE, adverse event.

Table 4-3. Effects on Daily Cigarette Consumption, Fagerström Test for Nicotine Dependence (FTND) Score, Minnesota Nicotine Withdrawal Scale (MNWS) and Immune System

Variable	Baseline, mean (SD)	Change [†], mean (SE)			Effect size [‡]		
		Week 4	Week 12	Week 24	Week 4	Week 12	Week 24
Cigarettes per day, n = 17	19 (11)	-11 (2) [***]	-7 (2) [**]	-5 (2) [*]	-1.37	-0.84	-0.57
FTDN score, n = 17	5.5 (2.3)	-3.1 (0.6) [***]	-1.6 (0.5) [**]	-2.1 (0.6) [**]	-1.23	-0.74	-0.90
MNWS, n = 36							
Negative affect	3.4 (3.3)	-0.4 (0.6)	-0.5 (0.7)	0.1 (0.7)	-0.12	-0.13	0.03
Restlessness	1.2 (1.2)	-0.1 (0.2)	-0.2 (0.2)	0 (0.2)	-0.09	-0.12	0.02
Insomnia	3.1 (2.7)	-0.2 (0.5)	-0.9 (0.4) [*]	0.4 (0.5)	-0.06	-0.36	0.12
Increased appetite	0.9 (1.2)	0.5 (0.2) [*]	0.25 (0.2)	0.2 (0.3)	0.36	0.20	0.13
Craving	2.6 (1.0)	-1.1 (0.2) [***]	-1.2 (0.3) [***]	-1.1 (0.2) [***]	-0.90	-0.78	-0.88

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CD4 count, cells/mm^3	601 (291)	-2 (22)	20 (23)	69 (20)**	-0.02	0.15	0.58
Log$_{10}$ viral load if detectable, n = 6	3.79 (0.73)	-0.20 (0.17)	-0.39 (0.25)	-0.64 (0.42)	-0.47	-0.64	-0.62

Cigarettes per day and FTDN score are measured in subjects who never quit during study only. † Change, calculated by comparing to baseline. ‡ Effect size, the change divided by its standard deviation: 0.2 to 0.3 to be small, 0.5 medium, and 0.8 large. FTND scores of 0-2 indicate very low nicotine dependence, 3-4 low, 5 medium, 6-7 high, and 8-10 very high. MNWS negative affect, includes depressed mood, irritability/ frustration/ anger, anxiety/ nervousness, and difficulty concentrating, with sub-score ranging 0-16; Restlessness 0-4; Insomnia, difficulty going to sleep and difficulty staying asleep, 0-8; Increased appetite, increased appetite or weight gain, 0-4; Craving, urge to smoke, 0-4. In each sub-domain, higher scores indicated more severe nicotine withdrawal symptoms. All the p values were obtained by general linear model for repeated measures, compared to baseline. * $p < 0.05$. ** $p < 0.01$. *** $p < 0.001$.

FTND, Fagerström Test for Nicotine Dependence. MNWS, Minnesota Nicotine Withdrawal Scale.

Table 4-4. Adverse Events (AEs) During Treatment (n = 36)

	Total reported, n (%)	Severe or intolerable, n (%)
Any AEs	28 (78)	12 (33)
Nausea	12 (33)	2 (6)
Abnormal dreams	11 (31)	2 (6)
Affect lability [†]	7 (19)	3 (8)
Insomnia	7 (19)	2 (6)
Headache	5 (14)	2 (6)
Gastroesophageal reflux disease	4 (11)	2 (6)
Abdominal pain	4 (11)	1 (3)
Nightmares	3 (8)	2 (6)
Sleep disorder	3 (8)	1 (3)
Weight gain	3 (8)	1 (3)
Vomiting	3 (8)	0
Constipation	3 (8)	0
Taste perversion	3 (8)	0
Fatigue	3 (8)	0
Depressed mood	2 (6)	1 (3)
Smell perversion	2 (6)	1 (3)
Gas	2 (6)	0
Sweat	2 (6)	0
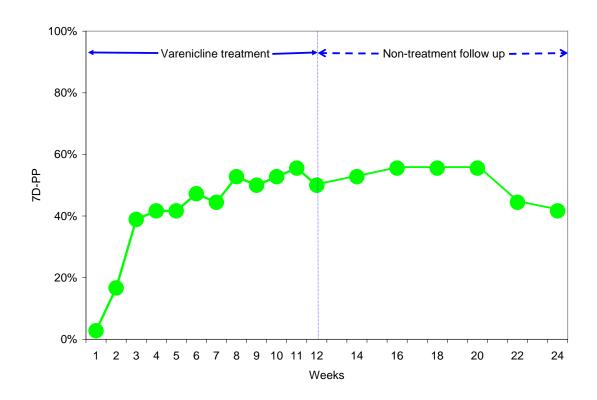
Increased appetite	1 (3)	1 (3)
Dry mouth	1 (3)	0
Dizziness	1 (3)	0
Hot flush	1 (3)	0
Muscle pain	1 (3)	0

[†] Affect lability, included irritability, mood swing, agitation and anger.

Figures

Figure 4-1. Self-reported Seven-day Point Prevalence (7D-PP) of Abstinence at Each Follow Up

Self-reported seven-day point prevalence of abstinence (7D-PP) at each follow up was

defined as the number of subjects who self-reported to be continuously abstinent from

smoking for the seven days preceding follow up divided by the total number of subjects

who participated in the study at baseline. Weekly follow up was taken during 12-week

varenicline treatment period, and biweekly follow up was taken during non-treatment

period until week 24.

Section 3:

Conclusions

Chapter 5:

Conclusions and future studies

Summary

In this concluding section, I summarize the previous chapters, discuss limitations of the three thesis studies, explore the role of smoking cessation in HIV prevention and care, and finish the thesis with a discussion of future studies.

Section 1: Introduction

Two in 1,000 Canadians are HIV-infected, and HIV prevalence in Ontario is disproportionally high compared with other provinces. One of the goals of this thesis was to identify special needs in this vulnerable population. Smoking is a distinct issue in HIV-positive people living in the anti-retroviral therapy (ART) era for three reasons: 1) HIV-infected people are living longer and chronic diseases are increasingly of concern; 2) Smoking prevalence is high in HIV-infected subjects; and 3) An elevated risk of death and smoking-related respiratory and cardiovascular diseases in smokers has been

identified. Although several studies have examined the role of smoking in terms of mortality, hospitalization, cardiovascular and respiratory diseases in HIV-infected people in the ART era [1-11], there is insufficient information on two aspects: 1) inconclusive or scarce studies examining the effect of smoking on lung function and immune function; and 2) limited Canadian data.

Smoking cessation is an urgent need for HIV-infected smokers and pharmacotherapy is an important component of smoking cessation. No study has tested varenicline, a promising smoking cessation aid, in HIV-positive smokers.

Methodological issues

I thoroughly discussed the issue of missing smoking data in OCS (Chapter 2), and explored three distinct methods to handle missingness, highlighting the weakness and strengths of each method. Specifically, I reviewed assumptions, theory and procedures for multiple imputation, and proposed different strategies to handle missing data in my thesis studies, based on the nature of missingness for data in this study. I described a validation study performed to address potential concerns regarding the quality of data in OCS, arising from the missing data issue. Correlated variables raise another methodological challenge. My approaches for dealing with correlated variable included either avoiding it, or finding an appropriate method, such as general linear models and generalized estimating equations, to accommodate repeated observations.

Section 2: Thesis studies

In Chapter 2, I described a retrospective cohort study, a 13-year province-wide secondary data analysis involving 3,211 adult HIV-positive volunteers. I demonstrated the consistent 2-3 times higher smoking prevalence in HIV-positive Ontarians between 1999 and 2007, compared to non-HIV Ontarians, despite an overall similar decreasing trend. By comparing smokers to non-smokers in my study, I found a 3 times higher mortality rate, 2 times higher IR of COPD and slightly higher IR of hospitalization in smokers. I also found smokers had a smaller increase in annual CD4 count over the years of follow-up, as compared with never smokers. My study was complicated by a large amount of missing smoking data in this OCS analysis, and I used three distinct methods to test the impact of missing data by excluding, grouping or multiply imputing missing data. My results showed multiple imputation yielded robust and more precise results, although all three methods showed comparable results for the association between smoking and death. I used a range of strategies to deal with missing data in this study, in addition to multiple imputation. I am aware that some strategies I used might be problematic, as I have discussed in Chapter 1, such as LOCF. In OCS, complete-case analysis showed amongst 480 non-smokers 11 (2%) started smoking during the study period, 18% (51/277) of former smokers relapsed, and 13% (142/1,124) of current smokers quit, suggesting the annual smoking prevalence might be biased, if I missed the status of change by using LOCF.

Chapter 3 described a cross-sectional study of respiratory symptoms and lung

function conducted in the SIS clinic at McMaster University Medical Centre involving 120 adult HIV-positive volunteers. I found increased cumulative cigarette consumption was associated with reduced lung function: $\%FEV_1$ was reduced by 2.1% for every 10 pack-years of smoking. I also found that current smokers had a 4-5 times higher risk of respiratory symptoms (cough, sputum and breathlessness) compared to non-smokers, while former smokers reported similar respiratory symptoms as non-smokers. I diagnosed 4 cases of COPD according to GOLD guidelines, all of whom were smokers. Notably COPD was likely underdiagnosed in this study: while four subjects were identified by pre- and post-salbutamol spirometry, another nine potential COPD subjects with abnormal pre-salbutamol testing declined post salbutamol testing.

In Chapter 4, I described a multi-centre open label study using varenicline (Champix) as a smoking cessation aid, involving 36 HIV-infected smokers. I found varenicline was as effective in our study as in published non-HIV studies, with comparable 4W-CAR through weeks 9-12 of 42% versus 51%. I also found comparable overall IR of adverse events, at 78% in my study, versus 82% in previous RCTs. The most common AE was nausea, as in generally healthy HIV-negative smokers, with comparable IR of 33% versus 32% in the literature. Varenicline did not adversely affect HIV treatment outcome, and all viral load suppressors were still undetectable throughout the study. Furthermore, study subjects experienced an improvement in CD4-T-lymphocyte count by 69 cells/μl at week 24. Representativeness is a major concern in this study. With small number of sample size and highly selective subjects, results from the

study may not be generalizable.

Putting these thesis studies into context, I can draw a picture of HIV-infected Ontarians and their smoking-related health. They are dominated by White middle-aged males, most of them with a post-secondary education. Approximately 50-60% HIV-infected people smoke, half of subjects use marijuana, and one in seven uses injection drugs. Smokers are more likely to use marijuana and other drugs. Smoking prevalence has been decreasing over the years, but remains very high. Mortality rate is decreasing, but smokers have a 3-fold higher risk of death. Lung function decreased with increased cigarette consumption, as in the general population. Approximately 13 or more subjects are diagnosed with COPD per 1,000 person-years, and smokers have double the risk of developing COPD. Smokers had a poorer improvement in CD4 counts over the years of follow-up and were more likely to be hospitalized. Smokers are likely to have a higher risk of developing lung cancer, TB, PVD and stroke, although these were not statistically significant in my study. Given this picture, the need for smoking cessation is urgent. HIV-infected smokers who use varenicline to help quitting smoking generally do well, although the majority experienced adverse events, mainly nausea. In summary, these studies show that cigarette smoking may be a profound health problem in HIV-infected people, and smoking cessation is feasible.

Limitations of thesis studies and future studies

These thesis studies have limitations. No study had a non-HIV control group,

therefore I am not able to assess HIV infection itself as a potential risk factor for those smoking-related outcomes. Although I used external non-HIV comparisons from the literature, the heterogeneity in different study populations in different studies might bias the results. Future studies may consider including contemporaneous non-HIV subjects. Similarly, in our smoking cessation study, as I did not have a placebo group, I was not able to ascertain whether the observed CD4 improvement of 69 cells/μl in 24 weeks was related to varenicline. Furthermore, due to the limited follow up period, I was not able to assess whether the increasing trend continues beyond 24 weeks. In other words, if I had been able to prove that taking varenicline was associated with an increased CD4 lymphocyte counts, smoking cessation might warrant even greater emphasis in HIV care. Future studies may consider larger cohorts or RCTs to examine the long-term effects of varenicline and of smoking cessation on CD4 counts.

Smoking is a serious, but modifiable risk factor for respiratory and cardiovascular diseases, and improved programs of smoking cessation in HIV-infected smokers are urgently needed [12,13]. Further studies should focus on improving smoking cessation in this vulnerable population. In my smoking cessation study, 23 (64%) subjects self-reported quitting for at least 7 days at least once during the entire 24-week study, however the abstinence rate was 22% less, at 42%, by the end of study, suggesting further supportive work may help these relapsing subjects keep abstinent for a longer time period, or for good. In addition to cigarette smoking, alcohol abuse and illicit drug use are common in HIV-positive people, and these co-dependencies as well as depression have to

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be considered in smoking cessation programs [14]. HIV-positive smokers have poorer adherence to medications than HIV non-smokers [15]. Using pharmacotherapy for nicotine addiction, the health provider must address adherence issues, as with prescribing and monitoring adherence to ART. Non-adherence can be prevented, measured and improved with appropriate interventions [16]. Smoking cessation at HIV clinics is practical [17], as well as integrating smoking cessation as a public health service into HIV care [18]. Health providers have not paid sufficient attention to smoking cessation, nor prioritized smoking cessation in their daily practice [19]. Future studies should address barriers from health care providers, as well as special needs of HIV-positive smokers, taking a comprehensive approach to help smokers quit and to prevent relapse [13,17,20]. ART has dramatically shifted the course of HIV infection course, and improved smoking cessation may be associated with another major improvement in outcome, with fewer smoking-related comorbidities and mortality in HIV-infected people.

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