Cardiovascular Disease Outcomes From Use Of Antiretroviral Agents Among HIV-Infected Individuals

By

Kunchok Dorjee

A dissertation submitted in partial satisfaction of the requirements

for the degree of

Doctor of Philosophy

In

Epidemiology

In the

Graduate Division

of the

University of California, Berkeley

Committee in Charge:

Professor Arthur L. Reingold, Chair
Professor Alan E. Hubbard
Professor Anand P. Chokkalingam
Professor Eva Harris

Spring 2016

Cardiovascular Disease Outcomes from Use of Antiretroviral Agents Among HIV-infected Individuals

By Kunchok Dorjee

Abstract

Cardiovascular Disease Outcomes from Use of Antiretroviral Agents Among HIV-infected Individuals

By

Kunchok Dorjee

Doctor of Philosophy in Epidemiology

University of California, Berkeley

Professor Arthur L. Reingold, Chair

Combination anti-retroviral therapy (ART) has improved the quality of life of Human Immunodeficiency Virus (HIV)-infected individuals, and has helped them to live longer. A better understanding of the adverse drug events associated with use of various antiretroviral (ARV) drugs can help in further improving the health outcomes of HIV-infected patients. In the past years, use of various individual and combinations of ARV agents have been shown to be associated with an increased risk of cardiovascular disease (CVD) among HIV-infected patients. Of the various ARV agents, the propensity of abacavir, a nucleoside reverse transcriptase inhibitor, to increase the risk of CVD has received particular attention because of its central role as an anchor drug in combination ART. This dissertation aims to investigate the risk of acute myocardial infarction (AMI) associated with use of various antiretroviral agents among HIV-infected individuals in the United States. It further presents the results of an in-depth analysis of the risk of CVD associated with use of abacavir among HIV-infected individuals. Additionally, the results of a systematic review and meta-analysis of epidemiologic studies that were carried out to assess the risk of CVD associated with abacavir use are also presented in this dissertation.

Because a number of covariates that could potentially confound the relationship between use of ARV agents and the risk of CVD may also lie on the causal pathway, and because these factors could potentially be influenced by receipt of the exposure in the past, marginal structural Cox proportional hazard models using stabilized inverse probability of treatment weights were used to investigate this relationship. Results from unweighted Cox regressions were additionally presented for comparison. For the systematic review and meta-analysis, random-effects models were used when there was heterogeneity of results across studies, and fixed-effects models were used when there was no heterogeneity. The fixed-effects and random-effects models were weighted by inverse of variance.

Chapter One of this dissertation describes the risk of AMI associated with use of several individual and combinations of ARV agents among HIV-infected patients receiving ART. The risk of AMI was elevated among HIV-infected individuals currently exposed to abacavir, lamivudine, didanosine, lopinavir, and darunavir. Of the ARV drug combinations, current exposure to combinations of abacavir+lamivudine+atazanavir, abacavir+lamivudine+darunavir, and tenofovir+emtricitabine+raltegravir was associated with an increased risk of developing AMI. There was a protective effect against AMI from exposures to tenofovir, emtricitabine, and efavirenz, as individual agents, and as a combination regimen. Chapter Two of this dissertation presents the results of a further investigation into the risk of ischemic CVD associated with use of abacavir among HIV-infected patients. The investigation showed an increased risk of CVD and AMI associated with current, recent, and cumulative exposure to abacavir. The results of a systematic review and a meta-analysis of the epidemiologic studies, presented in chapter three of this dissertation, confirmed that recent and cumulative exposure to abacavir were associated with an increased risk of CVD among HIV-infected individuals.

In sum, exposure to various individual and combinations of ARV agents was associated with an increased risk of CVD among HIV-infected individuals. Risk and benefits should be carefully weighed while formulating antiretroviral treatment regimens for HIV-infected patients. A trans-disciplinary approach in future studies with a translational focus to achieve a better understanding of the biological mechanisms underlying the observed risk of CVD from use of ARV agents shall help to inform future development of ARV drugs with better safety profile.

Dedication

I am dedicating my dissertation to my parents, my wife, and my two children. I am extremely grateful to my parents, Mr. Sonam Dorjee and Ms. Soekyi, for giving me so much love, for trying to fulfill my every wish, and for supporting me through school and beyond. The fact that they have supported my education by overcoming great difficulties during the early stages of their lives makes me admire them all the more. The values that they have shared with me and the lessons that I have learned from them about life guide me in my everyday life. They have never stopped to inspire, encourage, and support me as I go through the ups and downs of life. I thank my wife, Yangchen Lhamo, for having confidence in me, and for supporting me through every single day, as I worked to fulfill the requirements of my PhD degree over the past several years. I thank her for taking care of our two sons with so much love and affection. Her love and support have helped to make my experience as a PhD student at Berkeley an enjoyable one. I thank my sons, Geysem Dorjee and Kunden Dorjee, for loving us, for making us laugh, for being a source of fresh energy to us every day, and for being a positive influence all along.

Table of Contents

Acknowledgement

I express my deep thanks and gratitude to my adviser and teacher, Professor Art Reingold for all the support that I have received from him over the past several years. I thank Dr. Reingold for accepting me as his student, for providing me an excellent mentorship, and for his help in finding support for my education at Berkeley. Dr. Reingold has helped me to gain the maximum out of the resources at Berkeley, and his inputs helped me to optimize my output so that I could succeed in my academic endeavors, and make a useful contribution to the areas I chose to work on for my dissertation. I have learned from his teachings in class, and have benefited from the feedbacks he has provided me on my various academic works. He has inspired me as a teacher, a mentor, and a human being. The opportunities I have had to interact with him have helped me to grow as a person, and to understand better my fit to the world.

I extend my deep thanks to the other dissertation committee members, Professor Anand Chokkalingam, Professor Alan Hubbard, and Professor Eva Harris for all the support that I have received from them. I am thankful to them for the feedbacks they have provided me on my dissertation work. The education that I have received from them in class, and through in-person meetings have greatly benefitted me.

I am very thankful to Dr. Robertino Mera for the support I have received from him during my internship at Gilead Sciences. Dr. Mera has inspired me to apply causal inference methods to answer my study questions. I have learned much from Dr. Mera during my interactions with him. I am deeply thankful to Dr. Robertino Mera and Dr. Anand Chokkalingam of Gilead Sciences for providing access to the IMS' Pharmetrics Claims Data.

During my doctoral training at UC-Berkeley, I benefited very much from the support that I received from Dr. Philip Hopewell and his colleagues, Dr. Adithya Cattamanchi, and Dr. John Metcalfe at the University of California, San Francisco. I extend my deep thanks to them for going out of their way to support me, for being willing to work with me, and for providing me career advices, which all helped me to see through things more clearly.

I would like to extend my deep thanks to Mr. And Mrs. Chao for their support and generosity. I am also very thankful to Dr. Tom Lambert and Ms. Catherine McDonough for the kindness and support they extended to me when I first joined the PhD program at Berkeley.

I offer my deep thanks to Professor Richard Chaisson at Johns Hopkins University for inspiring me through the years, for working with me, and for his willingness to support me whenever possible, whether it is to write letters of recommendation for me, or to offer me precious words of advice. I also extend my deep thanks to Dr. Zorba Paster for supporting my work through miscellaneous ways. Dr. Paster always brims over with joyful energy that is delightfully contagious.

Last but not the least, I am very thankful to my class mates, fellow GSIs, and other students with whom I have worked at Berkeley, for all the things I have learned from them, for listening to me, for being so supportive over the years, and for the fun times and light moments. They have helped in completing my experience as a graduate student at Berkeley.

Introduction

The world has succeeded in meeting the United Nation's Millennium Development Goal (MDG) 6 for human immunodeficiency virus (HIV) infection–halt and reverse the spread of HIV-AIDS epidemic by 2015 (1). In Sub-Saharan Africa, the number of new HIV infections dropped 41%, from 2.3 million in 2000 to 1.4 million in 2014 (1). The number of AIDS-related deaths dropped 34% from 1.2 million in 2000 to 790,000 in 2014 (1). The Millennium Development Goals ended in 2015 and Sustainable Development Goals (SDGs) have been launched. With the SDGs, the world aims to end the HIV/AIDS epidemic by 2030 (1). As a step towards the SDGs, nations are now embarking on the UNAIDS' 90-90-90 Fast-Track strategy in which, by 2020, 90% of the HIV-infected individuals would know their HIV positive status; 90% of those who know their HIV positive status would have access to antiretroviral therapy (ART); and 90% of those on ART would be virally suppressed (1). However, these goals are ambitious and require a concerted global effort. Additionally, I believe that every individual can make a contribution to the realization of these goals. Therefore, I am dedicating my Epidemiology PhD Dissertation Research at the University of California, Berkeley to the study of the science of HIV-AIDS, with the hope that it will serve as a contribution towards the global goal of ending the HIV-AIDS epidemic by 2030.

In 2014, there were an estimated 36.9 million HIV-infected people in the world (1). Currently, approximately 15.8 million (43%) HIV-positive patients have access to ART (1). On the one hand, this finding is concerning because 22 million HIV-infected persons have yet to gain access to ART. However, on the other hand, this can be seen as an enormous achievement because this level of ART coverage has been achieved in a relatively short period of time, growing from less than 1% of HIV-infected people in low and middle income countries on ART in 2000 (1). Between 2014 and 2015 alone, the number of HIV-infected individuals having access to ART increased from 13.6 million to 15.8 million, an increase of 2.2 million in one year (1). The annual mortality among HIV-infected persons has decreased from more than 20% in the pre-ART era to ~3% currently (1, 2); an estimated 8 million HIV/AIDS-related deaths have been averted since 2000 (1). This enormous decline in mortality among HIV-infected individuals can be largely attributed to the implementation of highly active antiretroviral therapy (HAART). Ever since 1997-1998, when Hammer et al., Gulick et al., and Palella et al. first showed the benefits of combining classes of anti-retroviral drugs in treating HIV infection in terms of reducing the rates of progression to AIDS, HIV-related hospitalizations, and deaths, HAART has been implemented widely by clinicians worldwide to treat HIV infection (3-5). HAART has not only helped HIV-infected individuals live longer, but it has also helped them to have a vastly better quality of life (3-10). These advances have been achieved through a reduction in opportunistic infections among HIV-infected individuals and prevention of progression of HIV infection to clinical AIDS (5). As AIDS related mortality has declined and HIV-infected patients have begun to live longer, mortality from non-AIDS causes, such as cardiovascular disease (CVD), has become more common (11-13). CVD requires particular attention as a cause of mortality and morbidity among HIV-infected individuals because first, the risk factors for CVD have been shown to be more prevalent among HIV-positive patients, and second, some individual drugs and classes of

antiretroviral agents have been shown to be associated with an increased risk of CVD among HIV-infected patients (12, 14). The propensity of certain ARV drugs, (e.g. abacavir) to increase the risk of CVD has received particular attention because of its central role as an anchor drug in combination ART (15). It is important to understand the potential toxicities of various ARV drugs, so that treatment regimens with optimum potency and minimum side-effects can be devised, so as to optimize the health outcomes of HIV-infected individuals. Therefore, I dedicate my dissertation to the study of the risk of CVD associated with exposure to various antiretroviral drugs. Specifically, the three aims of this PhD dissertation are: 1) To document the risk and protective effects for acute myocardial infarction from exposure to various individual and combinations of antiretroviral agents; 2) To measure the risk of CVD associated with a current, recent, and cumulative exposure to abacavir among HIV-infected individuals; and 3) To conduct a systematic review and a meta-analysis of the epidemiologic studies that have investigated the relationship between exposure to abacavir and the risk of CVD among HIV-infected individuals.

References:

1. UNAIDS. AIDS by the numbers 2015 2015 [Available from: http://www.unaids.org/en/resources/campaigns/HowAIDSchangedeverything/factsheet.
2. Mocroft A, Vella S, Benfield TL, Chiesi A, Miller V, Gargalianos P, et al. Changing patterns of mortality across Europe in patients infected with HIV-1. EuroSIDA Study Group. Lancet. 1998;352(9142):1725-30.
3. Gulick RM, Mellors JW, Havlir D, Eron JJ, Gonzalez C, McMahon D, et al. Treatment with indinavir, zidovudine, and lamivudine in adults with human immunodeficiency virus infection and prior antiretroviral therapy. N Engl J Med. 1997;337(11):734-9.
4. Hammer SM, Squires KE, Hughes MD, Grimes JM, Demeter LM, Currier JS, et al. A controlled trial of two nucleoside analogues plus indinavir in persons with human immunodeficiency virus infection and CD4 cell counts of 200 per cubic millimeter or less. AIDS Clinical Trials Group 320 Study Team. N Engl J Med. 1997;337(11):725-33.
5. Palella FJ, Jr., Delaney KM, Moorman AC, Loveless MO, Fuhrer J, Satten GA, et al. Declining morbidity and mortality among patients with advanced human immunodeficiency virus infection. HIV Outpatient Study Investigators. N Engl J Med. 1998;338(13):853-60.
6. Dore GJ, Li Y, McDonald A, Ree H, Kaldor JM, National HIVSC. Impact of highly active antiretroviral therapy on individual AIDS-defining illness incidence and survival in Australia. J Acquir Immune Defic Syndr. 2002;29(4):388-95.
7. Egger M, Hirschel B, Francioli P, Sudre P, Wirz M, Flepp M, et al. Impact of new antiretroviral combination therapies in HIV-infected patients in Switzerland: prospective multicentre study. Swiss HIV Cohort Study. BMJ. 1997;315(7117):1194-9.
8. Hogg RS, Yip B, Kully C, Craib KJ, O'Shaughnessy MV, Schechter MT, et al. Improved survival among HIV-infected patients after initiation of triple-drug antiretroviral regimens. CMAJ. 1999;160(5):659-65.
9. Montaner JS, Reiss P, Cooper D, Vella S, Harris M, Conway B, et al. A randomized, double-blind trial comparing combinations of nevirapine, didanosine, and zidovudine for HIV-infected patients: the INCAS Trial. Italy, The Netherlands, Canada and Australia Study. JAMA. 1998;279(12):930-7.
10. Nakagawa F, May M, Phillips A. Life expectancy living with HIV: recent estimates and future implications. Curr Opin Infect Dis. 2013;26(1):17-25.
11. Antiretroviral Therapy Cohort C. Causes of death in HIV-1-infected patients treated with antiretroviral therapy, 1996-2006: collaborative analysis of 13 HIV cohort studies. Clin Infect Dis. 2010;50(10):1387-96.
12. Triant VA, Josephson F, Rochester CG, Althoff KN, Marcus K, Munk R, et al. Adverse Outcome Analyses of Observational Data: Assessing Cardiovascular Risk in HIV Disease. Clin Infect Dis. 2012;54(3):408-13.
13. Schneider MF, Gange SJ, Williams CM, Anastos K, Greenblatt RM, Kingsley L, et al. Patterns of the hazard of death after AIDS through the evolution of antiretroviral therapy: 1984-2004. AIDS. 2005;19(17):2009-18.

14. Triant VA, Lee H, Hadigan C, Grinspoon SK. Increased acute myocardial infarction rates and cardiovascular risk factors among patients with human immunodeficiency virus disease. J Clin Endocrinol Metab. 2007;92(7):2506-12.
15. Sabin CA, Worm SW, Weber R, Reiss P, El-Sadr W, Dabis F, et al. Use of nucleoside reverse transcriptase inhibitors and risk of myocardial infarction in HIV-infected patients enrolled in the D : A : D study: a multi-cohort collaboration. Lancet. 2008;371(9622):1417-26.

Chapter One: Risk and Protective Effects for Acute Myocardial Infarction from Use of Individual and Combinations of Anti-Retroviral Agents among HIV-infected Individuals in the United States

Abstract

Background

Individual antiretroviral (ARV) agents have been shown to be associated with an increased risk of cardiovascular disease (CVD) among human immunodeficiency virus (HIV)-infected individuals. However, ARV drugs are prescribed in combinations. Therefore, it is important to understand whether combinations of ARV drugs are also associated with an increased risk of CVD.

Methods

I assessed the risk of first episode of acute myocardial infarction (AMI) among 73,071 HIV-infected individuals above 18 years of age enrolled in the IMS' Pharmetrics Claims database in the United States, who were started on antiretroviral therapy (ART) between October 1, 2009 and December 31, 2014. Using marginal structural models with stabilized inverse probability of treatment weights generated as a function of time-fixed and time-dependent covariates, I assessed the risk from a current exposure to 13 individual and 10 combinations of ARV drugs.

Results

Over 114,417 person-years of exposure to ART among 73,071 subjects, 602 cases of AMI occurred an event rate of 5.26 (95% CI: 4.86, 5.70) per 1000 person-years. The median age of the participants was 45 years with 81.5% males. Of the individual ARV agents, I found an elevated risk of AMI (Hazard ratio; 95% Confidence Interval) with current use of abacavir (1.33; 1.06, 1.66), lamivudine (1.32; 1.09, 1.61), darunavir (1.54; 1.26, 1.94), didanosine (1.83; 1.13, 2.96), lopinavir (1.71; 1.31, 2.25) and raltegravir (1.39; 1.12, 1.74). Of the combinations of ARV drugs, current exposure to abacavir+lamivudine+atazanavir (1.55; 1.07, 2.25), abacavir+lamivudine+darunavir (1.95; 1.29, 2.96), and tenofovir+emtricitabine+raltegravir (1.39; 1.09, 1.74) was associated with an increased risk of developing AMI. I found a protective effect against AMI from exposures to tenofovir, emtricitabine, and efavirenz as individual agents and as a combination regimen.

Conclusion

Specific ARV drug combinations are associated with an increased risk of AMI. Careful assessment of the risks and benefits is necessary in formulating an ARV treatment regimen.

Introduction

There were approximately 37 million HIV-infected people globally in 2014 (1). The number of people receiving ART increased from 13.6 million in 2014 to 15.8 million in 2015 (1). Combination anti-retroviral therapy has helped reduce the mortality from HIV infection; the annual mortality among HIV-infected individuals has decreased from more than 20% in the pre-ART era to ~3% currently (1, 2). The health outcomes among HIV-infected patients can be further improved through a better understanding of the toxicities associated with the use of ARV agents. Recently, a number of individual and combinations of ARV agents from the three major drug classes, protease inhibitors (PI), nucleos(t)ide reverse transcriptase inhibitors (NRTI), and non-nucleotide reverse transcriptase inhibitors (NNRTI), have been shown to be associated with an increased risk of AMI and CVD (3-7). These effects may be related to the potential of ARV agents to induce dyslipidemia, insulin resistance, diabetes mellitus, hypertension, vascular inflammation, and platelet aggregation (8-16). However, there are controversies regarding these effects. Studies by Bozzette et al. and Klein et al. have failed to show an association between the use of PIs and the risk of AMI (17, 18). Newer generation PIs, such as atazanavir and darunavir, have been shown to have more favorable effects on blood lipids as compared to older PIs (19-23). Whether or not abacavir use is associated with the risk of an AMI is a subject of intense debate and substantial clinical significance (6, 24-27).

Because ARV drugs are prescribed in combinations, it is important to assess the relationship between exposure to combinations of ARV drugs and the risk of CVD. Most studies to date have investigated the effects of individual drugs. Desai et al. recently reported that four individual and five combinations of ARV drugs were significantly associated with an increased risk of CVD (5). However, several of their findings on the risk CVD associated with the use of individual ARV agents differed from those of the Data Collection on Adverse Events of Anti-HIV Drugs (D:A:D) study group's findings (6). To date, no study has investigated the risk of AMI from exposure to an integrase strand transfer inhibitor (InSTI) or newer PIs, such as darunavir. In this study, I use a large administrative health-plan dataset to investigate the relationship between exposure to various individual and combinations of ARV drugs from four major drug classes (NRTI, NNRTI, PI, InSTI) and the risk of AMI in the HIV-infected population in the United States.

Methods

Study population and data

I assessed the risk of AMI from exposure to individual and combinations of ARV drugs among 73,701 HIV-infected individuals in the U.S. who were started on ART between October 1, 2009 and December 31, 2014 and enrolled in the IMS' PharMetrics Plus database. A start date of October 1, 2009 was chosen because this is the earliest date of starting an ARV drug in the study cohort in the claims database. PharMetrics Plus is one of the largest health plan insurance claims databases in the U.S. containing adjudicated claims for more than 150 million unique enrollees from across the four regions (28). The data undergo a series of quality checks to minimize errors. I used a pre-defined algorithm (Figure 1) to extract and define my study population from the main claims databases. I restricted my study population to those greater than 18 years of age. Individuals are censored at either 1) the first occurrence of CVD after start of exposure, 2) last recorded date of ART receipt in the database, or 3) December 31, 2014, whichever occurred first. I didn't investigate ARV agents which were approved and introduced into the market after 2009.

Exposure, covariate, and outcome definitions

I identified individual ARV drugs in the database by their unique generic product identifier (GPI) codes. I generated a separate dataset for each exposure drug or drug combination. Any two prescriptions for an ARV agent separated by less than 30 days were combined to represent a single continuous exposure. I then compared the person time of any exposure to an individual drug or drug combinations to that of other ARV agents. I included individual drugs with greater than approximately 3,000 person years of exposure and drug combinations with greater than approximately 1,500 person years of exposure for investigation in my study. The data are longitudinal in nature, with each subject's follow up time divided into consecutive one month periods during which the treatment is allowed to vary. The values of covariates are updated at the start of each month and the outcome for an individual is defined as the first occurrence of an AMI after the start of the exposure. This temporal ordering of covariate, treatment, and outcome allows for a time-varying analysis, with an opportunity for a causal interpretation. Once an individual develops a health condition, he/she is assumed to have the condition for the remainder of the study. Current exposure to a drug or a drug combination is defined as exposure (yes/no) during each one-month observation period. Covariates and outcomes were ascertained using the International Classification of Disease, 9th Revision, Clinical Modification (ICD-9-CM) (Appendix table 1). The study outcome is acute myocardial infarction (AMI), defined by ICD-9-CM code 410.xx. Patients are censored at the occurrence of first AMI after the start of exposure.

Statistical Analysis

I assessed the risk of AMI from a current exposure to individual drugs or drug combinations using marginal structural models with stabilized inverse probability of treatment weights (sIPTW). I generated my treatment weights from four pooled logistic regression models, two each for the denominator and numerator of the sIPTW. For the denominator, I first modelled the probability of initiating exposure as a function of baseline and time dependent covariates. For this, I fit the logistic model to data up to the individual's first month of receiving the exposure or the end of follow up for those who were never exposed. I then modelled the probability of continuing exposure by fitting the model to data after the first month of starting the exposure. The treatment continuation model differs from the treatment initiation model in that it additionally contains a variable for past month's exposure status. The probabilities for the numerator of the sIPTW are modelled as a function of baseline (time-fixed) covariates only. The baseline covariates are sex, tobacco use (ever), substance or alcohol abuse (ever), hepatitis B & C, stroke, cancer, old myocardial infarction, and the baseline values of all the time dependent covariates. The time dependent covariates are age, body weight, chronic kidney disease (CKD), dyslipidemia, heart failure, cardiac dysrhythmia, atherosclerosis, diabetes mellitus, receipt of anti-hyperglycemic agents, hypertension, and receipt of medications for cardiovascular disease (aspirin, beta-blocker, angiotensin converting enzyme inhibitor, angiotensin receptor blocker, calcium channel blocker, and statins). Follow up time was allowed to be a function of natural cubic splines with three knots. In addition to adjusting for the sIPTW generated from the treatment model, the marginal model was further adjusted for the baseline or time fixed covariates listed above. Alongside the marginal structural results, I have reported corresponding unadjusted and adjusted results from conventional Cox models. I assumed uninformative censoring for my study. I extracted and processed my data from the main claims databases using TERADATA, SAS (Version 9.1), and STATA (13.1), and implemented the marginal structural models in STATA following the steps shown by Fewell et al (29). Additional description of the marginal structural model with the equations and the notations are provided in the appendix 1 of the dissertation chapter 2. The study was approved by the Committee for Protection of Human Subject (CPHS) at University of California, Berkeley.

Results

Over 114,417 person-years of exposure to ART among 73,071 subjects, 602 cases of AMI occurred at an event rate of 5.26 (95% CI: 4.86, 5.70) per 1000 person-years. The median age of the study population was 45 years with 81.5% males. Most of the traditional cardiovascular risk factors, such as higher age, male sex, hypertension, diabetes mellitus, dyslipidemia, smoking, substance abuse, prior CVD, and CKD were more prevalent among individuals who developed an AMI (Table 1). Thirteen individual and 10 ARV drug combinations met the criteria to be included for investigation (Table 2). Didanosine represented only 1,758 person-years of exposure, but I included it based on the prior controversial findings.

Of the 13 ARV drugs I investigated from the four major ARV drug classes, I found a significantly elevated risk (HR; 95% CI) of AMI from exposure to abacavir (1.33; 1.06, 1.66), lamivudine (1.32; 1.09, 1.61), didanosine (1.83; 1.13, 2.96), darunavir (1.54; 1.22, 1.94), lopinavir/r (1.71; 1.31, 2.25) and raltegravir (1.39; 1.12, 1.74) (Figure 2). The risk of AMI was also increased for zidovudine, but was not statistically significant (HR: 1.22; 95% CI: 0.94, 1.59). I found a protective effect (HR; 95% CI) for current exposure to tenofovir (0.70; 0.58, 0.85), emtricitabine (0.71; 0.59, 0.85), and efavirenz (0.66; 0.55, 0.79). Of the 10 ARV drug combinations that I investigated, I found a significantly increased risk (HR; 95% CI) from exposure to combinations of abacavir+lamivudine+atazanavir (1.55; 1.07, 2.25), abacavir+lamivudine+darunavir (1.95; 2.29, 2.96), and tenofovir+emtricitabine+raltegravir (1.38; 1.09, 1.73) (Figure 3). The risks (HR; 95% CI) of AMI from exposure to the combinations of abacavir+lamivudine+zidovudine (1.39; 0.87, 2.22) and tenofovir+emtricitabine+darunavir (1.41; 0.98, 2.03) was increased but not statistically significant. Exposure to a combination of tenofovir+emtricitabine+efavirenz (0.69; 0.58, 0.83) was associated with a protective effect against AMI. In conventional Cox models that adjusted for baseline and time varying covariates, I obtained similar results (Appendix tables 2 & 3).

Discussion

Findings of an increased risk of AMI as a result of exposure to various ARV agents, both singly and in various combinations, in recent studies have motivated me to conduct this investigation. The possibility of a channeling bias such that individuals with certain measured and unmeasured CVD risk factors may be more or less likely to receive specific ARV drugs or drug combinations, and a potential for a number of covariates, such as diabetes mellitus, hypertension, dyslipidemia, lipodystrophy, and renal dysfunction to act as both confounders and causal intermediates on the pathway between ART use and AMI motivated me to use marginal structural models to investigate my study questions. I observed a significantly increased risk of AMI from exposure to six individual ARV agents: abacavir, lamivudine, didanosine, darunavir, lopinavir, and raltegravir, and three combinations: abacavir+lamivudine+atazanavir, abacavir+lamivudine+darunavir, and tenofovir+emtricitabine+raltegravir. I observed a protective effect for AMI from exposure to tenofovir, emtricitabine and efavirenz, individually and in combination.

To date, most studies have investigated the relationship between ART exposure and the risk of AMI by modelling the risk associated with the use of individual ARV agents. However, ARV drugs are typically prescribed in combinations usually comprised of three or more drugs, and as a result, the findings from combinations of ARV drugs may be more relevant. I discuss below my study results concerning the risk of AMI associated with exposure to various individual and combinations of ARV drugs in the context of existing knowledge from an epidemiologic perspective, and when possible, from a biological perspective.

Nucleos(t)ide reverse transcriptase inhibitors

My findings of an increased risk of AMI from exposures to abacavir and didanosine agree with the findings of the D:A:D and other studies (5-7, 25, 27, 30-36). In two companion papers, one of which is a meta-analysis, I have discussed in more detail the results of my investigation on the risk of CVD associated with current, cumulative, and recent exposure to abacavir (Dissertation Chapters 3 & 4), and their fit with plausible biological mechanisms. It has been argued in the published literature that the observed risk of AMI associated with exposure to abacavir may be attributable to confounding by factors such as renal dysfunction and substance abuse (24, 26). I found an increased risk of AMI associated with exposure to abacavir after adjusting for these and other relevant covariates. Of the five abacavir based combinations, I found an increased risk of AMI associated with exposure to abacavir+lamivudine+atazanavir (HR: 1.55), abacavir+lamivudine+darunavir (HR: 1.95), and abacavir+lamivudine+zidovudine (HR: 1.39), although the result for the last combination was not statistically significant. Desai et al. also found an increased risk of CVD in association with exposure to abacavir+lamivudine+atazanavir and abacavir+lamivudine+zidovudine; they did not assess the risk from exposure to abacavir+lamivudine+darunavir. Of all the drug combinations that I investigated, I observed the highest risk for the abacavir+lamivudine+darunavir (HR: 1.95) combination, higher than that observed individually for abacavir (HR: 1.33), lamivudine (HR: 1.32), and darunavir (HR: 1.54). This finding is suggestive of a synergistic interaction between the drugs. I did not observe an increased risk from exposure to abacavir+lamivudine+efavirenz (HR: 0.64; 95% CI: 0.33, 1.26), while Desai et al. did find an increased risk of AMI for this combination. I do not know the reason for this difference in our results. They noted that all of the combinations that they found to be associated with an increased risk of CVD contained lamivudine. I also observed a significant association between the risk of AMI and current use of lamivudine. The D:A:D study reported an increased risk of AMI from recent exposure to lamivudine only after adjusting for past exposure, and they were unsure whether it was a real association or a false positive result (6). It is not clear what the biological mechanism is that would underlie an association between an increased risk of CVD and use of lamivudine. Interestingly I did not observe an increased risk of AMI associated with exposure to abacavir+lamivudine+raltegravir (HR: 0.95; 95% CI: 0.59, 1.53), although all of the three drugs, abacavir, lamivudine, and raltegravir were individually associated with an increased risk of AMI in my study. This finding begets the question as to whether the drugs, when taken together, interact in a unique way so as to have no effect on the risk of AMI in HIV-infected patients. Abacavir and lamivudine have recently been co-formulated with a novel InSTI, dolutegravir, and therefore, this finding of no increased risk of AMI associated with use of a similar combination containing an InSTI could have wider clinical significance. On the other hand, I found an increased risk of AMI associated with exposure to the combination of tenofovir+emtricitabine+raltegravir (HR: 1.38). The findings of differential effects of raltegravir depending upon which drugs it is combined with opens up important areas of research and suggests that researchers should attempt to replicated these findings in other study populations. I observed a protective effect for AMI against exposures to tenofovir, emtricitabine, and efavirenz, both as individual agents and as a combination. I am not aware of

a prior study that found an association between use of tenofovir/emtricitabine and an increased risk of AMI. Tenofovir has been shown to have an intrinsic lipid lowering effect and also to decrease the carotid intima media thickness, suggesting a possible cardiovascular and cerebrovascular protective effect (37, 38). Our observation of an increased risk of AMI associated with exposure to the tenofovir+emtricitabine+darunavir (HR: 1.41; 95% CI: 0.98, 2.03) combination may be attributable to the effects of darunavir discussed above.

Protease Inhibitors

Of the three PIs examined, I found an increased risk of AMI associated with exposure to lopinavir and darunavir. Use of darunavir was associated with an increased risk of AMI both when assessed individually and when combined with abacavir-lamivudine or tenofovir-emtricitabine, as discussed above. This is the first study to investigate the risk of AMI associated with exposure to darunavir. It is unclear whether a possible increased risk of AMI associated with exposure to darunavir may be mediated through its influence on blood lipid levels or through another mechanism. Although darunavir, like atazanavir, is a newer PI known to have more favorable effects on blood lipids, as compared to older PIs such as lopinavir (22, 23), a meta-analysis by Hill et al. has shown that ritonavir-boosted darunavir (darunavir/r) or atazanavir (atazanavir/r) did not differ from lopinavir/r or fosamprenavir/r in elevating LDL or HDL cholesterol levels (39). I did not observe an increased risk associated with exposure to atazanavir when assessed individually (HR: 0.99). However, I observed an increased risk of AMI when it was combined with abacavir-lamivudine (HR: 1.55) but not when it was combined with tenofovir-emtricitabine (HR: 1.08). I noted a greater magnitude of association between exposure to the abacavir+lamivudine+atazanavir combination and the risk of AMI than that observed for these drugs singly, which again suggests possible drug-drug interactive effects. Desai et al. observed similar findings with atazanavir (5). I observed an increased risk of AMI associated with exposure to lopinavir (HR: 1.71). The D:A:D study group also found an increased risk of AMI associated with exposure to lopinavir (7). Darunavir, atazanavir, and lopinavir are usually prescribed in combination with a low dose ritonavir or cobicistat as a booster; I did not endeavor to examine the effects, if any, of these booster agents on the risk of AMI in this study.

Non-nucleoside reverse transcriptase inhibitor

I found a protective effect for AMI associated with exposure to efavirenz when assessed individually and in combination with tenofovir-emtricitabine. The D:A:D study found no increased risk of AMI associated with exposure to efavirenz (7), while Desai et al. found an increased risk of CVD when efavirenz was taken in combination with abacavir-lamivudine (5). The meta-analysis by Hill et al. showed a smaller increase in triglyceride level and a greater increase in HDL cholesterol level when taking efavirenz, as compared to darunavir/r or atazanavir/r, suggestive of a relative cardioprotective effect from efavirenz (39). I didn't find an increased risk of AMI associated with exposure to nevirapine.

Integrase inhibitor

I observed an increased risk of AMI associated with exposure to raltegravir when combined with tenofovir-emtricitabine, but not when combined with abacavir-lamivudine. I do not know the mechanism whereby the tenofovir-emtricitabine-raltegravir combination would cause an increased risk of AMI. Apart from a gain in body fat, which has been reported from exposure to raltegravir, both when taken separately and when taken in combination with tenofovir-emtricitabine (40, 41), raltegravir has otherwise been shown to have a favorable effect of blood lipids (40-42). This is the first study to investigate the risk of AMI associated with exposure to an InSTI. It is important to investigate these relationships further in other populations. In addition, it is important to bear in mind that individuals who are prescribed novel ARV agents, such as darunavir and raltegravir, may have more advanced HIV disease, not captured in the data we have, that might put them at higher risk for various comorbid conditions, including CVD. However, the D:A:D study showed that adjustments for CD4 cell count and HIV viral load made little difference in the relationship between exposure to abacavir and the risk of AMI in their study population (6).

The main strength of my study is that I applied a robust and appropriate method to answer my study questions in a large U.S. health plan dataset containing longitudinal information on use of ART in more than 70,000 HIV-infected individuals receiving care across the country. The lack of availability of potentially important covariates, such as CD4 cell count, HIV viral load, race/ethnicity, and family history of AMI in the dataset is an important limitation. I have further enumerated the limitations applicable to claims data in general in the companion paper that explores in more depth the risk of CVD associated with exposure to abacavir (see dissertation Chapter 3).

Conclusion

I found a significantly increased risk of AMI associated with exposure to abacavir, lamivudine, lopinavir, didanosine, darunavir, raltegravir, and to the combinations of abacavir+lamivudine+atazanavir and tenofovir+emtricitabine+raltegravir. I report here for the first time an increased risk of AMI associated with darunavir and raltegravir. Last, I found a protective effect against AMI associated with exposure to tenofovir, emtricitabine, efavirenz, and the tenofovir+emtricitabine+efavirenz combination.

Tables and Figures

Table 1. Baseline characteristics of HIV-infected individuals above 18 years receiving ART (n=73,701)

Characteristics	All patients n(%)	Patients without an AMI n(%)	Patients with an AMI n(%)
Age, median (IQR)	45 (38-52)	45 (38-52)	53 (48-59)
Male	59,514 (81.5)	58,981 (81.4)	533 (88.5)
Region			
East	17,523 (24)	17,354 (24)	169 (28.1)
Mid-West	13,532 (18.5)	13,411 (18.5)	121 (20.1)
South	33,271 (45.5)	33,014 (45.6)	257 (42.7)
West	8,745 (12)	8,690 (12.0)	55 (9.1)
Year of ART initiation			
2009	24,435 (33.4)	24,127 (33.3)	308 (51.2)
2010	9,729 (13.3)	9,636 (13.3)	93 (15.5)
2011	10,274 (14.1)	10,190 (14.1)	84 (14.0)
2012	8,605 (11.8)	8,559 (11.8)	46 (7.6)
2013	7,830 (10.7)	7,794 (10.8)	36 (6.0)
2014	12,198 (16.7)	12,163 (16.8)	35 (5.8)
Ever substance abuse	13,395 (18.3)	13,152 (18.2)	243 (40.4)
Ever alcohol abuse	3,093 (4.2)	3,039 (4.2)	54 (9.0)
Ever tobacco use	11,849 (16.2)	11,587 (16.0)	262 (43.5)
Overweight or obese	1,511 (2.1)	1,503 (2.1)	8 (1.3)
Essential hypertension	7,450 (10.2)	7,336 (10.1)	114 (19.0)
Diabetes mellitus	4,128 (5.7)	4,052 (5.6)	76 (12.6)
Chronic Kidney Disease	729 (1)	701 (1.0)	28 (4.7)
Dyslipidemia	8,616 (11.8)	8,496 (11.7)	120 (19.9)
Lipodystrophy	224 (0.3)	220 (0.3)	4 (0.7)
[@#]CVD	1,754 (2.4)	1,688 (2.3)	66 (11.0)
[$]Medications for CVD	14,336 (19.6)	1,4074 (19.4)	262 (43.5)
Stroke	211 (0.3)	204 (0.3)	7 (1.2)
Symptomatic HIV disease	32,222 (44.1)	31,973 (44.1)	249 (41.4)
Hepatitis B	959 (1.3)	953 (1.3)	6 (1)
Hepatitis C	1,520 (2.1)	1,500 (2.1)	20 (3.3)
Any cancer	5,499 (7.5)	5,442 (7.5)	57 (9.5)

@Includes old myocardial infarction, heart failure, cardiac arrhythmia, and atherosclerosis. #cardiovascular disease.

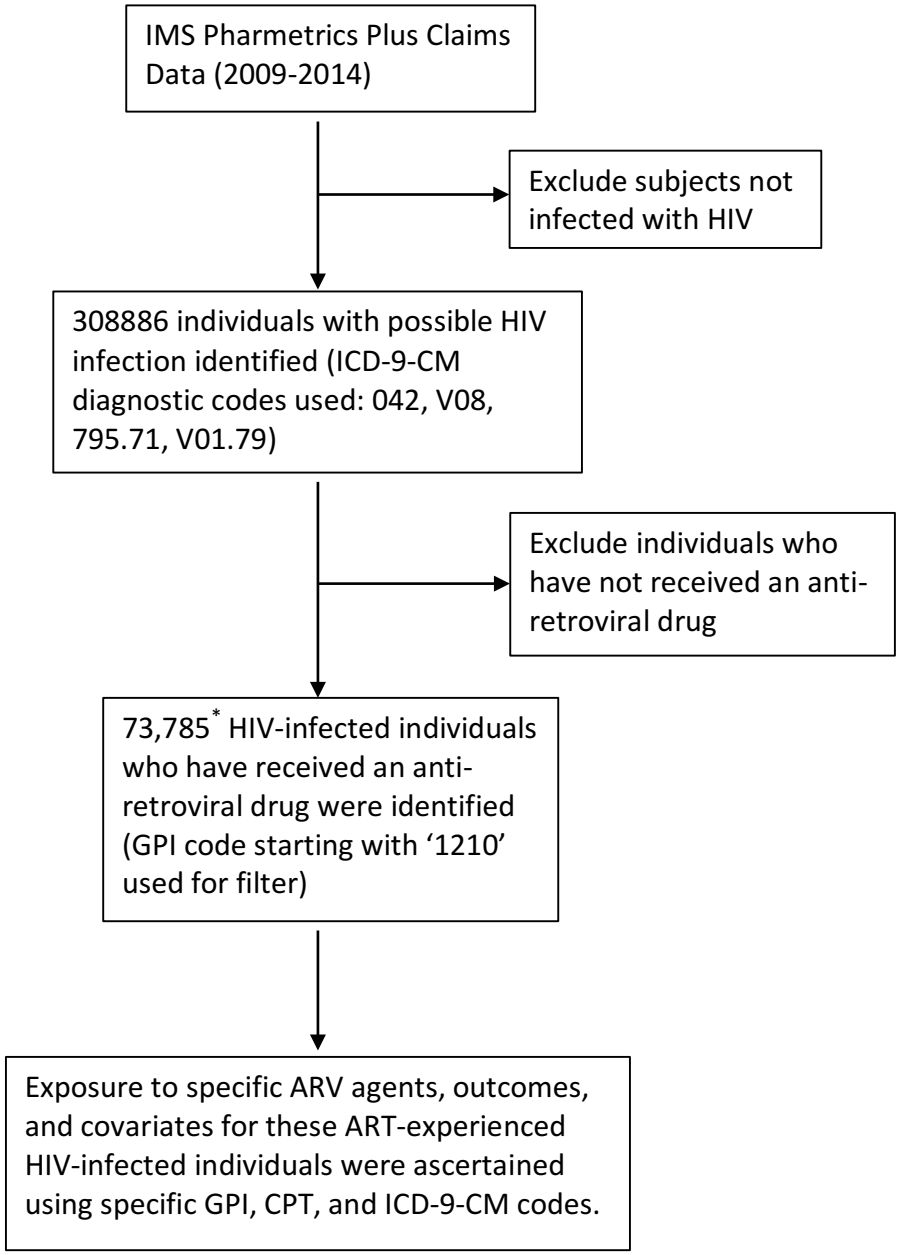
$ Includes aspirin, beta-blockers, calcium channel blockers, statins, angiotensin converting enzyme inhibitors, and angiotensin receptor blockers

Table 2. Median years of exposure and number of AMI events occurring over total person time of exposure to individual and combinations of anti-retroviral agents

ARV drug	Median no. of years of exposure (IQR)	No. of AMI events	Person years of exposure
Abacavir	2.67 (1.42, 4.08)	98	14,116
Tenofovir	2.67 (1.50, 4.17)	370	90,214
Lamivudine	2.67 (1.42, 4.17)	148	21,705
Zidovudine	2.67(1.50, 4.16)	68	10,586
Didanosine	2.50 (1.42, 3.92)	17	1,758
Emtricitabine	2.67 (1.42, 4.17)	342	85,051
Atazanavir	2.50 (1.33, 3.92)	72	15,409
Darunavir	2.25 (1.17, 3.50)	94	11,976
Lopinavir	2.50 (1.33, 3.83)	60	7,928
Fosamprenavir	2.50 (1.42, 4.25)	17	2,953
Efavirenz	2.83 (1.58, 4.25)	177	49,954
Nevirapine	2.92 (1.58, 4.33)	34	6,388
Raltegravir	2.42 (1.25, 3.83)	116	15,579
abc+3tc+atv	2.75 (1.58, 4.17)	30	3,951
abc+3tc+drv	2.83 (1.58, 4.08)	26	2,395
abc+3tc+zdv	2.92 (1.75, 4.33)	19	2,882
abc+3tc+efv	2.92 (1.75, 4.25)	9	2,541
abc+3tc+ral	3.08 (1.75, 4.12)	20	3,055
tdf+ftc+atv	2.83 1.67, 4.17)	63	13,620
tdf+ftc+drv	2.67 (1.50, 4.83)	62	10,981
tdf+ftc+efv	2.92 (1.75, 4.33)	170	49,493
tdf+ftc+fpv	3.17 (1.83, 4.42)	13	2,465
tdf+ftc+ral	2.83 (1.67, 4.17)	92	13,729

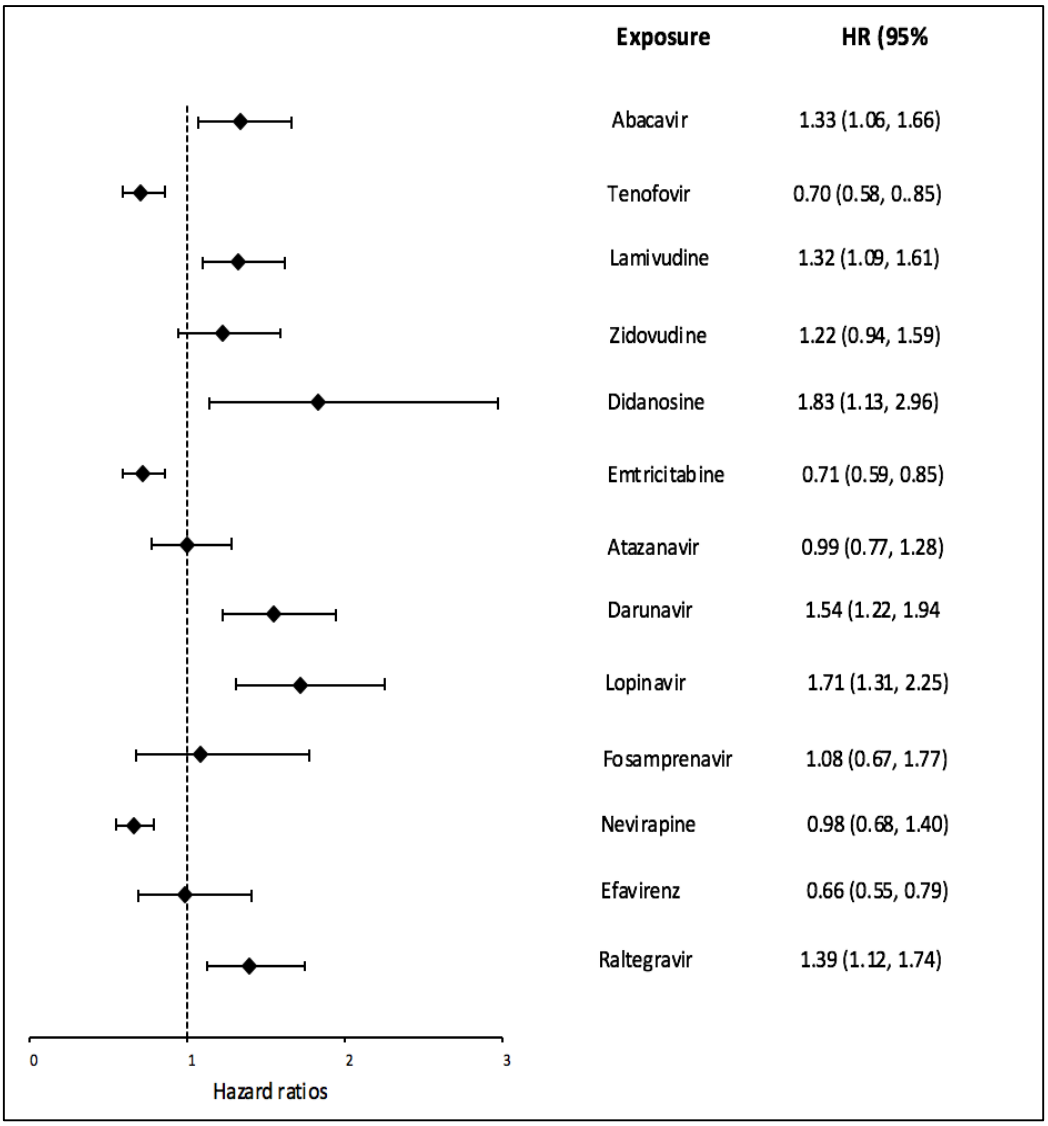
abc: abacavir; 3tc: lamivudine; atv: atazanavir; drv: darunavir; fpv: fosamprenavir; tdf: tenofovir; ftc: emtricitabine; efv: efavirenz; ral: raltegravir;

Figure 1. Algorithm for defining the study cohort.



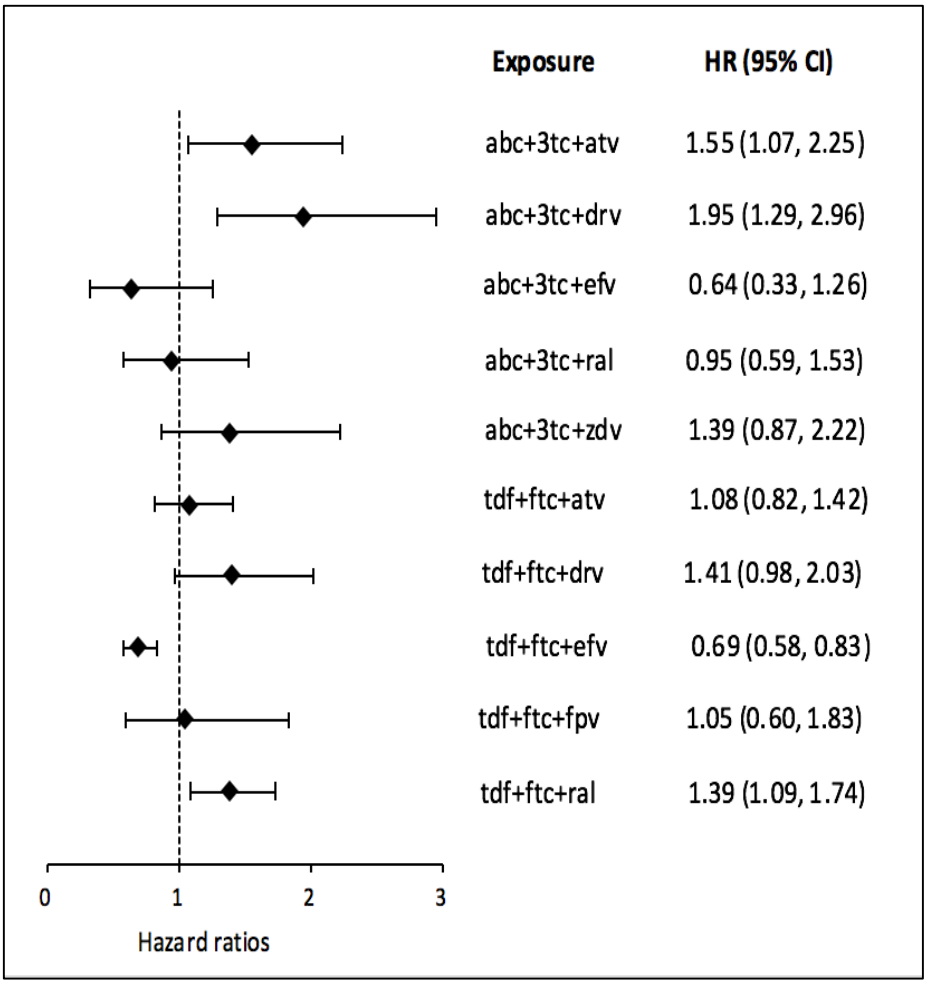
GPI: Generic Product Identifier; CPT: Current Procedural Terminology;
ICD-9-CM: International Classification of Disease, 9th Revision, Clinical Modification.
*Additional filter (age≥18) applied to obtain final cohort.

Figure 2. Risk of myocardial infarction from exposure to individual antiretroviral agents



Hazard ratios calculated from marginal structural models. See appendix table 2 for covariate adjustment.

Figure 3. Risk of myocardial infarction from exposure to combinations of ARV agents



Hazard ratios calculated from marginal structural models. See appendix table 3 for covariate adjustment. abc: abacavir; 3tc: lamivudine; atv: atazanavir; drv: darunavir; fpv: fosamprenavir; tdf: tenofovir; ftc: emtricitabine; efv: efavirenz; ral: raltegravir;

Appendices

Appendix table 1. ICD-9-CM codes used for defining various covariates and outcome	
Variable	ICD-9-CM Code
Acute myocardial infarction	410.xx
Tobacco use	305.1; v15.82
Substance abuse (dependent and non-dependent)	304.xx, 305.xx
Alcohol abuse (alcohol dependence and alcohol abuse)	303.xx, 305.0x
Overweight/obese	278.00, 278.01, 278.02
Diabetes mellitus	250.xx, 357.2x, 362.0x, 366.41
Essential hypertension	401.xx
Hypercholesterolemia	272.0x
Hypertriglyceridemia	272.1x
Mixed hyperlipidemia	272.2x
Other and unspecified hyperlipidemia	272.4x
Lipodystrophy	272.6x
Chronic kidney disease	585.xx
Heart failure	402.01, 402.91, 428%, 404.01, 404.03,404.11,404.13,404.91, 404.93
Cardiac dysrhythmia	427.xx
Old myocardial infarction	427.xx
Coronary atherosclerosis	414.xx
Stroke	434.xx
Hepatitis B virus infection	070.2x, 070.3x, V02.61
Hepatitis C virus infection	070.41, 070.44, 070.51, 070.54, 070.7x, v02.62
Any cancer	140-149, 150-159, 160-169, 170-179, 180-189, 190-199, 200-209, 210-229, 230-239

ICD-9-CM: International Classification of Disease, 9th Revision, Clinical Modification.

Appendix Table 2. Risk of myocardial infarction from current exposure to individual ARV agents as compared to others among HIV-infected individuals

Anti-retroviral drug	Unadjusted Cox Model HR (95% CI; p value)	Adjusted Cox Model[#] HR (95% CI; p value)	Marginal Structural Model[*] HR (95% CI; p value)
Abacavir	1.56 (1.25, 1.94; p=0.000)	1.22 (0.98, 1.52; p=0.08)	1.33 (1.06, 1.66; p=0.012)
Tenofovir	0.55 (0.46, 0.66; p=0.000)	0.76 (0.63, 0.91; p=0.003)	0.70 (0.58, .85; p=0.000)
Lamivudine	1.57 (1.30, 1.90; p=0.000)	1.19 (0.98, 1.45; p=0.083)	1.32(1.09, 1.61; p=0.005)
Zidovudine	1.39 (1.08, 1.80; p=0.010)	1.70 (0.90, 1.52; p=0.236)	1.22 (0.94, 1.59; p=0.128)
Didanosine	2.04 (1.26, 3.30; p=0.004)	1.78 (1.10, 2.88; p=0.019)	1.83 (1.13, 2.96 p=0.015)
Emtricitabine	0.54 (0.46, 0.65; p=0.000)	0.76 (0.64, 0.92; p=0.004)	0.71 (0.59, 0.85; p=0.000)
Atazanavir	0.97 (0.76, 1.24; p=0.813)	1.01 (0.79, 1.30; p=0.944)	0.99 (0.77, 1.28; p=0.947)
Darunavir	1.73 (1.38, 2.16; p=0.000)	1.42(1.13, 1.78; p=0.002)	1.54 (1.22, 1.94; p=0.000)
Lopinavir	1.63 (1.25, 2.14; p=0.000)	1.60 (1.22, 2.09; p=0.001)	1.71 (1.31, 2.25; p=0.000)
Fosamprenavir	1.20 (0.74, 1.94; p=0.466)	1.14 (0.70, 1.84; p=0.599)	1.08 (0.67, 1.77; p=0.744)
Efavirenz	0.61 (0.51, 0.73; p=0.000)	0.69 (0.58, 0.82; p=0.000)	0.66 (0.55, 0.79; p=0.000)
Nevirapine	1.12 (0.79, 1.58; p=0.528)	0.89 (0.63, 1.27; p=0.527)	0.98 (0.68, 1.40; p=0.894)
Raltegravir	1.72 (1.40, 2.11; p=0.000)	1.27 (1.03, 1.58; p=0.028)	1.39 (1.12, 1.74; p=0.003)

#Adjusted for baseline covariates: sex, tobacco use (ever), substances or alcohol abuse (ever), hepatitis B & C, stroke, cancer, old myocardial infarction, and time-dependent covariates: age, body weight, CKD, dyslipidemia, heart failure, cardiac dysrhythmia, atherosclerosis, diabetes mellitus, receipt of anti-hyperglycemic agents, hypertension, and receipt of medications for cardiovascular disease (aspirin, beta-blocker, angiotensin converting enzyme inhibitor, angiotensin receptor blocker, calcium channel blocker, and statins).

*In addition to adjusting for weights generated from the treatment model, the marginal model is adjusted for time-fixed covariates: sex, ever tobacco use, ever alcohol or substance abuse, and baseline covariates: age, stroke, cancer, hepatitis B & C, year of ART initiation, symptomatic HIV disease, CKD, dyslipidemia, old AMI, heart failure, cardiac dysrhythmia, atherosclerosis, diabetes mellitus, receipt of anti-hyperglycemic agents, hypertension, and receipt of medications for cardiovascular disease (aspirin, beta-blocker, angiotensin converting enzyme inhibitor, angiotensin receptor blocker, calcium channel blocker, and statins).

abc: abacavir; 3tc: lamivudine; atv: atazanavir; drv: darunavir; fpv: fosamprenavir; tdf: tenofovir; ftc: emtricitabine; efv: efavirenz; ral: raltegravir;

Appendix Table 3. Risk of myocardial infarction from current exposure to combinations of ARV agents as compared to others among HIV-infected individuals receiving ART

Anti-retroviral drug	Unadjusted Cox Model HR (95% CI; p value)	Adjusted Cox Model HR[#] (95% CI; p value)	Marginal Structural Model[*] HR (95% CI; p value)
abc+lmv+atv	1.63 (1.13, 2.36; p=0.009)	1.49 (1.03, 2.14; p=0.032)	1.55 (1.07, 2.25; p=0.02)
abc+lmv+drv	2.34 (1.58, 3.47; p=0.000)	1.69 (1.13, 2.53; p=0.010)	1.95 (1.29, 2.96; p=0.002)
abc+lmv+zdv	1.41 (0.89, 2.23; p=0.140)	1.24 (0.79, 1.94; p=0.356)	1.39 (0.87, 2.22; p=0.169)
abc+lmv+efv	0.74 (0.38, 1.43; p=0.375)	0.52 (0.27, 1.02; p=0.056)	0.64 (0.33, 1.26; p=0.197)
abc+lmv+ral	1.40 (0.90, 2.19; p=0.136)	0.88 (0.56, 1.39; p=0.583)	0.95 (0.59, 1.53; p=0.821)
tdf+ftc+atv	0.97 (0.75, 1.26; p=0.815)	1.09 (0.84, 1.42; p=0.504)	1.08 (0.82, 1.42; p=0.578)
tdf+ftc+drv	1.20 (0.92, 1.56; p=0.178)	1.16 (0.89, 1.51; p=0.282)	1.41 (0.98, 2.03; p=0.062)
tdf+ftc+efv	0.60 (0.50, 0.72; p=0.000)	0.73 (0.61, 0.88; p=0.001)	0.69 (0.58, 0.83; p=0.000)
tdf+ftc+fpv	1.11 (0.64, 1.93; p=0.710)	1.11 (0.64, 1.92; p=0.709)	1.05 (0.60, 1.83; p=0.860)
tdf+ftc+ral	1.47 (1.18, 1.84; p=0.001)	1.35 (1.07, 1.69; p=0.010)	1.38 (1.09, 1.74; p=0.006)

#Adjusted for baseline covariates: sex, tobacco use (ever), substances or alcohol abuse (ever), hepatitis B & C, stroke, cancer, old myocardial infarction, and time-dependent covariates: age, body weight, CKD, dyslipidemia, heart failure, cardiac dysrhythmia, atherosclerosis, diabetes mellitus, receipt of anti-hyperglycemic agents, hypertension, and receipt of medications for cardiovascular disease (aspirin, beta-blocker, angiotensin converting enzyme inhibitor, angiotensin receptor blocker, calcium channel blocker and statins).

*In addition to adjusting for weights generated from the treatment model, the marginal model is adjusted for time-fixed covariates: sex, ever tobacco use, ever alcohol or substance abuse, and baseline covariates: age, stroke, cancer, hepatitis B & C, year of ART initiation, symptomatic HIV disease, CKD, dyslipidemia, old AMI, heart failure, cardiac dysrhythmia, atherosclerosis, diabetes mellitus, receipt of anti-hyperglycemic agents, hypertension, and receipt of medications for cardiovascular disease (aspirin, beta-blocker, angiotensin converting enzyme inhibitor, angiotensin receptor blocker, calcium channel blocker, and statins).

abc: abacavir; 3tc: lamivudine; atv: atazanavir; drv: darunavir; fpv: fosamprenavir; tdf: tenofovir; ftc: emtricitabine; efv: efavirenz; ral: raltegravir;

References

1. UNAIDS. AIDS by the numbers 2015 2015 [Available from: http://www.unaids.org/en/resources/campaigns/HowAIDSchangedeverything/factsheet.
2. Mocroft A, Vella S, Benfield TL, Chiesi A, Miller V, Gargalianos P, et al. Changing patterns of mortality across Europe in patients infected with HIV-1. EuroSIDA Study Group. Lancet. 1998;352(9142):1725-30.
3. Group DADS, Friis-Moller N, Reiss P, Sabin CA, Weber R, Monforte A, et al. Class of antiretroviral drugs and the risk of myocardial infarction. N Engl J Med. 2007;356(17):1723-35.
4. Friis-Moller N, Sabin CA, Weber R, d'Arminio Monforte A, El-Sadr WM, Reiss P, et al. Combination antiretroviral therapy and the risk of myocardial infarction. N Engl J Med. 2003;349(21):1993-2003.
5. Desai M, Joyce V, Bendavid E, Olshen RA, Hlatky M, Chow A, et al. Risk of Cardiovascular Events Associated With Current Exposure to HIV Antiretroviral Therapies in a US Veteran Population. Clin Infect Dis. 2015;61(3):445-52.
6. Sabin CA, Worm SW, Weber R, Reiss P, El-Sadr W, Dabis F, et al. Use of nucleoside reverse transcriptase inhibitors and risk of myocardial infarction in HIV-infected patients enrolled in the D : A : D study: a multi-cohort collaboration. Lancet. 2008;371(9622):1417-26.
7. Worm SW, Sabin C, Weber R, Reiss P, El-Sadr W, Dabis F, et al. Risk of Myocardial Infarction in Patients with HIV Infection Exposed to Specific Individual Antiretroviral Drugs from the 3 Major Drug Classes: The Data Collection on Adverse Events of Anti-HIV Drugs (D:A:D) Study. J Infect Dis. 2010;201(3):318-30.
8. Behrens G, Dejam A, Schmidt H, Balks HJ, Brabant G, Korner T, et al. Impaired glucose tolerance, beta cell function and lipid metabolism in HIV patients under treatment with protease inhibitors. AIDS. 1999;13(10):F63-70.
9. Carr A, Samaras K, Burton S, Law M, Freund J, Chisholm DJ, et al. A syndrome of peripheral lipodystrophy, hyperlipidaemia and insulin resistance in patients receiving HIV protease inhibitors. AIDS. 1998;12(7):F51-8.
10. Carr A, Samaras K, Thorisdottir A, Kaufmann GR, Chisholm DJ, Cooper DA. Diagnosis, prediction, and natural course of HIV-1 protease-inhibitor-associated lipodystrophy, hyperlipidaemia, and diabetes mellitus: a cohort study. Lancet. 1999;353(9170):2093-9.
11. Periard D, Telenti A, Sudre P, Cheseaux JJ, Halfon P, Reymond MJ, et al. Atherogenic dyslipidemia in HIV-infected individuals treated with protease inhibitors. The Swiss HIV Cohort Study. Circulation. 1999;100(7):700-5.
12. Purnell JQ, Zambon A, Knopp RH, Pizzuti DJ, Achari R, Leonard JM, et al. Effect of ritonavir on lipids and post-heparin lipase activities in normal subjects. AIDS. 2000;14(1):51-7.
13. Walli R, Herfort O, Michl GM, Demant T, Jager H, Dieterle C, et al. Treatment with protease inhibitors associated with peripheral insulin resistance and impaired oral glucose tolerance in HIV-1-infected patients. AIDS. 1998;12(15):F167-73.
14. Baum PD, Sullam PM, Stoddart CA, McCune JM. Abacavir increases platelet reactivity via competitive inhibition of soluble guanylyl cyclase. AIDS (London, England). 2011;25(18):2243-8.
15. Hsue PY, Hunt PW, Wu Y, Schnell A, Ho JE, Hatano H, et al. Association of abacavir and impaired endothelial function in treated and suppressed HIV-infected patients. AIDS. 2009;23(15):2021-7.

16. Satchell CS, O'Halloran JA, Cotter AG, Peace AJ, O'Connor EF, Tedesco AF, et al. Increased Platelet Reactivity in HIV-1-Infected Patients Receiving Abacavir-Containing Antiretroviral Therapy. J Infect Dis. 2011;204(8):1202-10.
17. Bozzette SA, Ake CF, Tam HK, Chang SW, Louis TA. Cardiovascular and cerebrovascular events in patients treated for human immunodeficiency virus infection. N Engl J Med. 2003;348(8):702-10.
18. Klein D, Hurley LB, Quesenberry CP, Jr., Sidney S. Do protease inhibitors increase the risk for coronary heart disease in patients with HIV-1 infection? J Acquir Immune Defic Syndr. 2002;30(5):471-7.
19. Molina JM, Andrade-Villanueva J, Echevarria J, Chetchotisakd P, Corral J, David N, et al. Once-daily atazanavir/ritonavir versus twice-daily lopinavir/ritonavir, each in combination with tenofovir and emtricitabine, for management of antiretroviral-naive HIV-1-infected patients: 48 week efficacy and safety results of the CASTLE study. Lancet. 2008;372(9639):646-55.
20. Monforte A, Reiss P, Ryom L, El-Sadr W, Dabis F, De Wit S, et al. Atazanavir is not associated with an increased risk of cardio- or cerebrovascular disease events. AIDS. 2013;27(3):407-15.
21. Mills AM, Nelson M, Jayaweera D, Ruxrungtham K, Cassetti I, Girard PM, et al. Once-daily darunavir/ritonavir vs. lopinavir/ritonavir in treatment-naive, HIV-1-infected patients: 96-week analysis. AIDS. 2009;23(13):1679-88.
22. Overton ET, Arathoon E, Baraldi E, Tomaka F. Effect of darunavir on lipid profile in HIV-infected patients. HIV Clin Trials. 2012;13(5):256-70.
23. Ucciferri C, Falasca K, Vignale F, Di Nicola M, Pizzigallo E, Vecchiet J. Improved metabolic profile after switch to darunavir/ritonavir in HIV positive patients previously on protease inhibitor therapy. J Med Virol. 2013;85(5):755-9.
24. Bedimo RJ, Westfall AO, Drechsler H, Vidiella G, Tebas P. Abacavir Use and Risk of Acute Myocardial Infarction and Cerebrovascular Events in the Highly Active Antiretroviral Therapy Era. Clin Infect Dis. 2011;53(1):84-91.
25. Choi AI, Vittinghoff E, Deeks SG, Weekley CC, Li YM, Shlipak MG. Cardiovascular risks associated with abacavir and tenofovir exposure in HIV-infected persons. AIDS. 2011;25(10):1289-98.
26. Lang S, Mary-Krause M, Cotte L, Gilquin J, Partisani M, Simon A, et al. Impact of individual antiretroviral drugs on the risk of myocardial infarction in human immunodeficiency virus-infected patients: A case-control study nested within the French hospital database on HIV ANRS cohort CO4. Arch Intern Med. 2010;170(14):1228-38.
27. Palella Jr FJ, Althoff K, Moore R, Zhang J, Kitahata M, Gange S, et al., editors. Abacavir Use and Risk for Myocardial Infarction in the NA-ACCORD. CROI 2015; 2015 February 23-26, 2015; Seattle.
28. IMS Health. IMS' PharMetrics Plus Data Dictionary 2013 [Available from: http://tri.uams.edu/files/2015/08/Pharmetrics-Plus-Data-Dictionary-Jan-2013.pdf.
29. Fewell Z, Hernan MA, Wolfe F, Tilling K, Choi H, Sterne JAC. Controlling for time-dependent confounding using marginal structural models. The Stata Journal 2004;4(4):402-20.
30. Brouwer ES, Napravnik S, Eron Jr JJ, Stalzer B, Floris-Moore M, Simpson Jr RJ, et al. Effects of combination antiretroviral therapies on the risk of myocardial infarction among HIV patients. Epidemiology. 2014;25(3):406-17.

31. Durand M, Sheehy O, Lelorier J, Tremblay CL. Association between use of antiretroviral therapy and risk of acute myocardial infarction: A nested case control study using Quebec's Public Health Insurance Database (RAMQ). J Popul Ther Clin Pharmacol. 2011;18(2):e178-e9.
32. Marcus JL, Neugebauer RS, Leyden WA, Chao CR, Xu L, Quesenberry CP, et al. Use of abacavir and risk of cardiovascular disease among HIV-infected individuals. J Acquir Immune Defic Syndr. 2015.
33. Martin A, Bloch M, Amin J, Baker D, Cooper DA, Emery S, et al. Simplification of Antiretroviral Therapy with Tenofovir-Emtricitabine or Abacavir-Lamivudine: A Randomized, 96-Week Trial. Clin Infect Dis. 2009;49(10):1591-601.
34. Obel N, Farkas DK, Kronborg G, Larsen CS, Pedersen G, Riis A, et al. Abacavir and risk of myocardial infarction in HIV-infected patients on highly active antiretroviral therapy: A population-based nationwide cohort study. HIV Med. 2010;11(2):130-6.
35. Strategies for Management of Anti-Retroviral Therapy I, Groups DADS. Use of nucleoside reverse transcriptase inhibitors and risk of myocardial infarction in HIV-infected patients. AIDS (London, England). 2008;22(14):F17-24.
36. Young J, Xiao Y, Moodie EEM, Abrahamowicz M, Klein MB, Bernasconi E, et al. Effect of Cumulating Exposure to Abacavir on the Risk of Cardiovascular Disease Events in Patients From the Swiss HIV Cohort Study. J Acquir Immune Defic Syndr. 2015;69(4):413-21.
37. Santos JR, Saumoy M, Curran A, Bravo I, Llibre JM, Navarro J, et al. The lipid-lowering effect of tenofovir/emtricitabine: a randomized, crossover, double-blind, placebo-controlled trial. Clin Infect Dis. 2015;61(3):403-8.
38. Delaney JA, Scherzer R, Biggs ML, Shliplak MG, Polak JF, Currier JS, et al. Associations of antiretroviral drug use and HIV-specific risk factors with carotid intima-media thickness. AIDS. 2010;24(14):2201-9.
39. Hill A, Sawyer W, Gazzard B. Effects of first-line use of nucleoside analogues, efavirenz, and ritonavir-boosted protease inhibitors on lipid levels. HIV Clin Trials. 2009;10(1):1-12.
40. Eron JJ, Young B, Cooper DA, Youle M, Dejesus E, Andrade-Villanueva J, et al. Switch to a raltegravir-based regimen versus continuation of a lopinavir-ritonavir-based regimen in stable HIV-infected patients with suppressed viraemia (SWITCHMRK 1 and 2): two multicentre, double-blind, randomised controlled trials. Lancet. 2010;375(9712):396-407.
41. Young L, Wohl DA, Hyslop WB, Lee YZ, Napravnik S, Wilkin A. Effects of raltegravir combined with tenofovir/emtricitabine on body shape, bone density, and lipids in African-Americans initiating HIV therapy. HIV Clin Trials. 2015;16(5):163-9.
42. Lennox JL, Dejesus E, Berger DS, Lazzarin A, Pollard RB, Ramalho Madruga JV, et al. Raltegravir versus Efavirenz regimens in treatment-naive HIV-1-infected patients: 96-week efficacy, durability, subgroup, safety, and metabolic analyses. J Acquir Immune Defic Syndr. 2010;55(1):39-48.

Chapter Two: Risk of Cardiovascular Disease from Current, Recent, and Cumulative Exposure to Abacavir among HIV-infected Individuals Receiving Antiretroviral Therapy in the United States

Abstract

Background

There is an ongoing controversy on the association between abacavir use and cardiovascular disease (CVD) and a lack of understanding on how the risk may vary with an accumulating exposure to abacavir.

Methods

I assessed the risk of a first episode of CVD, defined as an acute myocardial infarction or having a coronary intervention procedure following exposure to abacavir among 72,733 HIV-infected individuals started on anti-retroviral (ARV) medications between 2009 and 2014 using a large administrative health plan dataset in the United States. I used marginal structural models with weights generated from inverse probability of receiving treatment. I adjusted for covariates age, sex, year of start of ART in the database, overweight or obesity, substance or alcohol abuse, tobacco use, presence of other forms of heart diseases, use of CVD-related medications, hypertension, diabetes mellitus, dyslipidemia, chronic kidney disease (CKD), lipodystrophy, stroke, hepatitis B and C infections, and cancer.

Results

Over 114,470 person-years of exposure to ART, 714 CVD events occurred at an incidence rate (IR) of 6.23 (95% CI: 5.80, 6.71) per 1000 person-years. Individuals exposed to abacavir had a higher IR of 9.74 (95% CI: 8.24, 11.52)/1000 person-years as compared to 5.75 (95% CI: 5.30, 6.24)/1000 person-years for exposure to other ARV agents. I observed increased hazard ratios [HR (95% CI)] for current [1.40 (1.15, 1.70)], recent [1.29 (0.95, 1.75)], and cumulative [1.16 (1.04, 1.28) per year] exposure to abacavir. The risk followed an inverted U-shaped pattern, levelling off only after 24 months of cumulative exposure. In sensitivity analyses, I saw a similar increased risk when the study population was restricted to those free of pre-existing heart disease and those not using illicit substance at baseline.

Conclusion

Use of abacavir is associated with an increased risk of CVD. The study results are most compatible with a gradual underlying biological mechanism.

Introduction

Cardiovascular disease (CVD) is responsible for around 16% of deaths among HIV-infected individuals (1). The risk factors for CVD are more prevalent among HIV-infected individuals (2), and use of various ARV drugs has been shown to be associated with an increased risk of CVD (3). In the history of ART use, whether and how abacavir leads to an increased risk of CVD has arguably been the most intensely debated subject. Abacavir, a guanosine analog nucleoside reverse transcriptase inhibitor (NRTI) that possess retroviral suppressive properties similar to tenofovir (4), is a commonly prescribed "anchor" ARV agent. However, the prescription of abacavir dropped after the Data Collection on Adverse Events of Anti-HIV Drugs (D:A:D) study group reported in 2008 an increased risk of acute myocardial infarction (AMI) among HIV-infected individuals exposed to abacavir (5). Independent investigations that were subsequently carried out both supported (6-15) and refuted (16-21) the D:A:D study's findings.

While studies conducted more recently mostly suggested an increased risk of CVD from abacavir exposure (6, 8, 10, 13), they were limited by having a relatively small number of outcomes, with results failing to reach statistical significance (6, 10). Failure to identify a clear underlying biological mechanism to explain the epidemiologic findings has added to the conundrum (22). There is a lack of consensus on whether the risk of CVD from exposure to abacavir reverses within a few months of stopping the drug (5, 15) and a lack of understanding on how the risk varies as exposure accumulates. In this study, I have endeavored to address these questions by investigating the risk of CVD from current, recent, and cumulative exposure to abacavir among HIV-infected individuals using marginal structural models, and interpret my findings in the context of possible underlying biological mechanism for such an increased risk.

Methods

Study population and data source

I assessed the risk of CVD among 72,733 HIV-infected individuals who started ARV drugs in the U.S. between October 1, 2009 and December 31, 2014 and were enrolled in the IMS' PharMetrics Plus database. A start date of October 1, 2009 was chosen based on availability of data. PharMetrics Plus is one of the largest health plan insurance claims databases in the U.S. comprised of adjudicated claims for more than 150 million unique enrollees from across the four regions of the country (23). The data undergo a series of quality checks to minimize errors. I used a pre-defined algorithm (Figure 1) to extract and define my study population of HIV-

infected individuals exposed to any ART in the database. I restricted my study population to those greater than 18 years of age. Individuals were censored at either 1) the first occurrence of CVD after start of exposure, 2) last recorded date of ART receipt in the database, or 3) December 31, 2014, whichever occurred first.

Exposure, covariate, and outcome definitions

Exposures to specific ARV agents were identified by their unique generic product identifier (GPI) codes. Person-time of exposure to abacavir was compared to exposure to ARV agents other than abacavir. Any two prescriptions for an ARV agent separated by less than 30 days were combined to represent a single continuous exposure. The data are longitudinal in nature, with each subject's follow up time divided into consecutive one-month periods during which the treatment is allowed to vary. The values of covariates are updated at the start of each month and the outcome for an individual is defined as the first occurrence of an AMI or receipt of a coronary intervention procedure after initiation of the exposure. This temporal ordering of covariate, treatment, and outcome allows for a time-varying analysis, with an opportunity for a causal interpretation. The first observation of a time-dependent covariate corresponds to its baseline value. Once an individual develops a health condition, he/she is assumed to have the condition for the remainder of the study. For example, an individual developing diabetes mellitus at a certain time point is assumed to have it for the rest of the study period. Current exposure to abacavir is defined as exposure (yes/no) during each one-month observation period. Recent exposure is defined as exposure (yes/no) in the last six months, including the current month. Cumulative exposure is defined as the total duration of exposure an individual had received at a particular time point, and is updated every month. Duration of exposure ceases to accumulate upon discontinuation of the drug but resumes if the drug is restarted. Covariates and outcomes were ascertained using International Classification of Disease, 9th Revision, Clinical Modification (ICD-9-CM) or Current Procedural Terminology (CPT) codes (Appendix table 1).

Statistical Analysis

I assessed the risk of CVD from a current, recent, and cumulative exposure to abacavir through marginal structural models using stabilized inverse probability of treatment weights (sIPTW). I used pooled logistic regression for my treatment and marginal models. For the denominator of the sIPTW, I modelled exposure to abacavir as a function of time-fixed covariates: sex, tobacco use (ever), substance or alcohol abuse (ever), serologic evidence of hepatitis B & C infections, stroke, cancer, and old myocardial infarction, and time dependent covariates: age, year of ART initiation, body weight, chronic kidney disease (CKD), dyslipidemia, heart failure, cardiac dysrhythmia, atherosclerosis, diabetes mellitus, receipt of anti-hyperglycemic agents, hypertension, and receipt of medications for cardiovascular disease (i.e. aspirin, beta-blocker, angiotensin converting enzyme inhibitor, angiotensin receptor blocker, calcium channel blocker, statins). The numerator of the stabilized weight was modelled as a function of baseline or time-fixed covariates only. Follow up time was modelled as a function of natural cubic

splines with three knots. I assumed that the patients remained on abacavir once they started it. In addition to adjusting for the stabilized weight generated from the treatment model, the marginal model was further adjusted for the baseline or time-fixed covariates. The same treatment weights were used for estimation of CVD risk from current, recent, and cumulative exposure to abacavir. I additionally categorized cumulative exposure to abacavir into six groups, i.e., never exposed, 1-6, 7-12, 13-18, 19-24, and 25-48 months of exposure, and assessed how the risk of CVD varied as a function of these time-periods of exposure. I assumed no exposure for exposures that occurred more than 48 months ago. I assessed the reversibility of the risk by comparing the risk of CVD between individuals who were exposed to abacavir for any duration prior to the last six months but not exposed to abacavir in the last six months (i.e. those who had stopped abacavir six months ago) to individuals who were never exposed to abacavir. In sensitivity analyses, I restricted the study population to individuals free of cardiovascular disease at baseline, i.e., those not having prior AMI, heart failure, atherosclerosis, and cardiac arrhythmia, and to individuals without a history of alcohol and substance abuse at baseline. In addition to the marginal structural results, I calculated corresponding unadjusted and adjusted results from conventional Cox models. I assumed uninformative censoring for my study. I extracted and processed my data from the main claims databases using TERADATA, SAS (Version 9.1), and STATA (13.1), and implemented the marginal structural models in STATA, following the steps shown by Fewell et al (24). Additional description of the marginal structural models with the equations and the notations are provided in the appendix 1. The study was approved by the Committee for Protection of Human Subject (CPHS) at University of California, Berkeley.

Results

On average, participants are exposed to ART for 2.8 years. The mean age of the study population was 46 years and 82% were males. There were 72,733 individuals in the study contributing 114,470 person-years of exposure to anti-retroviral agents, over which 714 CVD events occurred at an incidence rate of 6.23 (95% CI: 5.80, 6.71)/1000 person-years. Of the 714 outcomes, 137 were observed over 14,060 person-years of exposure to abacavir at an incidence rate of 9.74 (95% CI: 8.24, 11.52)/1000 person-years, as compared to 5.75 (95% CI: 5.30, 6.24)/1000 person-years for those exposed to other ARV drugs. The incidence rate of CVD was highest for those exposed to abacavir for 13-18 months (11.32/1000 person-years) (table 1). The overall incidence rate of AMI was 4.77 (95% CI: 4.39, 5.19)/1000 person-years and was highest for those > 70 years of age (table 2).

The prevalences of essential hypertension, diabetes mellitus, chronic kidney disease (CKD), dyslipidemia, lipodystrophy, heart diseases, and use of cardiovascular medications was higher among abacavir recipients at baseline (table 3). A pooled logistic regression for the treatment model showed that increasing age, CKD, symptomatic HIV infection, and lipodystrophy were associated with an increased probability of receiving abacavir whereas male sex, initiating ART after 2009, substance or alcohol abuse, any cancer, and receipt of medications for CVD were associated with a decreased probability of receiving abacavir (table 4). I found an increased risk

of CVD from a current exposure to abacavir from both a marginal structural model (HR: 1.40; 95% CI: 1.15, 1.70) and a corresponding extended Cox model (HR: 1.32; 95% CI: 1.09, 1.60). I also found an increased risk of CVD from a cumulative exposure to abacavir from both a marginal structural model (HR: 1.16 per year; 95% CI: 1.04-1.28 per year) and an extended Cox model (HR: 1.13; 95% CI: 1.02, 1.25) (table 5). This finding of an increased risk from an additional year of exposure prompted me to further assess the risk as a function of categories of increasing duration of cumulative exposure. I noted that the hazard ratio of CVD varied with duration of exposure in a U-shaped pattern (figure 2 and appendix table 2). The hazard of CVD continued to increase for up to 24 months of exposure, after which the risk decreased to non-significant levels. Through an intuitive modelling approach, I assessed the reversibility of risk by directly comparing the risk of CVD among those exposed to abacavir prior to but not in the last 6, 12, and 18 months to those who were never exposed. I observed a significantly increased risk (HR: 2.19; 95% CI: 1.18, 4.12) among those who had stopped abacavir six months previously as compared to those who were never exposed. There were few people who had received abacavir 12 or 18 months previously but had not received it the last 12 or 18 months (Appendix table 3).

In addition to the exposure variable, the factors that were significantly associated with CVD were increasing age, male sex, tobacco use, other heart diseases, prior AMI, use of CVD-related medications, diabetes mellitus, and dyslipidemia (Appendix table 4). Given the strong association of prior AMI and other heart diseases with the outcome, I conducted a sensitivity analysis by restricting the study to individuals without a prior AMI or heart diseases at baseline. I continued to observe an increased risk (HR: 1.49; 95% CI: 1.22, 1.84) in this subgroup (Appendix table 5). I assessed this relationship by excluding other cardiovascular diseases (heart failure, cardiac arrhythmia, atherosclerosis, or receipt of cardiovascular medications) from my adjustment set of covariates in both the treatment model for the marginal structural model and the extended Cox model. The results remained the same. I observed an increased risk (HR: 1.37; 95% CI: 1.13, 1.66) when the study population was restricted to individuals not using illicit substances or alcohol at baseline (Appendix table 6). Using extended Cox models, I assessed if the risk of CVD from current exposure to abacavir differed by the following baseline variables: sex, age, smoking, substance or alcohol abuse, overweight/obese, CKD, heart disease, diabetes mellitus, hypertension, dyslipidemia, and lipodystrophy. I did not find evidence of effect modification by these variables (Appendix table 6). I assessed the relationship between current abacavir exposure and the risk of AMI by excluding individuals whose CVD diagnoses were based on the presence of coronary intervention procedures; the risk was increased (HR: 1.33; 95% CI: 1.06, 1.66).

Discussion

I found an increased risk of AMI and CVD associated with exposure to abacavir using both conventional Cox models and marginal structural models. I found a trend of an increasing risk from a cumulative exposure for up to 24 months. The overall incidence rate of AMI in my study was higher (4.77/1000 person-years) than in the D:A:D study (3.3/1000 person-years). In

comparison, AMI incidences of 1.41/1000 people and 1.2/1000 people were seen in the general population in the Olmstead county in Minnesota in 2006 and in men 35-65 years of age in the Framingham study population, respectively (25, 26). The incidence rates of AMI associated with exposure to abacavir in this study (6.9/1000 person-years) and in the D:A:D study (6.1/1000 person-years) were ~4-5 fold higher than in the general population. I found a significantly increased HR from current and cumulative exposure to abacavir, and a statistically non-significant increased HR from a recent exposure to abacavir. Whereas the D:A:D study found a 90% increased risk of AMI associated with recent exposure, I found only a 40% increased risk associated with current exposure and a 29% increased risk associated with recent exposure, similar to findings reported by Desai et al. (HR: 1.50) (8) and Palella et al. (HR: 1.33) (13). My cumulative exposure model showed a 16% increased risk associated with an additional year of exposure, close to the 14% increased risk observed in the D:A:D study (5).

In an attempt to elucidate an underlying biological mechanism for the increase in the risk of CVD associated with abacavir use, I assessed how the risk of CVD varied with duration of exposure. I found that both the incidence rate of AMI and the hazard ratio increased with increasing duration of exposure in an inverted U-shaped pattern, peaking between 13-24 months of exposure and levelling off thereafter. This finding differs from the results of the D:A:D study, which found that the risk was increased only in the first 6 months of receiving abacavir, suggesting an underlying acute inflammatory mechanism is involved in the pathogenesis. Whereas my finding does not support an acute underlying process leading to AMI only in around the first six months of receiving abacavir, it also is not consistent with a dyslipidemia-related traditional atherogenic mechanism, which would be expected to be associated with an increase in the risk with increasing duration of exposure, and not levelling off after 24 months. One of the possible underlying biological mechanisms put forward is previously abacavir-induced platelet hyper-reactivity and aggregation through an active metabolite, carbovir-triphosphate (22, 27, 28). It is possible that abacavir may trigger an acute platelet response leading to endothelial injury with a longer lasting impact. An acute inflammatory response is another proposed underlying mechanism for an increased risk of CVD as a result of exposure to abacavir (14). Whether or not individuals with pre-existing coronary artery disease, such as atherosclerosis, a prior AMI, and coronary interventions may develop CVD more rapidly as a result of exposure to abacavir is unclear. While I did not find a difference in the time to development of CVD in these risk groups from my test of interactions, Choi et al. found a significant difference in the risk of CVD among sub-groups defined by the presence or absence of dyslipidemia in their study (7). Future studies should explore these areas further in a trans-disciplinary fashion from both a basic science and an epidemiologic perspective. While a finding of an increased risk of AMI within the first few months of receiving abacavir may connote an equally rapid reversal of the risk after stopping abacavir, this may not always be true, because an initial acute insult on the coronary vasculature could have an impact such that it takes longer time to reverse also. Through an intuitive exposure representation in my study, I showed that the risk of CVD associated with exposure to abacavir remains elevated six months after stopping abacavir. Due to inadequate exposure-time and very few outcomes, this study could not further determine exactly when the risk reverses after stopping exposure beyond six months. Young et al. also recently reported an elevated risk of CVD among individuals who had

received abacavir continually for the past four years (15). My study results suggest a reversible but more gradual underlying mechanism or an acute underlying process with a longer lasting impact that takes a longer time to regress after removal of the exposure.

I used marginal structural models for my study because first, individuals with certain risk factors for CVD such as CKD, hypertension, diabetes mellitus, and dyslipidemia, may be preferentially channeled into (or away from) receiving abacavir based on its known toxicity in the presence of these conditions, and such individuals may differ from the referent group in other unmeasured characteristics (e.g. race and socio-economic status) as well. Causal inference methods such as marginal structural models provide a valuable tool for balancing exposure groups through an inverse of the probability of receiving treatment after making a reasonable convenience assumption to identify a causal path between the exposure and the outcome (29, 30). Second, adjusting through traditional methods for covariates that may simultaneously serve as confounders and causal intermediates, can lead to biased results (30). After balancing the various covariates between the exposure groups, I found an increased risk of CVD from exposure to abacavir. Marcus et al. and Desai et al. both used marginal structural models for their analyses (8, 10). However, Marcus et al. reported estimates without having adjusted for any baseline/time-fixed covariates in the marginal model (10). Cole and Hernan have discussed the importance of adjusting for baseline covariates in the marginal model to make up for the compromise of the IPTW through the weight stabilization process (31). Desai et al. have included time-dependent covariates in their marginal model (8); Robins et al. have stated that inclusion of time-dependent covariates in the marginal model will bias the result (30).

In a sensitivity analysis, I observed an increased risk of CVD among individuals free of heart disease at baseline. Informed by a finding by Lang et al. (17) that the risk of CVD is not elevated among individuals not using illicit substances, I restricted my study population to those without a history of substance abuse at baseline. I saw a significantly increased risk in this sub-group also. My study data are after 2009, i.e. after the publication of the D:A:D study in 2008, and therefore, I did not conduct a sub-group analysis based on year of initiation of abacavir as other studies have done.

Key strength of my study is that I applied a robust method to answer my study questions in a large U.S. health plan dataset containing longitudinal information on usage of ART in more than 70,000 HIV-infected individuals receiving care across the country. The similarity of my results to those from prior studies, the reproducibility of the results in the sensitivity analyses, and the finding of a background incidence rate of AMI comparable to that found in the D:A:D study are reassuring aspects of my results. A limitation of my study is that the ICD-9 and CPT diagnostic codes used in my study may be prone to coding errors; however, such errors are likely to affect the exposure groups equally and should not bias my study results. The ICD-9 code for AMI has been previously validated in another claims database (32). It is possible that information on covariates for which re-imbursement may not be sought could be under-reported in this database and hence be under reported in the study population. Again, this problem should exist non differentially across both exposure groups. Information on race/ethnicity, CD4 cell count, and HIV viral load were not available in the claims database, and these could be relevant

risk factors. However, the D:A:D study showed that adjustment for CD4 cell count and HIV viral load made little difference to the relative rate of AMI (5). There is potential for bias in my study results from residual confounding that may arise from the binary categorization of most variables in my study, rather than having a graded or a finer response. I assessed the influence such categorization would have for two variables. First, I assessed if the study results changed after including CKD as six separate indicator variables (CKD stage 1-6) rather than a single dichotomous CKD variable. Then I assessed if the results changed after including various lipid disorders as separate entities rather than collapsing them into one dyslipidemia variable. My results did not change for either of the variables.

Conclusion

There is an increased risk of CVD and AMI associated with exposure to abacavir; I recommend a holistic patient evaluation with careful analysis of risks and benefits while formulating an anti-retroviral treatment regimen.

Tables and Figures

Table 1. Incidence rate (IR) of cardiovascular disease* among HIV-infected individuals exposed to abacavir for various periods of time

Duration of exposure to abacavir (months)	Person-years	No. of CVD	IR per 100,000 people (95% CI)
Never exposed	99,384	566	570 (525, 618)
1-6 (recent exposure)	4,757	51	1,072 (815, 1,411)
7-12	3,125	31	992 (698, 1,411)
13-18 months	2,208	25	1,132 (765, 1,676)
19-24 months	1,663	18	1,082 (682, 1,718)
25-48 months	3,333	23	690 (459, 1,039)

*Includes AMI and coronary intervention procedures

Table 2. Age specific incidence rate (IR) of acute myocardial infarction among HIV-infected individuals receiving anti-retroviral therapy			
Age group	Person-years	No. of AMI	IR per 1000 people (95% CI)
18-39	27,869	33	1.18 (0.84, 1.67)
40-49	46,677	149	3.19 (2.72, 3.75)
50-59	32,852	259	7.88 (6.98, 8.90)
60-69	6,779	97	14.31 (11.73, 17.46)
>=70	562	10	17.80 (9.58, 33.08)
Overall	114,738	548	4.78 (4.39, 5.19)

Table 3. Demographic and clinical characteristics at baseline of HIV-infected individuals receiving antiretroviral agents		
Characteristic	Exposed to Abacavir	Exposed to Other ARV agents (reference group)
Age, median (IQR)	48 (43-54)	46 (39-52)
Male, n(%)	6,889 (80.76)	52,402 (81.62)
Region, n(%)		
East	2,057 (24.11)	15,336 (23.89)
Mid-West	1,370 (16.06)	12,104 (18.85)
South	3,986 (46.73)	29,179 (45.45)
West	1,117 (13.09)	7,584 (11.81)
Year of ART initiation in the database, n(%)		
2009	3,590 (42.09)	20,440 (31.84)
2010	1,120 (13.13)	8,578 (13.36)
2011	1,147 (13.45)	9,121 (14.21)
2012	801 (9.39)	7,824 (12.19)
2013	643 (7.54)	7,259 (11.39)
2014	1229 (14.41)	10,981 (17.10)
Ever substance abuse, %	1,290 (15.12)	11,837 (18.44)
Ever alcohol abuse	273 (3.20)	2,750 (4.28)
Ever tobacco use, %	1,198 (14.04)	10,385 (16.18)
Overweight or obese, %	116 (1.36)	1,130 (1.76)
Essential hypertension, %	766 (8.98)	5,026 (7.83)
Diabetes mellitus, %	366 (4.29)	2,049 (3.19)
Chronic Kidney Disease, %	265 (3.11)	492 (0.77)
Dyslipidemia, %	820 (9.61)	5,552 (8.65)
Lipodystrophy	36 (0.42)	129 (0.20)
Heart disease	242 (2.84)	1,768 (2.75)
Medications used for cardiovascular disease	819 (9.60)	4,816 (7.50)
Stroke	25 (0.29)	160 (0.25)
Symptomatic HIV disease	2313 (27.12)	18,839 (29.34)
Hepatitis B	69 (0.81)	612 (0.95)
Hepatitis C	141 (1.65)	896 (1.40)
Cancer	438 (5.13)	4,152 (6.47)

Table 4. Factors associated with initiation of abacavir among HIV-infected individuals (Treatment model)		
Variable	Hazard Ratio (95% CI)	P value
Male sex[$]	0.90 (0.86, 0.96)	0.000
Age[†]	1.03 (1.029, 1.034)	0.000
Year of initiating ART[†]		
2009	Reference	
2010	0.84 (0.78, 0.90)	0.000
2011	0.80 (0.75, 0.86)	0.000
2012	0.70 (0.65, 0.75)	0.000
2013	0.63 (0.58, 0.68)	0.000
2014	0.68 (0.63, 0.73)	0.000
Ever smoking	1.03 (0.94, 1.14)	0.504
Ever substance/alcohol abuse	0.83 (0.76, 0.91)	0.000
Symptomatic HIV infection at baseline[$]	1.09 (1.03, 1.15)	0.002
Any cancer[$]	0.85 (0.78, 0.94)	0.001
Chronic Kidney Disease[†]	4.22 (3.73, 4.77)	0.000
Receipt of medications for CVD[†@]	0.89 (0.83, 0.95)	0.001
Lipodystrophy[†]	1.75 (1.37, 2.24)	0.000
Dyslipidemia[†]	1.03 (0.96, 1.11)	0.398
Old AMI[$]	1.21 (0.77, 1.88)	0.412
Heart failure/cardiac arrhythmia/atherosclerosis[†]	0.92 (0.81, 1.04)	0.174
Essential hypertension[†]	1.06 (0.98, 1.16)	0.132
Diabetes mellitus/receipt of anti-hyperglycemic agents[†]	0.99 (0.90, 1.10)	0.909
Ever tobacco use	1.03 (0.94, 1.14)	0.506
Hepatitis B[$]	0.87 (0.69, 1.10)	0.250
Hepatitis C[$]	1.16 (0.98, 1.37)	0.078
Overweight/obese[†]	0.95 (0.81, 1.11)	0.503
Stroke[$]	0.91 (0.61, 1.37)	0.655

[†]Time-dependent variables. The first observation of a time-dependent covariate corresponds to its baseline value.

[@]aspirin, beta-blocker, statins, angiotensin converting enzyme inhibitor, angiotensin receptor blocker, calcium channel blocker.

[$]Baseline covariates.

Table 5. Cardiovascular Disease outcomes from current and cumulative exposure to abacavir among HIV-infected individuals			
Exposure	Unadjusted Cox Model HR (95% CI; p value)	Adjusted Cox Model HR[#] (95% CI; p value)	Marginal Structural Model HR[*] (95% CI; p value)
[$]Current	1.70 (1.41, 2.05; p=0.000)	1.32 (1.09, 1.60; p=0.004)	1.40 (1.15, 1.70; p=0.001)
[@]Recent	1.67 (1.23, 2.25; p=0.001)	1.23 (0.91, 1.66; p=0.177)	1.29 (0.95, 1.75; p=0.107)
[@]Cumulative (per year)	1.24 (1.12, 1.37; p=0.000)	1.13 (1.02, 1.25; p=0.024)	1.16 (1.04, 1.28; p=0.006)

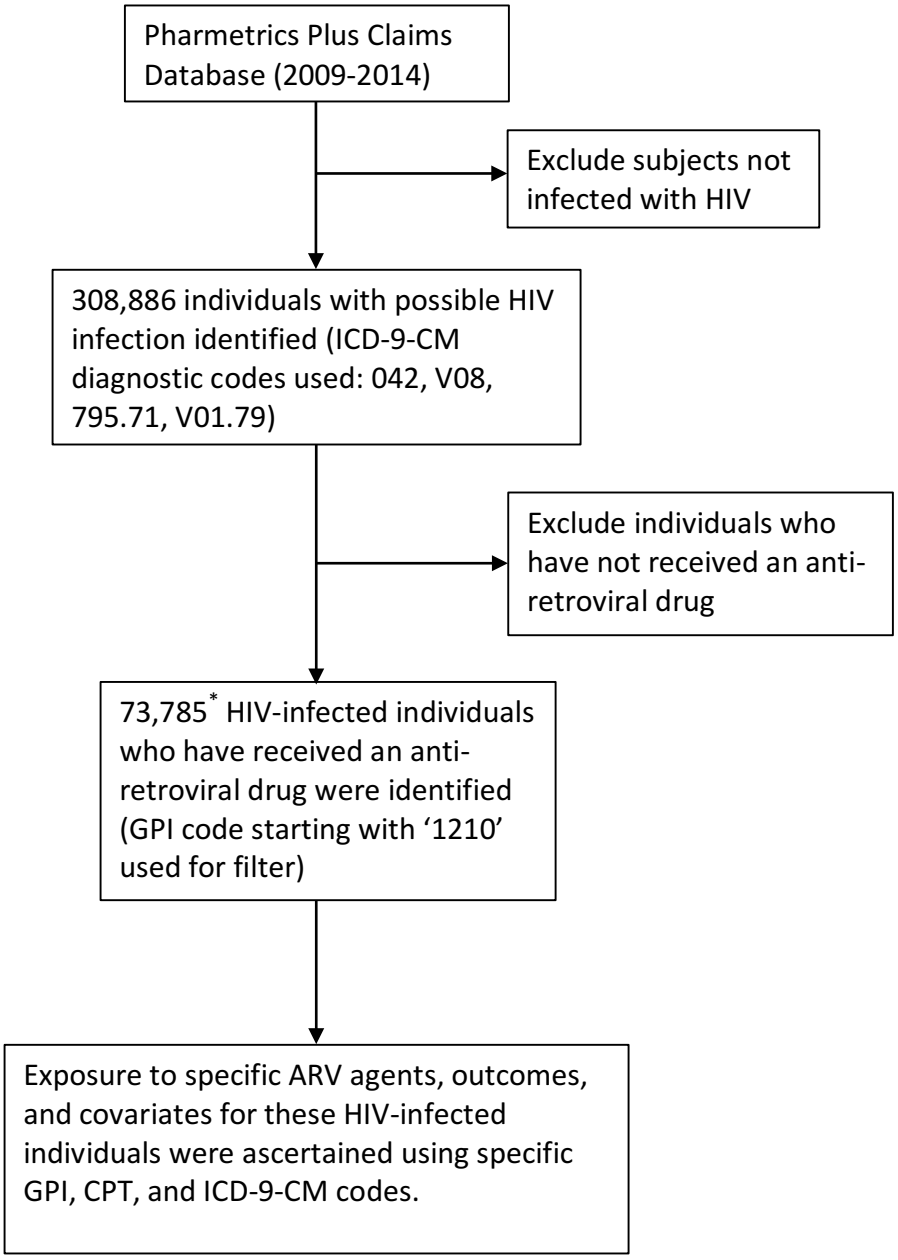
#Models for current, recent, and cumulative exposures are adjusted for baseline covariates: sex, tobacco use (ever), substances or alcohol abuse (ever), hepatitis B & C, stroke, cancer, old myocardial infarction, and time-dependent covariates: age, body weight, CKD, dyslipidemia, heart failure, cardiac dysrhythmia, atherosclerosis, diabetes mellitus, receipt of anti-hyperglycemic agents, hypertension, and use of CVD related medications (aspirin, beta-blocker, angiotensin converting enzyme inhibitor, angiotensin receptor blocker, calcium channel blocker).

*In addition to adjusting for weights generated from the treatment model, the marginal models for both the current and cumulative exposures are adjusted for time-fixed covariates: sex, ever tobacco use, ever alcohol or substance abuse, and baseline covariates: age, stroke, cancer, hepatitis B & C, year of ART initiation, symptomatic HIV disease, CKD, dyslipidemia, old AMI, heart failure, cardiac dysrhythmia, atherosclerosis, diabetes mellitus, receipt of anti-hyperglycemic agents, hypertension, and receipt of medications for cardiovascular disease (aspirin, beta-blocker, angiotensin converting enzyme inhibitor, angiotensin receptor blocker, calcium channel blocker).

@Referent group is those never exposed to abacavir.

$Referent group is those not currently exposed to abacavir, but may be exposed in the past.

Figure 1. Algorithm for defining the study cohort.



GPI: generic product identifier; CPT: current procedural terminology; ICD-9-CM: International Classification of Disease, 9th Revision, Clinical Modification.
*Additional filter (age≥18) applied to obtain final cohort.

Figure 2. Risk of cardiovascular disease from an increasing duration of exposure to abacavir as compared to those never exposed.

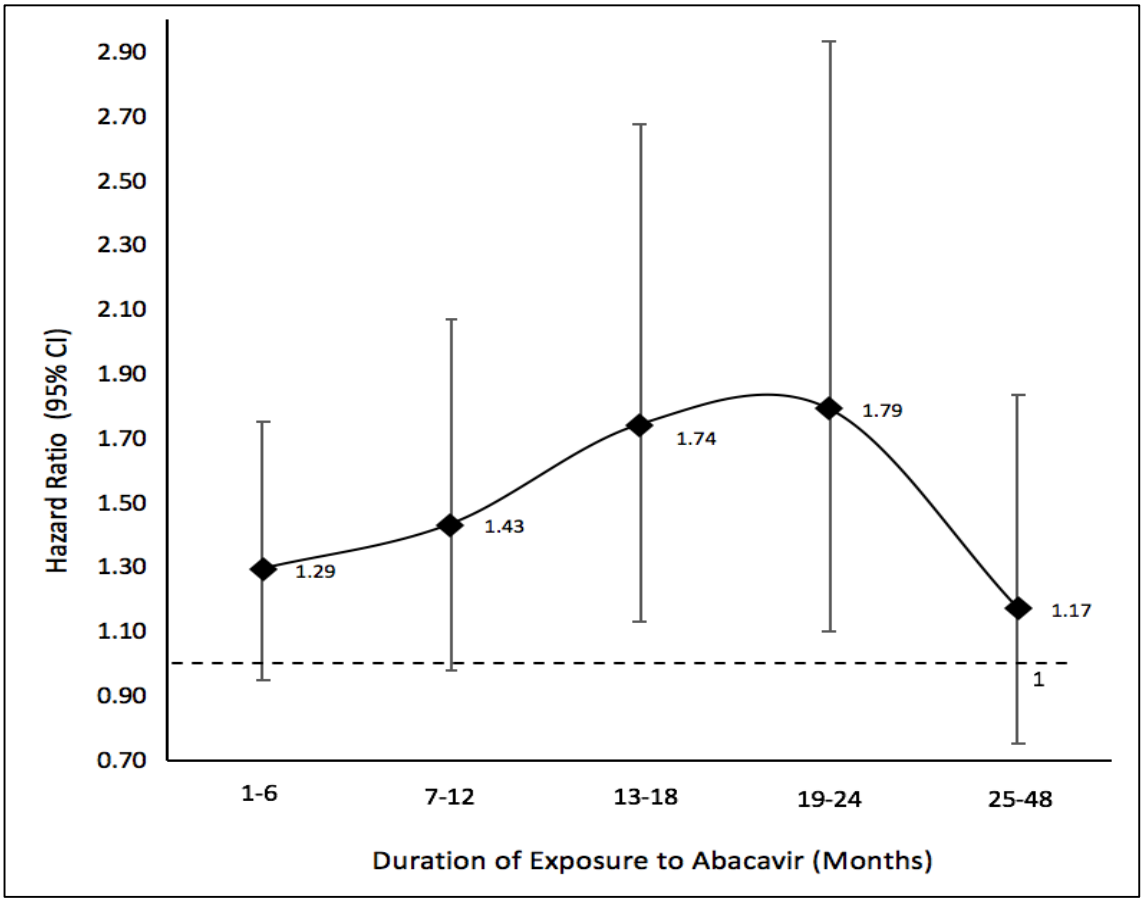
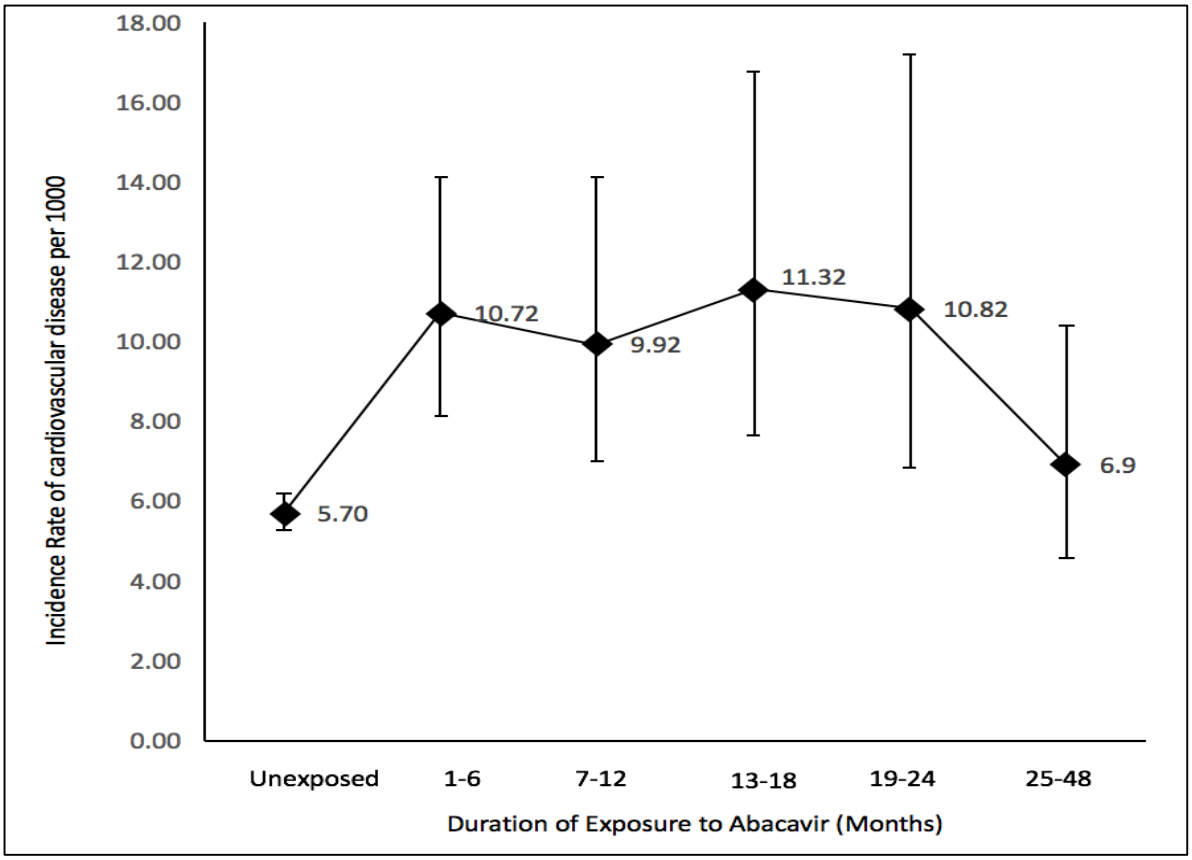


Figure 3. Incidence rates of cardiovascular disease from an increasing duration of exposure to abacavir



Appendix 1.

Marginal Structural Model

Because the estimates generated by traditional methods could be biased in the presence of confounders that lie on the causal pathway between the exposure and the outcome, and were also predicted by past exposure to the ARV agent of interest, I have used a causal inference approach that estimates so-called marginal structural models, which can account for the issue of time-dependent confounding. In this study, the relationship between abacavir use and risk of CVD may be confounded by covariates such as diabetes mellitus, hypertension, dyslipidemia, lipodystrophy, and chronic kidney disease, and the values of these covariates could be influenced by past exposure to abacavir or the comparator ARV agents, such as PIs. The specified MSM will model the hazard of AMI had everyone in the study population received the ARV agent of interest compared to if everyone had not received the exposure. A usual approach for modeling the effect of a time dependent exposure/covariate on the effect of survival is to use the time dependent Cox proportional hazard model. However, as stated above, in the presence of time dependent confounders, which are affected by past treatment, the estimate of the effect is biased, as is the case in this study. Therefore, in this context of time-dependent covariates, although the effect estimate from the usual Cox proportional hazards model is an unbiased estimate of the associational parameter, it is a biased estimator of the causal effect of the specified exposure on survival among HIV-infected patients receiving ART. Therefore, I will employ the marginal structural Cox proportional hazards method (Hernan et al., 2000). Adopting the notations used by Hernan et al, I define T to be the patient's time to AMI/CVD or the censoring date, with time measured in months, and $A(t) = 1$ if the subject received the specified exposure at a given time t, where $0 \leq u \leq t$. $\bar{A}(t)$ represents patient's treatment history up to time t. V represents the vector of time independent baseline covariates. $L(t)$ represents the vector of time dependent covariates at time t, and $T_{\bar{a}}$ represents the counterfactual random variable that represents patient's time to outcome, had he/she experienced the exposure history from the start of follow up rather than his/her observed history. We observe $T_{\bar{a}}$ only for those patients' exposure histories $\bar{a}$, where the subject actually received the exposure, in our case, the specified ARV agent, from start of follow up until the development of AMI/CVD or the censor date. Then $T_{\bar{a}}$ equals T, and for each $\bar{a}$, an example of a marginal structural cox proportional hazard model is given by:

$$\lambda_{T_{\bar{a}}}(t|V) = \lambda_0(t) \exp(\beta_1 a(t) + \beta_2 V) \quad (1)$$

where $\lambda_{T_{\bar{a}}}(t|V)$ was the hazard of AMI/CVD among subjects with baseline covariates V had, contrary to fact, all subjects followed the specified exposure history through $\bar{a}$. This model is a marginal structural model as it assumes a smooth (parametric) function relating the subset of covariates and counterfactual levels of treatment to the hazard. I obtained the parameters of

MSM defined in (1) through a pooled logistic regression that essentially models the hazard of AMI/CVD by fitting the model

$$\lambda_T(t|\bar{A}(t), V) = \lambda_0(t)\exp\left(\beta_1^* A(t) + \beta_2^* V\right) \quad (2)$$

using a stabilized weight

$$sw_i = \prod_{k=0}^{int(t)} \frac{pr(A(k)=a_i(k)|\bar{A}(k-1)=\bar{a}_i(k-1), V=v_i)}{pr\left(A(k)=a_i(k)|\bar{A}(k-1)=\bar{a}_i(k-1), V=v_i, \bar{L}(k)=\bar{l}_i(k)\right)} \quad (3)$$

where $\bar{A}(-1)$ is defined to be 0 and $int(t)$ is the largest integer less than or equal to t and k denotes months since start of follow up. Here, in fitting the treatment model, I assumed that once an HIV-infected individual is started on the specified ARV agent, he/she remained on that drug. Assuming sufficiency of the measured time dependent covariates for identifiability of the causal question of ART use and risk of AMI/CVD, the use of stabilized weights sw_i effectively generates, in a risk set at time t, a pseudo-population in which $\bar{L}(t)$ no longer predicts the specified exposure use and hence $\bar{L}(t)$ is no longer a confounder (Robins et al., 2000) and the causal parameter would be same as that in the original study population (Robins et al., 2000). The stabilized weight was generated using a pooled logistic regression as follows.

Estimation of the weights

Numerator: The numerator of the stabilized weight was modelled using a logistic model

$$Logit\ pr(A_k = 1|\bar{A}_{k-1} = \bar{a}_{k-1}, V) = \alpha_0^* + \alpha_1^* a_{k-1} + \alpha_2^* V + \alpha_3^* k \quad (4)$$

where I allowed the probability of current treatment to be a function of the baseline/time-fixed covariates, exposure at time *k-1,* and time modelled as a smoothed function of natural cubic splines with three internal knots at 25th, 50th, and 75th percentile corresponding to month 6, month 14, and month 26.

Denominator: The denominator of the stabilized weight was modelled using the logistic model

$$Logit\ pr(A_k = a_k|\bar{A}_{k-1} = \bar{a}_{k-1}, \bar{L}_k = \bar{l}_k, V) = \alpha_0 + \alpha_1 a_{k-1} + \alpha_2 l_k + \alpha_3 V + \alpha_4 k \quad (5)$$

where the probability of receiving current treatment depended upon the last month's treatment history, time-dependent and time-fixed covariates, and time modelled as a function of natural cubic splines as that of the numerator. We then obtained the predicted probabilities of receiving treatment for each individual from the logistic models of (4) and (5), which we then use to calculate the stabilized weight as

$$SW_i = \frac{\prod_{k=0}^{K}(\hat{p}_{ki}^{*})^{a_{ki}}(1-\hat{p}_{ki}^{*})^{(1-a_{ki})}}{\left\{\prod_{k=0}^{K}(\hat{p}_{ki})^{a_{ki}}(1-\hat{p}_{ki})^{(1-a_{ki})}\right\}} \quad (6)$$

Hence, by using the stabilized weights, we obtained an unbiased estimate of the causal parameter β_1. e^{β_1} is the causal hazard ratio for the effect estimate of the hazard of AMI in HIV-infected patients receiving the specified exposure, compared to HIV-infected patients receiving the reference exposure. The stabilized weights help to create a pseudo-population in which the treatment is not confounded by the covariates, and the causal parameters generated from the pseudo-population are the same as those of the true population (Robins et al., 2000). Robins et al. also showed that the stabilized weights have smaller variance as compared to the IPTW weights, leading to narrower confidence intervals. We also assessed effect modification by including interaction terms between time independent baseline covariates and the treatment variable in the MSM.

Inference: 95% confidence interval will be calculated using the Huber White robust or sandwich estimator of the variance for β1 given by $\hat{\beta}_1 \pm 1.96\sqrt{var(\hat{\beta}_1)}$.

Appendix 2.

Appendix 2 table 1. ICD-9-CM, and CPT codes for defining various covariates and outcomes	
Variable	ICD-9-CM Code
Acute myocardial infarction	410.xx
Percutaneous coronary intervention (CPT)	92920-92921, 92924-92925, 92928-92929, 92933-92934, 92937-92938, 92941, 92943-92944, 92980-92981, 92984, 92996
Coronary artery bypass graft (CPT)	33510-33514, 33516-33519, 33521-33523, 33533-33536
Tobacco use	305.1; v15.82
Substance abuse (dependent and non-dependent)	304.xx, 305.xx
Alcohol abuse (alcohol dependence and alcohol abuse)	303.xx, 305.0x
Overweight/obese	278.00, 278.01, 278.02
Diabetes mellitus	250.xx, 357.2x, 362.0x, 366.41
Essential hypertension	401.xx
Hypercholesterolemia	272.0x
Hypertriglyceridemia	272.1x
Mixed hyperlipidemia	272.2x
Other and unspecified hyperlipidemia	272.4x
Lipodystrophy	272.6x
Chronic kidney disease	585.xx
Heart failure	402.01, 402.91, 428%, 404.01, 404.03,404.11,404.13,404.91, 404.93
Cardiac dysrhythmia	427.xx
Old myocardial infarction	427.xx
Coronary atherosclerosis	414.xx
Stroke	434.xx
Hepatitis B virus infection	070.2x, 070.3x, V02.61
Hepatitis C virus infection	070.41, 070.44, 070.51, 070.54, 070.7x, v02.62
Any cancer	140-149, 150-159, 160-169, 170-179, 180-189, 190-199, 200-209, 210-229, 230-239

ICD-9-CM: International Classification of Disease, 9th Revision, Clinical Modification; CPT: Current Procedural Terminology

Appendix 2 table 2. Risk of cardiovascular disease among HIV-infected individuals exposed to abacavir for various time-periods			
Duration of exposure (months)	HR (95% CI; p value) Unadjusted Cox Model	Adjusted Cox Model HR[#] (95% CI; p value)	Marginal Structural Model HR* (95% CI; p value)
1-6 (recent)	1.66 (1.23, 2.25; p=0.001)	1.24 (0.92, 1.67; p=0.164)	1.29 (0.95, 1.75; p=0.101)
7-12	1.69 (1.15, 2.47; p=0.007)	1.27 (0.87, 1.86; p=0.221)	1.43 (0.98, 2.07; p=0.065)
13-18	2.28 (1.47, 3.53; p=0.000)	1.71 (1.10, 2.65; p=0.017)	1.74 (1.13, 2.67; p=0.011)
19-24	2.09 (1.26, 3.46; p=0.004)	1.62 (0.98, 2.69; p=0.061)	1.79 (1.10, 2.93; p=0.020)
25-48	1.40 (0.90, 2.18; p=0.131)	1.16 (0.75, 1.82; p=0.499)	1.17 (0.75, 1.83; p=0.500)
Never exposed	Referent	Referent	Referent

#Adjusted for baseline covariates: sex, tobacco use (ever), substances or alcohol abuse (ever), hepatitis B & C, stroke, cancer, old myocardial infarction, and time-dependent covariates: age, body weight, CKD, dyslipidemia, heart failure, cardiac dysrhythmia, atherosclerosis, diabetes mellitus, receipt of anti-hyperglycemic agents, hypertension, and receipt of medications for cardiovascular disease (aspirin, beta-blocker, angiotensin converting enzyme inhibitor, angiotensin receptor blocker, calcium channel blocker, statins).

*In addition to adjusting for weights generated from the treatment model, the marginal model is adjusted for time-fixed covariates: sex, ever tobacco use, ever alcohol or substance abuse, and baseline covariates: age, stroke, cancer, hepatitis B & C, year of ART initiation, symptomatic HIV disease, CKD, dyslipidemia, old AMI, heart failure, cardiac dysrhythmia, atherosclerosis, diabetes mellitus, receipt of anti-hyperglycemic agents, hypertension, and receipt of medications for cardiovascular disease (aspirin, beta-blocker, angiotensin converting enzyme inhibitor, angiotensin receptor blocker, calcium channel blocker, statins).

Appendix 2 table 3. Risk of CVD from abacavir exposure among HIV-infected individuals who have received abacavir prior to but not in the last various periods of time				
[@]Last receipt of abacavir	Person-years of exposure	Outcomes/total no. of outcomes	Adjusted Cox Model HR[#] (95% CI; p value)	Marginal Structural Model HR[*] (95% CI; p value)
6 months ago	800	12/407	2.14 (1.20, 3.81; p=0.010)	2.19 (1.19, 4.02; p=0.011)
12 months ago	534	7/286	1.90 (0.89, 4.07; p=0.099)	1.82 (0.82, 4.02; p=0.138)
18 months ago	346	4/208	1.79 (0.65, 4.93; p=0.257)	1.64 (0.58, 4.62; p=0.351)

@The reference group for for the model for each exposure category is those never exposed to abacavir.

#Adjusted for baseline covariates: sex, tobacco use (ever), substances or alcohol abuse (ever), hepatitis B & C, stroke, cancer, old myocardial infarction, and time-dependent covariates: age, body weight, CKD, dyslipidemia, heart failure, cardiac dysrhythmia, atherosclerosis, diabetes mellitus, receipt of anti-hyperglycemic agents, hypertension, and receipt of medications for cardiovascular disease (aspirin, beta-blocker, angiotensin converting enzyme inhibitor, angiotensin receptor blocker, calcium channel blocker, statins).

*In addition to adjusting for weights generated from the treatment model, the marginal model is adjusted for time-fixed covariates: sex, ever tobacco use, ever alcohol or substance abuse, and baseline covariates: age, stroke, cancer, hepatitis B & C, year of ART initiation, symptomatic HIV disease, CKD, dyslipidemia, old AMI, heart failure, cardiac dysrhythmia, atherosclerosis, diabetes mellitus, receipt of anti-hyperglycemic agents, hypertension, and receipt of medications for cardiovascular disease (aspirin, beta-blocker, angiotensin converting enzyme inhibitor, angiotensin receptor blocker, calcium channel blocker, statins).

Appendix 2 table 4. The influence of various risk factors on the development of cardiovascular disease among HIV-infected individuals receiving anti-retroviral therapy		
Variable	Adjusted Cox Model[#] HR (95% CI)	P-value
Age (per year)[†]	1.06 (1.05, 1.07)	0.000
Male sex[$]	1.83 (1.42, 2.36)	0.000
Tobacco use (ever)	1.53 (1.19, 1.98)	0.000
Substance/alcohol abuse (ever)	1.10 (0.85, 1.42)	0.456
Year of ART initiation[†]		
2009	Referent	-
2010	0.92 (0.61, 1.38)	0.691
2011	0.95 (0.63, 1.44)	0.814
2012	0.80 (0.52, 1.22)	0.293
2013	0.74 (0.48, 1.13)	0.160
2014	0.91 (0.59, 1.39)	0.649
Overweight/obese[†]	0.86 (0.62, 1.19)	0.369
Symptomatic HIV infection[$]	0.89 (0.74, 1.07)	0.205
Heart diseases (Heart failure, cardiac arrhythmia, atherosclerosis)[†]	4.13 (3.45, 4.95)	0.000
Old myocardial infarction[$]	3.38 (2.02, 1.75)	0.000
Use of CVD related medication[†]	1.46 (1.22, 1.75)	0.000
Diabetes Mellitus[†]	1.32 (1.09, 1.60)	0.005
Essential Hypertension[†]	1.17 (0.97, 1.42)	0.095
Dyslipidemia[†]	1.35 (1.13, 1.61)	0.001
Lipodystrophy[†]	1.24 (0.86, 1.79)	0.251
Chronic Kidney Disease[†]	1.04 (0.79, 1.36)	0.793
Hepatitis B[$]	0.79 (0.32, 1.96)	0.615
Hepatitis C[$]	1.61 (0.99, 2.64)	0.057
Cancer[$] (any)	0.77 (0.55, 1.07)	0.118

$ baseline variables; † time dependent variables

#Cox proportional hazard model adjusted for baseline covariates: sex, tobacco use (ever), substances or alcohol abuse (ever), hepatitis B & C, stroke, cancer, old myocardial infarction, and time-dependent covariates: age, body weight, CKD, dyslipidemia, heart failure, cardiac dysrhythmia, atherosclerosis, diabetes mellitus, receipt of anti-hyperglycemic agents, hypertension, and receipt of medications for cardiovascular disease (aspirin, beta-blocker, angiotensin converting enzyme inhibitor, angiotensin receptor blocker, calcium channel blocker).

Appendix 2 table 5. Risk of CVD from exposure to abacavir among HIV-infected individuals free of cardiovascular disease at baseline		
Unadjusted Cox Model HR (95% CI; p value)	Adjusted Cox Model HR[#] (95% CI; p value)	Marginal Structural Model HR[*] (95% CI; p value)
1.81 (1.48, 2.23; p=0.000)	1.41 (1.15, 1.74; p=0.001)	1.49 (1.22, 1.84; p=0.000)

#Adjusted for baseline covariates: sex, tobacco use (ever), substance or alcohol abuse (ever), hepatitis B & C, stroke, cancer, and time dependent covariates: age, body weight, CKD, dyslipidemia, congestive heart failure, cardiac dysrhythmia, atherosclerosis, diabetes mellitus, receipt of anti-hyperglycemic agents, hypertension, and receipt of medication for cardiovascular disease (aspirin, beta-blocker, angiotensin converting enzyme inhibitor, angiotensin receptor blocker, calcium channel blocker).

*In addition to adjusting for weights generated from the treatment model, the marginal model is adjusted for time-fixed covariates: sex, ever tobacco use, ever alcohol or substance abuse, and baseline covariates age, stroke, cancer, hepatitis B & C, year of ART initiation, symptomatic HIV disease, CKD, dyslipidemia, diabetes mellitus, receipt of anti-hyperglycemic agents, essential hypertension, and receipt of medication for cardiovascular disease.

Appendix 2 table 6. Risk of cardiovascular disease from a current exposure to abacavir among HIV-infected individuals without a prior history of substance or alcohol abuse at baseline		
Unadjusted Cox Model	Adjusted Cox Model	Marginal Structural Model
HR (95% CI; p value)	HR[#] (95% CI; p value)	(95% CI; p value)
1.69 (1.40, 2.04; p=0.000)	1.30 (1.08, 1.58; p=0.007)	1.37 (1.13, 1.66; p=0.002)

#Adjusted for baseline covariates: sex, tobacco use (ever), hepatitis B & C, stroke, cancer, prior AMI, congestive heart failure, and time-dependent covariates: age, body weight, CKD, dyslipidemia, heart failure, cardiac dysrhythmia, atherosclerosis, diabetes mellitus, receipt of anti-hyperglycemic agents, hypertension, and receipt of medication for cardiovascular disease (aspirin, beta-blocker, statin, angiotensin converting enzyme inhibitor, angiotensin receptor blocker, calcium channel blocker).

*In addition to adjusting for weights generated from the treatment model, the marginal model is adjusted for time-fixed covariates: sex, ever tobacco use, and baseline covariates age, stroke, cancer, hepatitis B & C, year of ART initiation, symptomatic HIV disease, CKD, dyslipidemia, old AMI, heart failure, cardiac dysrhythmia, atherosclerosis, diabetes mellitus, receipt of anti-hyperglycemic agents, essential hypertension, and receipt of medication for cardiovascular disease.

Appendix 2 table 7. Risk of cardiovascular disease from current exposure to abacavir in sub-groups of variables at baseline (test of interactions)		
Variable	#Hazard Ratio (95% CI)	P value for test of interaction
Age>45 Age<=45	1.23 (1.01, 1.51) 1.97 (1.23, 3.15)	 0.072
Female Male	0.97 (0.48, 1.97) 1.36 (1.11, 1.65)	 0.371
Chronic Kidney Disease (CKD) No CKD	1.06 (0.43, 2.58) 1.33 (1.10, 1.62)	 0.622
*CVD No CVD	1.41 (1.15, 1.74) 0.98 (0.62, 1.54)	 0.143
Dyslipidemia No dyslipidemia	1.18 (0.69, 2.02) 1.35 (1.11, 1.66)	 0.636
Diabetes mellitus No diabetes mellitus	1.05 (0.54, 2.07) 1.35 (1.11, 1.64)	 0.876
Lipodystrophy No lipodystrophy	5.70 (0.50, 64.49) 1.31 (1.08, 1.58)	 0.239
Hypertension No Hypertension	1.34 (0.80, 2.25) 0.90 (0.67, 1.22)	 0.942
Substance abuse No substance abuse	1.54 (0.60, 3.94) 1.31 (1.08, 1.59)	 0.741
Alcohol abuse No alcohol abuse	3.08 (0.67, 14.22) 1.31 (1.08, 1.58)	 0.274
Overweight/obese Not overweight/obese	0.81 (0.11, 6.18) 1.32 (1.09, 1.60)	 0.636
Smoker Non smoker	2.42 (1.02, 5.76) 1.28 (1.06, 1.56)	 0.160

*Includes individuals receiving medications for cardiovascular disease.
#A separate Cox model containing the corresponding interaction term is run for each variable after adjusting for a uniform set of covariates as defined previously for other models.

References

1. Antiretroviral Therapy Cohort C. Causes of death in HIV-1-infected patients treated with antiretroviral therapy, 1996-2006: collaborative analysis of 13 HIV cohort studies. Clin Infect Dis. 2010;50(10):1387-96.
2. Triant VA, Lee H, Hadigan C, Grinspoon SK. Increased acute myocardial infarction rates and cardiovascular risk factors among patients with human immunodeficiency virus disease. J Clin Endocrinol Metab. 2007;92(7):2506-12.
3. Bavinger C, Bendavid E, Niehaus K, Olshen RA, Olkin I, Sundaram V, et al. Risk of Cardiovascular Disease from Antiretroviral Therapy for HIV: A Systematic Review. PLoS One. 2013;8(3).
4. Cruciani M, Mengoli C, Malena M, Serpelloni G, Parisi SG, Moyle G, et al. Virological efficacy of abacavir: systematic review and meta-analysis. J Antimicrob Chemother. 2014;69(12):3169-80.
5. Sabin CA, Worm SW, Weber R, Reiss P, El-Sadr W, Dabis F, et al. Use of nucleoside reverse transcriptase inhibitors and risk of myocardial infarction in HIV-infected patients enrolled in the D : A : D study: a multi-cohort collaboration. Lancet. 2008;371(9622):1417-26.
6. Brouwer ES, Napravnik S, Eron Jr JJ, Stalzer B, Floris-Moore M, Simpson Jr RJ, et al. Effects of combination antiretroviral therapies on the risk of myocardial infarction among HIV patients. Epidemiology. 2014;25(3):406-17.
7. Choi AI, Vittinghoff E, Deeks SG, Weekley CC, Li YM, Shlipak MG. Cardiovascular risks associated with abacavir and tenofovir exposure in HIV-infected persons. AIDS. 2011;25(10):1289-98.
8. Desai M, Joyce V, Bendavid E, Olshen RA, Hlatky M, Chow A, et al. Risk of Cardiovascular Events Associated With Current Exposure to HIV Antiretroviral Therapies in a US Veteran Population. Clin Infect Dis. 2015;61(3):445-52.
9. Durand M, Sheehy O, Lelorier J, Tremblay CL. Association between use of antiretroviral therapy and risk of acute myocardial infarction: A nested case control study using Quebec's Public Health Insurance Database (RAMQ). J Popul Ther Clin Pharmacol. 2011;18(2):e178-e9.
10. Marcus JL, Neugebauer RS, Leyden WA, Chao CR, Xu L, Quesenberry CP, et al. Use of abacavir and risk of cardiovascular disease among HIV-infected individuals. J Acquir Immune Defic Syndr. 2015.
11. Martin A, Bloch M, Amin J, Baker D, Cooper DA, Emery S, et al. Simplification of Antiretroviral Therapy with Tenofovir-Emtricitabine or Abacavir-Lamivudine: A Randomized, 96-Week Trial. Clin Infect Dis. 2009;49(10):1591-601.
12. Obel N, Farkas DK, Kronborg G, Larsen CS, Pedersen G, Riis A, et al. Abacavir and risk of myocardial infarction in HIV-infected patients on highly active antiretroviral therapy: A population-based nationwide cohort study. HIV Med. 2010;11(2):130-6.
13. Palella Jr FJ, Althoff K, Moore R, Zhang J, Kitahata M, Gange S, et al., editors. Abacavir Use and Risk for Myocardial Infarction in the NA-ACCORD. CROI 2015; 2015 February 23-26, 2015; Seattle.

14. Strategies for Management of Anti-Retroviral Therapy I, Groups DADS. Use of nucleoside reverse transcriptase inhibitors and risk of myocardial infarction in HIV-infected patients. AIDS (London, England). 2008;22(14):F17-24.
15. Young J, Xiao Y, Moodie EEM, Abrahamowicz M, Klein MB, Bernasconi E, et al. Effect of Cumulating Exposure to Abacavir on the Risk of Cardiovascular Disease Events in Patients From the Swiss HIV Cohort Study. J Acquir Immune Defic Syndr. 2015;69(4):413-21.
16. Bedimo RJ, Westfall AO, Drechsler H, Vidiella G, Tebas P. Abacavir Use and Risk of Acute Myocardial Infarction and Cerebrovascular Events in the Highly Active Antiretroviral Therapy Era. Clin Infect Dis. 2011;53(1):84-91.
17. Lang S, Mary-Krause M, Cotte L, Gilquin J, Partisani M, Simon A, et al. Impact of individual antiretroviral drugs on the risk of myocardial infarction in human immunodeficiency virus-infected patients: A case-control study nested within the French hospital database on HIV ANRS cohort CO4. Arch Intern Med. 2010;170(14):1228-38.
18. Cruciani M, Zanichelli V, Serpelloni G, Bosco O, Malena M, Mazzi R, et al. Abacavir use and cardiovascular disease events: a meta-analysis of published and unpublished data. AIDS. 2011;25(16):1993-2004.
19. Ding X, Andraca-Carrera E, Cooper C, Miele P, Kornegay C, Soukup M, et al. No association of abacavir use with myocardial infarction: findings of an FDA meta-analysis. J Acquir Immune Defic Syndr. 2012;61(4):441-7.
20. Ribaudo HJ, Benson CA, Zheng Y, Koletar SL, Collier AC, Lok JJ, et al. No risk of myocardial infarction associated with initial antiretroviral treatment containing abacavir: Short and long-term results from ACTG A5001/ALLRT. Clin Infect Dis. 2011;52(7):929-40.
21. Brothers CH, Hernandez JE, Cutrell AG, Curtis L, Ait-Khaled M, Bowlin SJ, et al. Risk of Myocardial Infarction and Abacavir Therapy: No Increased Risk Across 52 GlaxoSmithKline-Sponsored Clinical Trials in Adult Subjects. J Acquir Immune Defic Syndr. 2009;51(1):20-8.
22. Gresele P, Falcinelli E, Momi S, Francisci D, Baldelli F. Highly active antiretroviral therapy-related mechanisms of endothelial and platelet function alterations. Rev Cardiovasc Med. 2014;15 Suppl 1:S9-20.
23. IMS Health. IMS' PharMetrics Plus Data Dictionary 2013 [Available from: http://tri.uams.edu/files/2015/08/Pharmetrics-Plus-Data-Dictionary-Jan-2013.pdf.
24. Fewell Z, Hernan MA, Wolfe F, Tilling K, Choi H, Sterne JAC. Controlling for time-dependent confounding using marginal structural models. The Stata Journal 2004;4(4):402-20.
25. Parikh NI, Gona P, Larson MG, Fox CS, Benjamin EJ, Murabito JM, et al. Long-term trends in myocardial infarction incidence and case fatality in the National Heart, Lung, and Blood Institute's Framingham Heart study. Circulation. 2009;119(9):1203-10.
26. Roger VL, Weston SA, Gerber Y, Killian JM, Dunlay SM, Jaffe AS, et al. Trends in incidence, severity, and outcome of hospitalized myocardial infarction. Circulation. 2010;121(7):863-9.
27. Falcinelli E, Francisci D, Belfiori B, Petito E, Guglielmini G, Malincarne L, et al. In vivo platelet activation and platelet hyperreactivity in abacavir-treated HIV-infected patients. Thromb Haemost. 2013;110(2):349-57.
28. Gresele P, Francisci D, Falcinelli E, Belfiori B, Petito E, Fierro T, et al. Possible role of platelet activation in the cardiovascular complications associated with HIV infection: Differential effects of abacavir (ABC) vs. tenofovir (TDF). J Thromb Haemost. 2011;9:799.

29. Petersen ML, van der Laan MJ. Causal models and learning from data: integrating causal modeling and statistical estimation. Epidemiology. 2014;25(3):418-26.
30. Robins JM, Hernan MA, Brumback B. Marginal structural models and causal inference in epidemiology. Epidemiology. 2000;11(5):550-60.
31. Cole SR, Hernan MA. Constructing inverse probability weights for marginal structural models. Am J Epidemiol. 2008;168(6):656-64.
32. Kiyota Y, Schneeweiss S, Glynn RJ, Cannuscio CC, Avorn J, Solomon DH. Accuracy of Medicare claims-based diagnosis of acute myocardial infarction: estimating positive predictive value on the basis of review of hospital records. Am Heart J. 2004;148(1):99-104.

Chapter Three: Risk of Cardiovascular Disease From Exposure To Abacavir Among HIV-infected Individuals: A Systematic Review And Meta-analysis Of Results From Fourteen Epidemiologic Studies

Abstract

Background

There is controversy regarding abacavir's potential to cause ischemic cardiovascular disease (CVD) among HIV-infected individuals. Studies have continued to show conflicting results.

Objective

To conduct a systematic review and meta-analysis of the existing evidence to assess the risk of ischemic CVD from exposure to abacavir among HIV-infected individuals.

Methods

I searched Medline, Embase, Web of Science, and abstract books of 2014-15 Conference on Retroviruses and Opportunistic Infections (CROI) to identify studies for my meta-analysis. I quantified the risk of CVD separately for recent and cumulative exposure to abacavir using random effects models weighted by inverse of the variance.

Results

Out of 374 unique citations identified, I reviewed the full-text of 62 research articles. All fourteen studies that met my inclusion criteria assessed the risk of CVD from recent exposure to abacavir; three studies assessed the risk from recent exposure among anti-retroviral therapy (ART) naive individuals. Six studies assessed the risk from a cumulative exposure. I obtained relative rate (95% CI) summary estimates of 1.45 (1.33, 1.72) for recent exposure, 1.81 (1.26-2.60) for recent exposure in an ART-naïve population, and 1.09 (1.03, 1.14) per year for cumulative exposure to abacavir.

Conclusion

My findings suggest an increased risk of CVD from both recent and cumulative exposure to abacavir.

Introduction

The Data Collection on Adverse Events of Anti-HIV Drugs (D:A:D) study groups first reported in 2008 that abacavir use is associated with an increased risk of acute myocardial infarction (AMI) among HIV-infected individuals (1). Studies conducted subsequently to investigate this risk have yielded conflicting results (2-9). Bedimo et al. have argued that the observed increase in risk of cardiovascular disease (CVD) may be due to potential confounding by renal dysfunction, because individuals with renal dysfunction, a known risk factor for AMI, may be preferentially prescribed abacavir instead of tenofovir, given the latter's potential to cause nephrotoxicity (2). Lang et al. found no increased risk of AMI associated with exposure to abacavir when the study population was restricted to individuals not using cocaine or injection drugs (6). Three meta-analyses of randomized controlled trials (RCT) found no association between abacavir use and AMI (9-11). While the results of these studies argued against an increased risk of CVD associated with abacavir exposure, results of several other studies have agreed with the findings of the D:A:D study (4, 5, 7, 8, 12-15). In the face of this controversy, prescription of abacavir to HIV-infected patients has declined since the publication of the D:A:D study results in 2008 (16-18).

The D:A:D study reported an increased risk of AMI from both recent and cumulative exposure to abacavir (1). They further reported that the risk of AMI reverses after six months of stopping abacavir, and that after adjusting for recent exposure, there is no increase in the risk of AMI associated with cumulative exposure to abacavir, which led them to posit that abacavir may act through an acute inflammatory process to cause AMI, rather than triggering a gradual atherogenic process by altering blood lipid levels, as is known to be caused by some protease inhibitors (19). However, studies conducted to identify an underlying biological mechanism to explain the observed increase in risk of AMI associated with abacavir exposure have yielded conflicting results (20).

Bavinger et al. published a systematic review and meta-analysis in 2014, in which they suggested that recent abacavir exposure is associated with an increased risk of CVD (21). However, the summary estimate (RR: 1.91, 95% CI: 1.50-2.42) was obtained by meta-analysis of only two studies. Inadequacy of studies prevented the authors from drawing a conclusion regarding the risk of CVD from a cumulative exposure to abacavir. Four studies investigating the risk of CVD associated with either recent or cumulative exposure to abacavir have been published since that meta-analysis (12, 13, 15). Therefore, I decided to perform a systematic review and meta-analysis to summarize the relationship between recent and cumulative exposure to abacavir and the risk of CVD among HIV-infected individuals. I discuss the biases potentially affecting the study results and the methodological challenges and inconsistencies that I observed across studies with regard to study design and analysis, and interpretation of the results. Additionally, I briefly discuss the existing evidence on plausible biological mechanisms underlying the risk.

Methods

I have searched Medline, Embase, Web of Science, abstract books of 2014 and 2015 CROI, and the bibliographies of three published reviews to identify studies that investigated the risk of cardiovascular disease associated with exposure to abacavir (Figure 1). I used the terms, 'abacavir', 'cardiovascular disease', 'myocardial infarction', and 'heart disease' for the search and included comparative studies in English with the exposure defined as abacavir or an abacavir-based ART regimen and outcome of AMI or CVD. I required CVD to be defined as an ischemia-driven cardiac event/procedure such as AMI, angina pectoris, or percutaneous coronary intervention. I included studies that assessed ischemic stroke as a component of the CVD definition, but excluded results that assessed only stroke as an outcome. I included conference abstracts if the data were unique (i.e. not included in the research articles chosen for the meta-analysis). I excluded studies that 1) are not in English, 2) assessed aa class of ARV agents but not specifically abacavir as the exposure, and 3) assessed CVD risk in a pediatric population.

I performed meta-analyses separately on results for 1) recent exposure to abacavir, usually defined as exposure within last six months, including current exposure, and 2) cumulative exposure, defined as an accumulating sum of the total duration of exposure at a particular time point. In addition to pooling results across all studies that assessed the risk of CVD from recent exposure, I performed a meta-analysis separately for studies that assessed the risk in ART naïve individuals. Where studies reported more than one result for either cumulative or recent exposure from multiple models containing either or both of these exposure terms, I used the result from the model containing just one exposure term. In a subgroup analysis, I performed a meta-analysis to assess the risk of CVD associated with recent exposure by excluding the 2008 D:A:D study that led to the other studies. In the presence of heterogeneity across studies, I used a random effects model to perform the meta-analysis. I assessed heterogeneity among studies by using a Chi-squared test of homogeneity. I performed the meta-analyses using Microsoft Excel.

Results

I identified 374 unique articles/abstracts from the searches of Medline, Embase, Web of Science, and abstract books of CROI 2014 & 2015. Of these, I reviewed the full texts of 62 research articles (Figure 1). In the end, 14 studies meeting my inclusion criteria were used for the meta-analysis. All of the 14 studies assessed the risk of CVD associated with recent exposure, and six studies also assessed the risk associated with cumulative exposure to abacavir. There were 11 cohort studies, two case control studies, and one randomized controlled trial. Table 1 summarizes the salient features of these studies. I observed heterogeneity in results for both recent and cumulative exposure; I therefore used a random effects model to perform the meta-analyses. I did not observe heterogeneity in results for studies that assessed the risk of CVD in ART-naïve patients, and hence I report my result for this population using a fixed effects model.

Recent exposure

Ten out of 14 studies showed a significantly increased risk of CVD from recent exposure to abacavir. I obtained a relative rate (95% CI) summary estimate of 1.45 (1.33, 1.72) for recent exposure (Figure 2). Of these 14 studies, three included an ART-naïve study population. I conducted a separate meta-analysis for these three studies obtaining a relative rate summary estimate (95% CI) of 1.81 (1.26-2.60) (Figure 3). Palella et al. had calculated the risk of CVD associated with recent exposure separately in a full (ART-naïve and experienced) and a restricted (ART-naïve only) NA-ACCORD study population (17). I used the full cohort result for my meta-analysis for overall risk associated with recent exposure to abacavir (Figure 2) and the restricted cohort result for the analysis in the ART-naïve population (Figure 3). Desai et al. obtained the following results [HR (95% CI)] for the risk of CVD associated with exposure to abacavir-based ARV drug combinations: abacavir+atazanavir+lamivudine: 2.08 (1.41-3.06); abacavir+efavirenz+lamivudine: 1.94 (1.34-2.79); abacavir+lamivudine+zidovudine: 1.60 (1.21-2.11); abacavir+lamivudine+lopinavir: 1.44 (0.91-2.28); and abacavir+lamivudine+nevirapine: 1.49 (0.81-2.73) (12). Dorjee et al. obtained the following results [HR (95% CI)] for the risk of AMI associated with exposure to various abacavir-based ARV drug combinations: abacavir+lamivudine+atazanavir: 1.55 (1.07-2.25); abacavir+lamivudine+darunavir: 1.95 (1.29-2.96); abacavir+lamivudine+zidovudine: 1.39 (0.87-2.22); abacavir+lamivudine+efavirenz: 0.64 (0.33-1.26); and abacavir+lamivudine+raltegravir: 0.95 (0.59-1.53). I have not included these results of Desai et al. and Dorjee et al. into this meta-analysis because each of these abacavir-based combinations represents a unique exposure, and it would be in appropriate to pool the risk of CVD associated with a specific drug combination with the results of other studies, which all assessed the risk associated with exposure to abacavir as an individual agent. In a sub-group analysis, in which I assessed the risk of CVD associated with a recent exposure to abacavir by excluding the 2008 D:A:D study, which triggered the subsequent studies, I continued to find a significantly increased risk (HR: 1.39; 95% CI: 1.19, 1.64). In the study conducted by Martin et al., both the sample size (n=357) and the number of CVD cases (n=9) were limited, giving rise to a wide confidence interval. Excluding this study from the meta-analysis examining recent exposure made no difference to the meta-analysis result (HR: 1.43; 95% CI: 1.24, 1.65). Similarly, excluding the study by Lundren et al., with a relatively wide confidence interval, did not change the summary estimate (HR: 1.42; 95% CI: 1.23, 1.65).

Cumulative exposure

Three out of six studies reported an increased risk of CVD associated with cumulative exposure to abacavir. I obtained a RR (95 % CI) summary estimate of 1.09 (1.03, 1.14) per one-year increase in exposure to abacavir (Figure 4). While the D:A:D study groups reported an increased RR (95% CI) of 1.14 (1.08, 1.21) for AMI from cumulative exposure to abacavir, they did not see an increased risk after adjusting for recent exposure (1). The D:A:D study groups also reported that the risk was not significantly increased among those who had stopped abacavir six months earlier. Young et al. reported an increased risk of AMI (HR: 2.06; 95% CI: 1.43, 2.98) from

continued cumulative exposure for up to four years (15), while Dorjee et al. reported an increased risk of CVD associated with a cumulative exposure to abacavir (HR: 1.16; 95% CI: 1.04, 1.28) and further showed that the risk remained elevated for up to 24 months after stopping abacavir.

Discussion

My meta-analysis including 14 studies found a 45% increased risk of CVD among HIV-infected individuals who were recently exposed to abacavir. Bavinger et al. also found an increased risk of CVD associated with recent abacavir exposure in their meta-analysis (21). Bavinger et al. reviewed nine studies, but reported a summary estimate (HR: 1.92; 95% CI: 1.50, 2.42) that pooled results across only two studies, stating that the presence of heterogeneity among studies was the reason for not combining other studies. I accounted for heterogeneity among the studies by using a random effects model. I combined odds ratios and rate ratios because the former approximate the latter well for rare outcomes (22). I combined rate ratios and hazard ratios because most studies in this meta-analysis had discrete time data and the estimate from a logistic or a Poisson model for such data is an approximation of an hazard ratio from a Cox model (23, 24). Because there may be important differences between ART-naïve and ART-experienced populations, I performed a separate meta-analysis for studies that assessed the risk of CVD in ART-naïve HIV-infected individuals. The summary estimate (RR: 1.81, 95% CI:1.26-2.60) among ART-naïve HIV-infected individuals was not affected by confounding by prior ART use, and hence may be construed as providing an estimate that is closest to the causal effect from exposure to abacavir.

Bavinger et al. were not able to draw any conclusions regarding the risk of CVD associated with cumulative exposure to abacavir because the results of only three studies were available for them to review, and the results were inconsistent across those studies. Three more studies that assessed the risk of CVD associated with cumulative exposure to abacavir have since been published. Combining results across six studies, I obtained a summary estimate that indicates a 9% increase in the risk of CVD for every additional year of exposure to abacavir. When a study had reported more than one effect estimate for the relationship between the risk of CVD and cumulative abacavir exposure–as is the case when results are reported first from a model containing cumulative exposure only and then from a model containing cumulative exposure and recent/past exposure–I used for my meta-analysis the result from the model containing a minimum number of exposure terms. Bavinger et al. explained that when a model contains both cumulative exposure and recent exposure, the parameter for cumulative exposure captures the risk only among the exposed group (21). I add here that this is true if the referent group for the recent exposure variable comprises individuals never exposed to abacavir. However, if the referent group comprises individuals who were not recently exposed, which may include both never-exposed individuals and individuals who are exposed prior to last six months, then the parameter for cumulative exposure becomes virtually uninterpretable. For example, it is difficult to understand what is meant when the D:A:D study groups reported that after adjustment for recent exposure to abacavir, there was no risk of AMI associated with

cumulative exposure to the drug, as the referent group for recent exposure was those individuals not recently exposed (2008 D:A:D study, page 1421, paragraph 2). Our understanding of how the risk of CVD varies as exposure to abacavir accumulates is limited. Whereas the D:A:D study groups reported that the risk of AMI associated with abacavir exposure was limited to those recently exposed to abacavir, and that the risk was reversible six months after the exposure stopped, Young et al. reported in the Swiss HIV cohort an increased risk (HR: 2.06) from a continued exposure over the prior 4 years. Dorjee et al. also found an increased risk of CVD associated with cumulative abacavir exposure, and further reported that the risk of CVD remained elevated for up to 24 months of cumulative exposure, after which the risk started to level off (Dorjee et al.). Dorjee et al. clearly showed that the risk of CVD remained elevated beyond six months of stopping abacavir. While the D:A:D study result suggested that exposure to abacavir may lead to an acute inflammatory process resulting in AMI, results from Young et al. and Dorjee et al. may suggest a more gradual biological mechanism underlying the risk of CVD associated with taking abacavir. There is a need for more studies to better understand this relationship.

Three meta-analyses, with significant overlap in the data they included, that have now assessed the risk of CVD using RCT data all showed no increase in risk of CVD associated with abacavir exposure (9-11). However, as described by the authors of these meta-analyses themselves, and by Bavinger et al., these studies were of limited duration, were lacking in generalizability, and had low power, owing to their primary objective being to assess the efficacies of various ARV drugs (21). Inclusion of data from these studies in our meta-analyses would be inappropriate. One RCT, conducted by Martin et al., specifically assessed the risk of CVD as a study outcome; it reported an increased risk of CVD in the abacavir group (14). Lang et al., in a case control study using a French hospital database, reported a significantly increased of AMI (HR: 1.62, 95% CI: 0.93, 2.81) associated exposure to abacavir; however, they did not see the effect when the study population was restricted to those not using cocaine and injection drugs (HR: 1.27, 95% CI: 0.64, 2.49) (6). This finding of no increased risk of CVD by Lang et al. was not reproduced in other studies, which continued to show an increased risk of CVD in association with abacavir exposure after adjusting for substance use (4, 5, 15).

In 2011, Bedimo et al. first showed in a population of patients receiving care in the U.S. Veterans Administration hospitals that renal dysfunction is a significant risk factor for AMI (HR: 3.85; 95% CI: 2.74, 5.42); they reported that the HR for AMI associated with current exposure to abacavir decreased from 0.73 (p=0.013) to 0.67 (p=0.07) after adjusting for renal dysfunction (2). They argued that the 2008 D:A:D study results linking abacavir exposure to AMI could be due to a channeling bias, whereby individuals having renal dysfunction were preferentially put on abacavir to avoid additional nephrotoxicity from tenofovir. However, the D:A:D study groups in 2014 showed through separate pre- and post-March 2008 analyses that the risk of CVD continued to remain elevated (HR: 1.98; 95% CI: 1.72-2.29) after adjusting for pertinent covariates, including CKD (18). They demonstrated that individuals at moderate and high risk for CVD were, in fact, channeled away from abacavir use after 2008 (18). The observation of an elevated risk despite a reverse channeling bias led them to conclude that the observed increase in the risk of CVD associated with use of abacavir cannot be due to a channeling bias. Other

studies have also shown an elevated risk of CVD associated with abacavir use after adjusting for renal dysfunction (4, 5, 7, 12-15). Interestingly, Choi et al., who studied a VA population, showed that recent exposure to abacavir was associated with an increased risk of CVD (HR: 1.48; 95% CI: 1.08, 2.04) (4). However, the studies conducted by Bedimo et al. and Choi et al. differed in the patients included in their cohorts and in their definitions of the exposure and outcome (Table 1), as described by Desai et al (12).

In many studies examining the association between abacavir use and CVD, authors have cited channeling bias as the main reason for the need to use causal inference methods to investigate this study question. While I support the use of causal inference methods, I question this reasoning; a channeling bias, which is in essence, confounding by indication, does not require the use of causal inference methods any more or less than any do other potential confounders, such as hypertension, dyslipidemia, and diabetes mellitus. A discussion regarding why it is necessary to use causal inference methods needs to focus on understanding the dynamics of the relationship between the observed data (covariates, exposure, and outcome) and the unmeasured factors possibly influencing the observed data, and whether a causal path between the exposure and the outcome can be identified, after assuming independence between these unmeasured factors, and after adjustment of the measured covariates (25). For example, if individuals with renal dysfunction are more likely have characteristics such as a given level of socio-economic status or race/ethnicity, which may be unmeasured in a study (as is the case for many studies in this review), and could potentially influence both the treatment and the outcome, then using causal inference methods is necessary in order to obtain an estimate that better approximates the causal effect in the population. A reason to specifically use marginal structural models in this particular instance is that covariates, such as CKD, hypertension, diabetes mellitus, and dyslipidemia, which are potential confounders, could also potentially lie on the causal pathway between ART use and development of CVD. As Robins et al. have shown, adjusting for such factors through traditional methods can give rise to biased effect estimates (26). There is reason to be concerned over the effect estimates generated by Desai et al. and Marcus et al. using marginal structural models (12, 13). Robins et al. have stated that inclusion of time-dependent covariates in the marginal model, as done by Desai et al., gives rise to biased estimate (26). Marcus et al. reported estimates without including any baseline/time-fixed covariates in the marginal model (13). Cole and Hernan have shown that because while using stabilized IPTW, it is necessary to adjust for baseline/time fixed covariates in the marginal structural model (27). In the data analyzed to assess the risk of CVD from exposure to various ARV agents for our two companion papers, the effect estimate from a marginal structural model varied significantly, depending on inclusion/exclusion of baseline covariates (Dorjee et al).

Plausible biological mechanisms

The D:A:D study result that showed a reversal of risk of CVD within six months of discontinuation of abacavir prompted investigators to search for a rapidly acting underlying biological mechanism for the risk of CVD associated with abacavir exposure. While the SMART/INSIGHT study investigators, Kristoffersen et al., and Hileman et al. (8, 28, 29), showed

evidence for a possible role of inflammatory biomarkers, [e.g. increased levels of high sensitivity c-reactive protein (hsCRP) and interleukin-6 (IL-6)] in causing CVD among abacavir users, several other studies showed that levels of biomarkers such as hsCRP, IL-6, selectin P and E , D-dimer, vascular adhesion molecule-1, intercellular adhesion molecule-1, and tumor necrosis factor alpha are not elevated among as a result of exposure to abacavir (30-42).

Studies that evaluated abacavir's role in causing endothelial dysfunction have also yielded mixed results (38, 43, 44). Endothelial dysfunction, induced by both traditional cardiovascular risk factors and chronic inflammation (20), increases the risk of CVD by promoting atherosclerosis (45). Hsue et al. have reported in their study that abacavir use independently predicted lower brachial artery flow mediated vasodilation, a measure of endothelial dysfunction (44). Sinn et al. observed lower arterial stiffness and improvement in Framingham risk score when individuals on abacavir were switched to tenofovir (43). However, in a randomized controlled trial, Wohl et al. found no evidence of endothelial dysfunction from abacavir use as compared to tenofovir (38).

Baum et al., Satchel et al., and Falcinelli et al. showed that abacavir increases platelet aggregation and reactivity, that could potentially lead to thrombosis and myocardial infarction (46-48). Satchel et al. showed that among abacavir recipients, platelet aggregation increased upon exposure to various platelet agonists, such as, adenosine di-phosphate, collagen, epinephrine, and thrombin receptor-activating peptide (48). Baum et al. further showed that abacavir causes platelet hyper-reactivity by competitive inhibition of a nitric oxide-induced soluble guanylyl cyclase via its active metabolite, carbovir-triphosphate, leading to a decreased production of cyclic guanosine monophosphate, an inhibitor of platelet aggregation and secretion (20, 46). Falcinelli et al., confirmed these findings in both in-vivo and ex-vivo settings (47).

From this review, I can more confidently conclude that both recent and cumulative exposure to abacavir may lead to an increased risk of cardiovascular disease. The finding of increased risk of CVD associated with abacavir use among ART-naïve population is particularly suggestive of the causal nature of this relationship. While this risk appears to be reversible upon discontinuation of abacavir, it may take longer than six months for the risk to reverse. Platelet aggregation and reactivity appear to be a plausible underlying biological mechanism for an abacavir-induced cardiotoxicity; confirmation of this platelet-mediated biological mechanism in various HIV cohorts would be reassuring.

Conclusion

In view of the increased risk of CVD associated with exposure to abacavir among HIV-infected individuals, anti-retroviral treatment regimens need to be carefully selected, taking into account existing risk factors for CVD, a detailed history of prior exposure to ART, the patient's clinical status, and the availability of other ARV drugs.

Tables and Figures

Figure 1. Flow diagram showing the search strategy and algorithm for identification of studies.

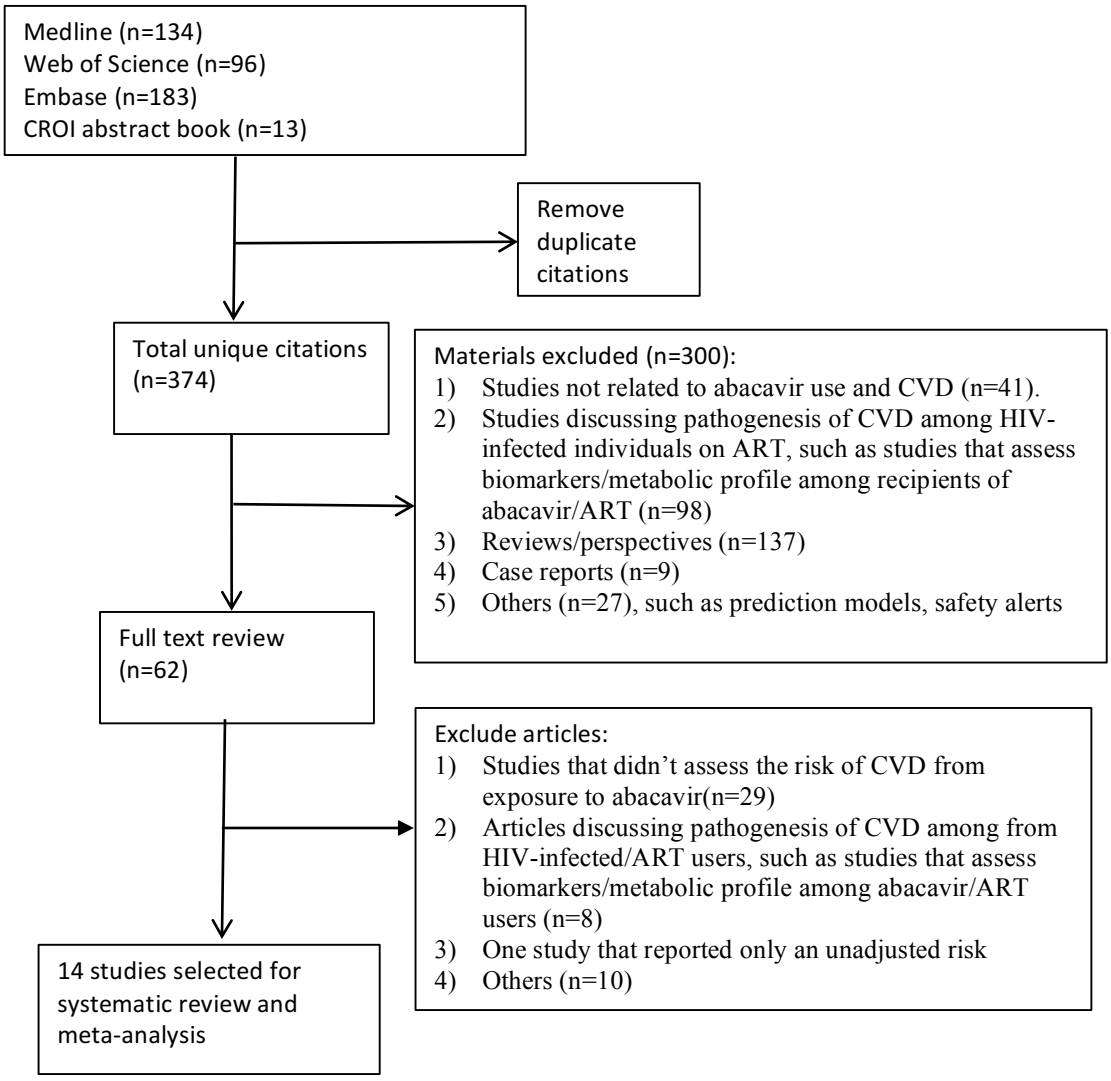


Table 1. Studies assessing the risk of acute myocardial infarction(AMI) or cardiovascular disease (CVD) from recent or cumulative exposure to abacavir

Author, year of publication	Study period	Cohort/ Location	Sample (n)	Outcome (n)	Exposure (Recent/cumulative)	Test statistic: point estimate (95% CI)	Remarks
D:A:D study Groups, 2008	1999-2007	Multi-national	33347	AMI (517)	Recent Cumulative	RR: 1.90 (1.47, 2.45) RR: 1.14 (1.08, 1.21)	Detected an increased risk from both recent and cumulative exposure to abacavir. They reported the risk reverses after 6 months of stopping abacavir. Showed in 2014 in the same cohort with accrued person time that channeling bias from CKD is not a concern.
Lundgren et al., 2008	2002-2007	Multi-national (SMART study)	4544	AMI (19)	Recent	HR: 4.25 (1.39, 13)	Reported an HR (95% CI) of 1.80 (1.04, 3.11) for major CVD defined as any AMI, stroke, surgery for coronary artery disease, and CVD related death.
Choi et al., 2011	1997-2007	VA HIV Clinical Case Registry, U.S.	10931	CVD (501)	Recent Cumulative	HR: 1.48 (1.08, 2.04) HR: 0.93 (0.79, 1.10)	Detected an increased risk from recent but not cumulative exposure to abacavir. Reported that adjustment for CKD made little difference to the result.
Bedimo et al., 2011	1996-2004	VA HIV Clinical Case Registry, U.S.	19424	AMI (267) AMI (278)	Recent Cumulative	HR: 0.67 (0.43, 1.03) HR: 1.18 (0.92, 1.50)	Reported no increased risk from both current and cumulative exposure to abacavir when compared to individuals receiving ART other than abacavir and tenofovir. The model for current exposure is adjusted only for CKD. Argued that the finding of increased risk in the 2008 D:A:D study could be due to a channeling bias from renal dysfunction.
Obel et al., 2010	1995-2005	Danish HIV Cohort Study	2952	AMI (67)	Recent	HR: 2.00 (1.10, 3.64)	Reported an increased risk overall. Also reported in sub-group analyses increased risk among those who have stopped abacavir 6 months ago, and among those initiating abacavir two years after ART initiation, and conjectured against a channeling bias as the reason for the observed risk.
Brouwer et al., 2014	2002-2008	North Carolina Medicaid Beneficiaries	3481	AMI (38)	Recent	HR: 2.05 (0.72, 5.86)	Using IPTW, reported a non-significantly increased risk as compared to those receiving tenofovir in ART-naïve individuals. Exposure is time-fixed.
Desai et al., 2015	1996-2009	VA HIV Clinical Case Registry, U.S.	24510	CVD (934)	Recent	HR: 1.50 (1.26, 1.79)	Reported an increased risk using marginal structural models. Investigators have used time-dependent covariates in the marginal model, and hence the result could be biased (Robins 2009).

Young et al., 2015	2000-2012	Swiss HIV Cohort Study	11856	CVD (365)	Recent Cumulative	HR: 1.63 (1.14, 2.32) HR: 1.04 (0.97, 1.10)	Using marginal structural models, they reported an increased risk from recent exposure. Risk from cumulative exposure was not statistically significant. Using a new marginal structural model with flexible cumulative exposure modelling, they reported an increased risk from continued exposure to abacavir in the past four years. 2.06 (1.43, 2.98), suggesting a more gradual process for pathogenesis of CVD from abacavir exposure.
Palella et al., 2015	1995-2010	NA-ACCORD, North America	16733* 6485#	AMI (301) AMI (93)	Recent Recent	HR: 1.34 (0.96, 1.88) HR: 1.95 (1.18, 3.45)	Reported a non-significantly increased risk from recent exposure in the full* study population (ART naïve and experienced) and a significantly increased risk in a restricted# study population (ART naïve).
Dorjee et al., (unpublished)	2009-2014	U.S. Insurance Claims Data	72733	CVD (714)	Recent Cumulative	HR: 1.40 (1.15, 1.69) HR: 1.16 (1.04, 1.28)	Using marginal structural models, reported an increased risk from both current and cumulative exposure. Showed that the risk reverses and became comparable to referent group after 24 months of stopping abacavir.
Durand et al., 2011	1985-2007	RAMQ and Med-Echo Databases, Quebec	1209	AMI (125)	Recent	OR: 1.72 (1.10, 2.71)	Reported an increased risk from recent exposure.
Lang et al., 2010	2000-2006	French Hospital Database	1173	AMI (289)	Recent Cumulative	OR: 1.62 (0.93, 2.81) OR: 0.97 (0.87, 1.10)	They found a significantly increased risk (OR: 2.01; 95% CI: 1.11, 3.64) from short-term recent exposure (<1 year of exposure & recent use). When the study population was restricted to those not using cocaine or intravenous drugs (87% of cohort), no increased risk was detected (OR: 1.27; 95% CI: 0.64, 2.49). Cumulative exposure was not implicated.
Martin et al., 2009	2005-2008	STEAL study, Australia	357	CVD (9)	Recent	HR: 8.33 (1.40, 49.58)	Reported an increased risk in a 96-week RCT comparing ABC/3TC to TDF/FTC. Authors reported, "ABC-3TC was associated with greater increases in total, low-density lipoprotein, and HDL cholesterol levels than TDF-FTC by intent-to-treat analysis".
Marcus et al., 2015	1998-2011	Kaiser (KPNC&KPSC), California	8154	CVD (178)	Recent	HR: 2.10 (0.90, 5.00)	Used a marginal structural model to analyze the risk. Authors didn't mention whether baseline covariates are included into the treatment model. Authors have not reported corresponding estimates from extended Cox model.

Figure 2.

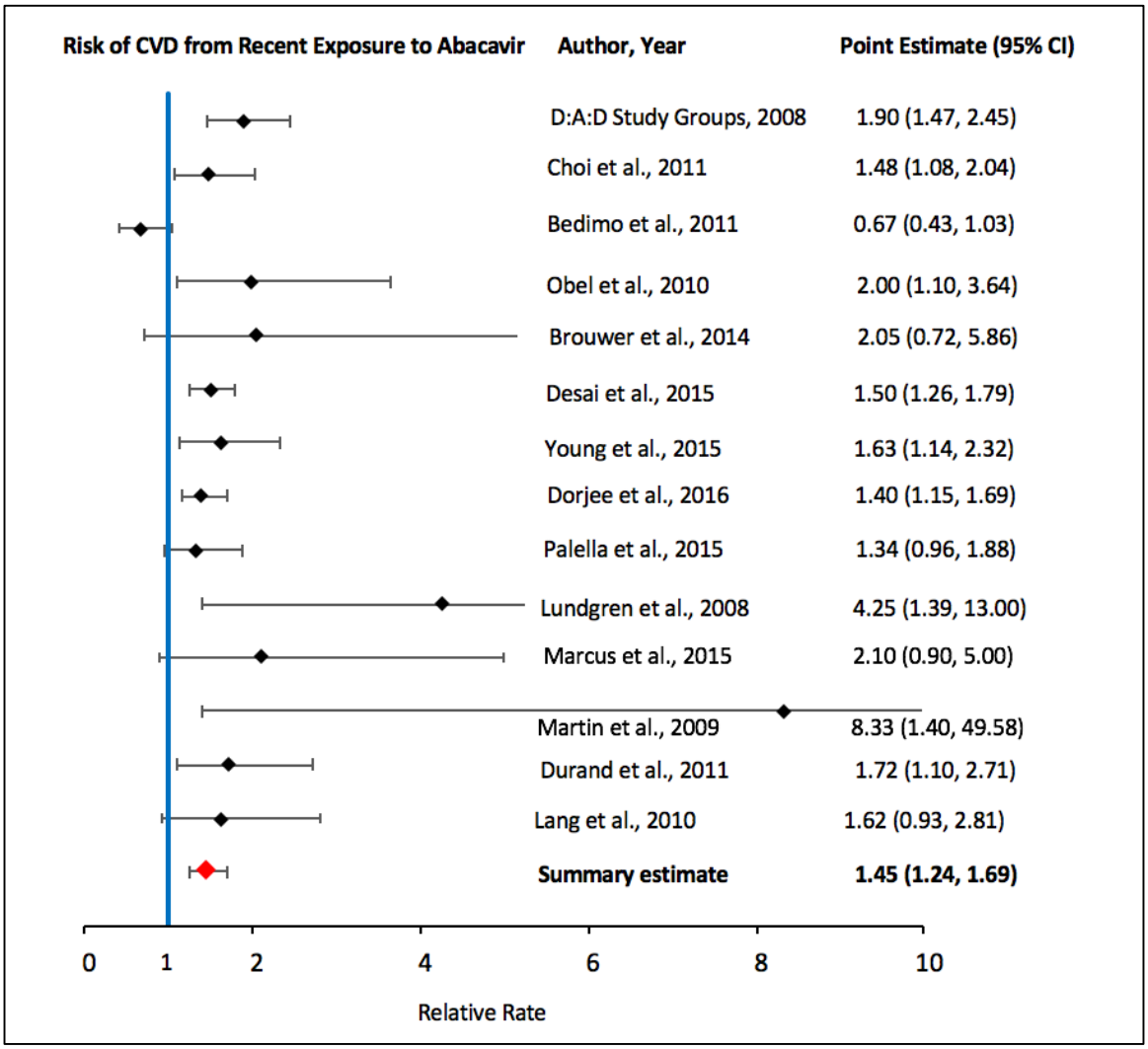

Risk of CVD from Recent Exposure to Abacavir
Author, Year
Point Estimate (95% CI)
D:A:D Study Groups, 2008
1.90 (1.47, 2.45)
Choi et al., 2011
1.48 (1.08, 2.04)
Bedimo et al., 2011
0.67 (0.43, 1.03)
Obel et al., 2010
2.00 (1.10, 3.64)
Brouwer et al., 2014
2.05 (0.72, 5.86)
Desai et al., 2015
1.50 (1.26, 1.79)
Young et al., 2015
1.63 (1.14, 2.32)
Dorjee et al., 2016
1.40 (1.15, 1.69)
Palella et al., 2015
1.34 (0.96, 1.88)
Lundgren et al., 2008
4.25 (1.39, 13.00)
Marcus et al., 2015
2.10 (0.90, 5.00)
Martin et al., 2009
8.33 (1.40, 49.58)
Durand et al., 2011
1.72 (1.10, 2.71)
Lang et al., 2010
1.62 (0.93, 2.81)
Summary estimate
1.45 (1.24, 1.69)
0
1
2
4
6
8
10
Relative Rate

Figure 3.

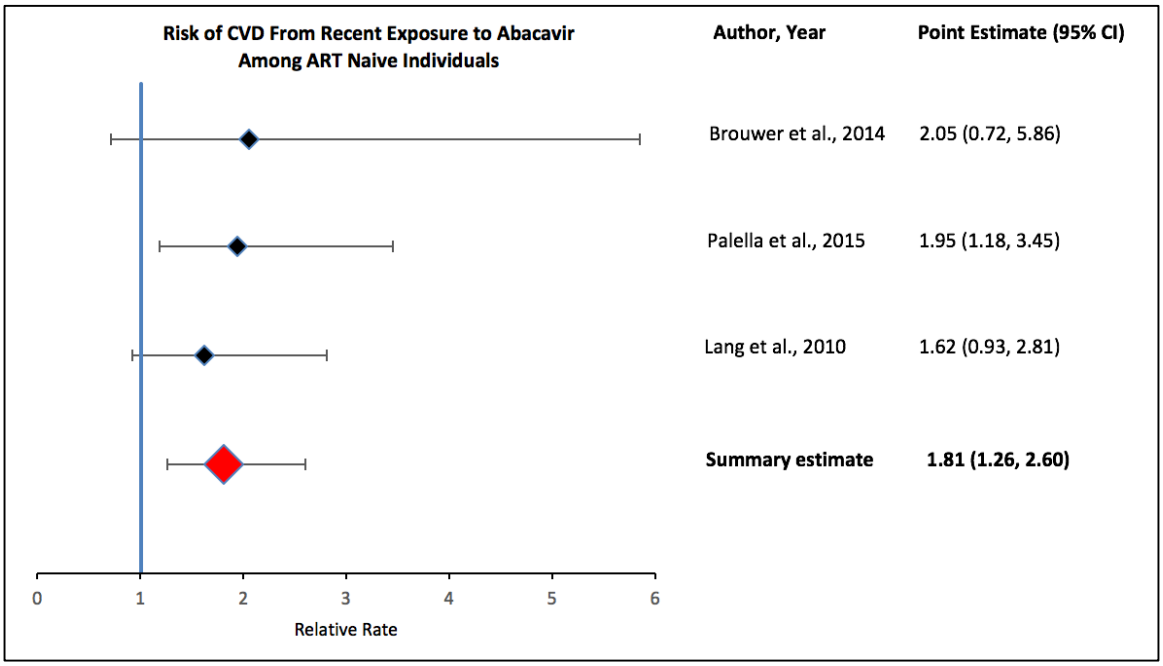
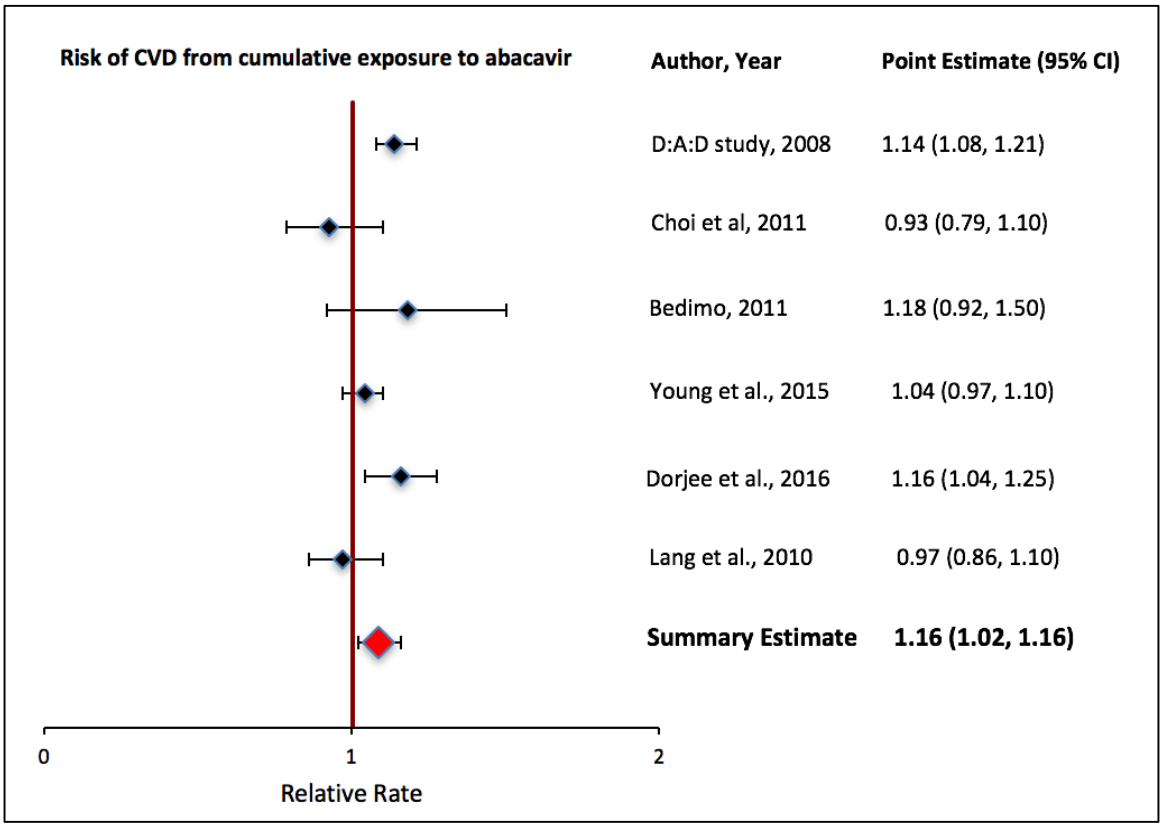

Risk of CVD From Recent Exposure to Abacavir Among ART Naive Individuals
Author, Year
Point Estimate (95% CI)
Brouwer et al., 2014
2.05 (0.72, 5.86)
Palella et al., 2015
1.95 (1.18, 3.45)
Lang et al., 2010
1.62 (0.93, 2.81)
Summary estimate
1.81 (1.26, 2.60)
0
1
2
3
4
5
6
Relative Rate

Figure 4.


Risk of CVD from cumulative exposure to abacavir
Author, Year
Point Estimate (95% CI)
D:A:D study, 2008
1.14 (1.08, 1.21)
Choi et al, 2011
0.93 (0.79, 1.10)
Bedimo, 2011
1.18 (0.92, 1.50)
Young et al., 2015
1.04 (0.97, 1.10)
Dorjee et al., 2016
1.16 (1.04, 1.25)
Lang et al., 2010
0.97 (0.86, 1.10)
Summary Estimate
1.16 (1.02, 1.16)
0
1
2
Relative Rate

References

1. Sabin CA, Worm SW, Weber R, Reiss P, El-Sadr W, Dabis F, et al. Use of nucleoside reverse transcriptase inhibitors and risk of myocardial infarction in HIV-infected patients enrolled in the D : A : D study: a multi-cohort collaboration. Lancet. 2008;371(9622):1417-26.
2. Bedimo RJ, Westfall AO, Drechsler H, Vidiella G, Tebas P. Abacavir Use and Risk of Acute Myocardial Infarction and Cerebrovascular Events in the Highly Active Antiretroviral Therapy Era. Clin Infect Dis. 2011;53(1):84-91.
3. Brouwer ES, Napravnik S, Eron Jr JJ, Stalzer B, Floris-Moore M, Simpson Jr RJ, et al. Effects of combination antiretroviral therapies on the risk of myocardial infarction among HIV patients. Epidemiology. 2014;25(3):406-17.
4. Choi AI, Vittinghoff E, Deeks SG, Weekley CC, Li YM, Shlipak MG. Cardiovascular risks associated with abacavir and tenofovir exposure in HIV-infected persons. AIDS. 2011;25(10):1289-98.
5. Durand M, Sheehy O, Lelorier J, Tremblay CL. Association between use of antiretroviral therapy and risk of acute myocardial infarction: A nested case control study using Quebec's Public Health Insurance Database (RAMQ). J Popul Ther Clin Pharmacol. 2011;18(2):e178-e9.
6. Lang S, Mary-Krause M, Cotte L, Gilquin J, Partisani M, Simon A, et al. Impact of individual antiretroviral drugs on the risk of myocardial infarction in human immunodeficiency virus-infected patients: A case-control study nested within the French hospital database on HIV ANRS cohort CO4. Arch Intern Med. 2010;170(14):1228-38.
7. Obel N, Farkas DK, Kronborg G, Larsen CS, Pedersen G, Riis A, et al. Abacavir and risk of myocardial infarction in HIV-infected patients on highly active antiretroviral therapy: A population-based nationwide cohort study. HIV Med. 2010;11(2):130-6.
8. Strategies for Management of Anti-Retroviral Therapy I, Groups DADS. Use of nucleoside reverse transcriptase inhibitors and risk of myocardial infarction in HIV-infected patients. AIDS (London, England). 2008;22(14):F17-24.
9. Ding X, Andraca-Carrera E, Cooper C, Miele P, Kornegay C, Soukup M, et al. No Association of Abacavir Use With Myocardial Infarction: Findings of an FDA Meta-Analysis. Jaids-Journal of Acquired Immune Deficiency Syndromes. 2012;61(4):441-7.
10. Cruciani M, Zanichelli V, Serpelloni G, Bosco O, Malena M, Mazzi R, et al. Abacavir use and cardiovascular disease events: a meta-analysis of published and unpublished data. AIDS. 2011;25(16):1993-2004.
11. Ribaudo HJ, Benson CA, Zheng Y, Koletar SL, Collier AC, Lok JJ, et al. No risk of myocardial infarction associated with initial antiretroviral treatment containing abacavir: Short and long-term results from ACTG A5001/ALLRT. Clin Infect Dis. 2011;52(7):929-40.
12. Desai M, Joyce V, Bendavid E, Olshen RA, Hlatky M, Chow A, et al. Risk of Cardiovascular Events Associated With Current Exposure to HIV Antiretroviral Therapies in a US Veteran Population. Clin Infect Dis. 2015;61(3):445-52.
13. Marcus JL, Neugebauer RS, Leyden WA, Chao CR, Xu L, Quesenberry CP, et al. Use of abacavir and risk of cardiovascular disease among HIV-infected individuals. J Acquir Immune Defic Syndr. 2015.

14. Martin A, Bloch M, Amin J, Baker D, Cooper DA, Emery S, et al. Simplification of Antiretroviral Therapy with Tenofovir-Emtricitabine or Abacavir-Lamivudine: A Randomized, 96-Week Trial. Clin Infect Dis. 2009;49(10):1591-601.
15. Young J, Xiao Y, Moodie EEM, Abrahamowicz M, Klein MB, Bernasconi E, et al. Effect of Cumulating Exposure to Abacavir on the Risk of Cardiovascular Disease Events in Patients From the Swiss HIV Cohort Study. J Acquir Immune Defic Syndr. 2015;69(4):413-21.
16. Dellamonica P, Katlama C, Lévy-Bachelot L, Daures JP, Finkielsztejn L. Abacavir (Ziagen®) use between 2003 and 2008 in France according to the electronic medical record NADIS®. Med Mal Infect. 2013;43(11-12):467-74.
17. Palella Jr FJ, Althoff K, Moore R, Zhang J, Kitahata M, Gange S, et al., editors. Abacavir Use and Risk for Myocardial Infarction in the NA-ACCORD. CROI 2015; 2015 February 23-26, 2015; Seattle.
18. Sabin C, Reiss P, Ryom P, De Wit S, Kirk O, Weber R, et al., editors. Is There Continued Evidence for an Association Between Abacavir and Myocardial Infarction Risk? CROI 2014; 2014 March 3-6, 2014; Boston.
19. Sax PE, Kumar P. Tolerability and safety of HIV protease inhibitors in adults. Journal of acquired immune deficiency syndromes (1999). 2004;37(1):1111-24.
20. Gresele P, Falcinelli E, Momi S, Francisci D, Baldelli F. Highly active antiretroviral therapy-related mechanisms of endothelial and platelet function alterations. Rev Cardiovasc Med. 2014;15 Suppl 1:S9-20.
21. Bavinger C, Bendavid E, Niehaus K, Olshen RA, Olkin I, Sundaram V, et al. Risk of Cardiovascular Disease from Antiretroviral Therapy for HIV: A Systematic Review. PLoS One. 2013;8(3).
22. Greenland S, Thomas DC. On the need for the rare disease assumption in case-control studies. Am J Epidemiol. 1982;116(3):547-53.
23. D'Agostino RB, Lee ML, Belanger AJ, Cupples LA, Anderson K, Kannel WB. Relation of pooled logistic regression to time dependent Cox regression analysis: the Framingham Heart Study. Stat Med. 1990;9(12):1501-15.
24. Thompson WA, Jr. On the treatment of grouped observations in life studies. Biometrics. 1977;33(3):463-70.
25. Petersen ML, van der Laan MJ. Causal models and learning from data: integrating causal modeling and statistical estimation. Epidemiology. 2014;25(3):418-26.
26. Robins JM, Hernan MA, Brumback B. Marginal structural models and causal inference in epidemiology. Epidemiology. 2000;11(5):550-60.
27. Cole SR, Hernan MA. Constructing inverse probability weights for marginal structural models. Am J Epidemiol. 2008;168(6):656-64.
28. Kristoffersen US, Kofoed K, Kronborg G, Benfield T, Kjaer A, Lebech AM. Changes in biomarkers of cardiovascular risk after a switch to abacavir in HIV-1-infected individuals receiving combination antiretroviral therapy. HIV Med. 2009;10(10):627-33.
29. Hileman CO, Wohl DA, Tisch DJ, Debanne SM, McComsey GA. Short Communication Initiation of an Abacavir-Containing Regimen in HIV-Infected Adults Is Associated with a Smaller Decrease in Inflammation and Endothelial Activation Markers Compared to Non-Abacavir-Containing Regimens. AIDS Res Hum Retroviruses. 2012;28(12):1561-4.

30. Young B, Squires KE, Ross LL, Santiago L, Sloan LM, Zhao HH, et al. Inflammatory Biomarker Changes and Their Correlation with Framingham Cardiovascular Risk and Lipid Changes in Antiretroviral-Naive HIV-Infected Patients Treated for 144 Weeks with Abacavir/Lamivudine/Atazanavir with or without Ritonavir in ARIES. AIDS Res Hum Retroviruses. 2013;29(2):350-8.
31. Martinez E, Larrousse M, Podzamczer D, Perez I, Gutierrez F, Lonca M, et al. Abacavir-based therapy does not affect biological mechanisms associated with cardiovascular dysfunction. AIDS. 2010;24(3):F1-9.
32. Palella FJ, Gange SJ, Benning L, Jacobson L, Kaplan RC, Landay AL, et al. Inflammatory biomarkers and abacavir use in the Women's Interagency HIV Study and the Multicenter AIDS Cohort Study. AIDS. 2010;24(11):1657-65.
33. Padilla S, Masia M, Garcia N, Jarrin I, Tormo C, Gutierrez F. Early changes in inflammatory and pro-thrombotic biomarkers in patients initiating antiretroviral therapy with abacavir or tenofovir. BMC Infect Dis. 2011;11.
34. De Luca A, Donati KD, Cozzi-Lepri A, Colafigli M, De Curtis A, Capobianchi MR, et al. Exposure to Abacavir and Biomarkers of Cardiovascular Disease in HIV-1-Infected Patients on Suppressive Antiretroviral Therapy: A Longitudinal Study. Jaids-Journal of Acquired Immune Deficiency Syndromes. 2012;60(3):E98-E101.
35. Kim C, Gupta SK, Green L, Taylor BM, Deuter-Reinhard M, Desta Z, et al. Abacavir, didanosine and tenofovir do not induce inflammatory, apoptotic or oxidative stress genes in coronary endothelial cells. Antivir Ther. 2011;16(8):1335-9.
36. Patel P, Bush T, Overton T, Baker J, Hammer J, Kojic E, et al. Effect of abacavir on acute changes in biomarkers associated with cardiovascular dysfunction. Antivir Ther. 2012;17(4):755-61.
37. Rasmussen TA, Tolstrup M, Melchjorsen J, Frederiksen CA, Nielsen US, Langdahl BL, et al. Evaluation of cardiovascular biomarkers In HIV-infected patients switching to abacavir or tenofovir based therapy. BMC Infect Dis. 2011;11.
38. Wohl DA, Arnoczy G, Fichtenbaum CJ, Campbell T, Taiwo B, Hicks C, et al. Comparison of cardiovascular disease risk markers in HIV-infected patients receiving abacavir and tenofovir: the nucleoside inflammation, coagulation and endothelial function (NICE) study. Antivir Ther. 2014;19(2):141-7.
39. Hammond E, McKinnon E, Mallal S, Nolan D. Longitudinal evaluation of cardiovascular disease-associated biomarkers in relation to abacavir therapy. AIDS. 2008;22(18):2540-3.
40. O'Halloran J, Dunne E, Tinago W, Denieffe S, Kenny D, Mallon P, editors. Effect of Switch From Abacavir To Tenofovir DF On Platelet Function Markers: A SWIFT Trial Substudy. CROI 2014; 2014 March 3-6, 2014; Boston.
41. Martin A, Amin J, Cooper DA, Carr A, Kelleher AD, Bloch M, et al. Abacavir does not affect circulating levels of inflammatory or coagulopathic biomarkers in suppressed HIV: a randomized clinical trial. AIDS. 2010;24(17):2657-63.
42. De Luca A, de Gaetano Donati K, Cozzi-Lepri A, Colafigli M, De Curtis A, Capobianchi MR, et al. Exposure to abacavir and biomarkers of cardiovascular disease in HIV-1-infected patients on suppressive antiretroviral therapy: a longitudinal study. J Acquir Immune Defic Syndr. 2012;60(3):e98-101.

43. Sinn K, Richardson R, Carr A. Lower arterial stiffness and Framingham score after switching abacavir to tenofovir in men at high cardiovascular risk. AIDS. 2010;24(15):2403-5.
44. Hsue PY, Hunt PW, Wu Y, Schnell A, Ho JE, Hatano H, et al. Association of abacavir and impaired endothelial function in treated and suppressed HIV-infected patients. AIDS. 2009;23(15):2021-7.
45. Gresele P, Momi S, Migliacci R. Endothelium, venous thromboembolism and ischaemic cardiovascular events. Thromb Haemost. 2010;103(1):56-61.
46. Baum PD, Sullam PM, Stoddart CA, McCune JM. Abacavir increases platelet reactivity via competitive inhibition of soluble guanylyl cyclase. AIDS (London, England). 2011;25(18):2243-8.
47. Falcinelli E, Francisci D, Belfiori B, Petito E, Guglielmini G, Malincarne L, et al. In vivo platelet activation and platelet hyperreactivity in abacavir-treated HIV-infected patients. Thromb Haemost. 2013;110(2):349-57.
48. Satchell CS, O'Halloran JA, Cotter AG, Peace AJ, O'Connor EF, Tedesco AF, et al. Increased Platelet Reactivity in HIV-1-Infected Patients Receiving Abacavir-Containing Antiretroviral Therapy. J Infect Dis. 2011;204(8):1202-10

Conclusion and Future Directions

Through this dissertation research, I have first described the risk of AMI from exposure to darunavir, raltegravir, abacavir+lamivudine+darunavir, tenofovir+emtricitabine+raltegravir and tenofovir+emtricitabine+darunavir. In addition, I found an increased risk of AMI from exposure to abacavir, didanosine, lamivudine, zidovudine, lopinavir, abacavir+lamivudine+atazanavir, and abacavir+lamivudine+zidovudine, which were described in prior studies as well. Upon further investigation of the risk of CVD from exposure to abacavir in a cohort study and a meta-analysis, I found an elevated risk of CVD from a current, recent, and a cumulative exposure to abacavir. I found a protective effect for CVD from exposure to tenofovir, emtricitabine, efavirenz, and tenofovir+emtricitabine+efavirenz.

This dissertation research enabled me to address gaps in our current understanding of the risk of CVD from exposure to various antiretroviral agents. For example, I was able to show that abacavir is associated with an increased risk of CVD among HIV infected individuals; this has been a subject of intense debate over the past several years. Moreover, I was able to uncover areas that need further investigation. For example, the increased risk of AMI from exposure to darunavir and raltegravir needs further exploration. The finding of differential effects on AMI from exposure to raltegravir based on whether it is combined with abacavir-lamivudine or tenofovir-emtricitabine calls for further exploration. The scope of the dissertation permitted me to assess the risk of AMI from only a current exposure to various antiretroviral agents and their combinations, except for abacavir. As a next step, I would like to further investigate how the the risk of CVD from exposure to various ARV drugs varies as a function of time, as I have done here for abacavir. Moreover, I would like to assess whether the increased risk of AMI that I found with lamivudine can be reproduced in a cohort of patients with hepatitis B virus infection, since lamivudine is also used for treating hepatitis B virus infection also.

From a methodological perspective, I have used marginal structural models to describe the risk. In the review of literature, I observed a lack of consistency across published studies in terms of the application of various methods and also in the definition of the final marginal model for studies that use marginal structural models. Additionally, I observed a tendency for a confusion regarding why we would need to use causal inference methods to investigate these relationships. I have tried my level best to discuss these and other concerns in the relevant chapters of my dissertation in accordance with the established science and knowledge. I hope that this study shall contribute towards a better understanding and adoption of the appropriate statistical methods in answering questions in the field of HIV-AIDS and beyond.

In sum, I would like to re-iterate that despite the findings of an increased risk of CVD from exposure to various antiretroviral agents, the overall prevalence of CVD in HIV infected population remains low and it is important to carefully weigh the risk and benefits in choosing antiretroviral drugs for a patient. I hope this dissertation shall contribute towards improving the health outcomes of HIV infected individuals globally, and inform the development of efficacious

and safe antiretroviral agents in the future until the world sees an end to the HIV-AIDS epidemic.