HIV-ASSOCIATED COPD: AN EXPLORATION OF RISK FACTORS AND IMPACTS

by

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ABSTRACT

With recent advances in highly active antiretroviral therapy (HAART) and HIV-patient care, the longevity of individuals living with human immunodeficiency virus (HIV) infection has drastically increased. However, long term complications like chronic obstructive pulmonary disease (COPD) are synchronously more prevalent among this population. The fact that clinical manifestations of COPD develop in young HIV-infected individuals (20 to 50 years of age) compared older uninfected smokers (>50 years of age) suggests HIV-associated COPD may reflect a state of accelerated aging. In this thesis, we presented the clinical and HIV-related variables associated with telomere length, a measure of cellular age, in an HIV-infected cohort and explored the variables that determine poorer respiratory related health quality in this cohort.

By using a novel technique to determine telomere length in peripheral leukocytes of HIV-infected and uninfected male subjects, we found that: firstly, telomere lengths were significantly shorter with HIV-infection even after adjusting for age, BMI, and smoking; secondly, telomere lengths were significantly and independently associated with age, FEV1(% of predicted), and soluble cluster of differentiation (sCD14); and thirdly, shorter telomere lengths were seen with worse emphysema scores as enumerated by computed tomography (CT) scans. Furthermore, the advantage of this new telomere length assay allowed us to determine that participants in our study demonstrated a “telomere age” roughly 40 years older than their biologic age, yet their respective slopes of decline with age remained parallel, when compared to an uninfected cohort.

The St. George's Respiratory Questionnaire was used to assess the influence of respiratory symptoms on a patient’s self-perceived health, social, and psychological status. Strikingly, we found HIV-infected patients are plagued with considerable respiratory limitations when compared to uninfected and even COPD counterparts. Also, we found that FEV1%pred, CD4 cell count, interleukin-6 (IL-6), but not telomere length were associated with worse respiratory health.


**Preface**

A modified version of Chapter 2 of this thesis, “Shorter Leukocyte Telomeres in Patients with Human Immunodeficiency Virus-Associated COPD,” has been submitted for review and publication (as of June 2014):


Chapter 3 of this thesis is based on the manuscript, “The Determinants of Poor Respiratory Health Status in Adults Living with Human Immunodeficiency Virus Infection,” [1] which has been published in the journal *AIDS Patient Care and STDs*:


*Equal contribution.*
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**LIST OF ABBREVIATIONS AND ACRONYMS**

AIC: Akaike Information Criterion

A1AT: alpha-1 antitrypsin

cART: combination antiretroviral therapy

COPD: chronic obstructive pulmonary disease

CRP: C-reactive protein

CT: computed tomography

FEV1: forced expiratory volume in 1 second

FEV1 %Pred: forced expiratory volume in 1 second percent predicted

FVC: forced vital capacity

FVC %Pred: forced vital capacity percent predicted

GzmB: granzyme B

HIV: human immunodeficiency virus

IL-6: interleukin-6

PJP: *Pneumocystis jirovecii* pneumonia

SGRQ: St. George's Respiratory Questionnaire

SGRQ-T: St. George's Respiratory Questionnaire Total Score

sCD14: soluble cluster of differentiation-14

SP-D: surfactant protein-D

VL: viral load
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My profound gratitude for my research supervisor, Dr. Don Sin, cannot be overstated. For three years, Dr. Sin has given me the prodigious opportunity and privilege to study and work under his guidance, which has led to the completion of this thesis, allowed me to publish multiple manuscripts and to leave a mark in the scientific community, and taught me more than just science. I especially thank Dr. Paul Man for bringing me under his wing in the study of HIV-associated COPD, for always being patient and understanding during any ‘hiccups’ along these three years, and for encouraging the team’s successes along the way. The chance to work under the concurrent supervision of both Dr. Sin and Dr. Man has been the most fortuitous blessing for me and will have a hand in any success I have in the future. Furthermore, I sincerely thank Dr. Richard Harrigan for the insightful (and at times humorous) advice, guidance, and expertise in science and in the field of HIV. Because of you, I will never host a meeting without handouts ever again – thank you. Dr. Palepu has given knowledgeable comments and feedback which have greatly improved this thesis.

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I Dedicate This Thesis To:

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For your unwavering love and support

Dr. Albert Chang
For the genius that always kept me on track
and striving to keep up with you

Dr. Andy Huang
For being the life changer that I'll never forget
What more can I say?

And to the someone or something up there
For making my every step successful – I do not know you yet, but thank you
I. **INTRODUCTION**

With the advent of combination antiretroviral therapy (cART), the life expectancy of patients with human immunodeficiency virus (HIV) in the developed world has lengthened considerably.[1] By 2015, it is estimated that over half of the HIV population in the United States will be 50 years or older.[2] In parallel to this observation, HIV-infected individuals increasingly experience age-related co-morbidities including cancers, cardiovascular diseases, and metabolic syndromes.[3-6] More specifically, recent reports suggest that HIV-infected patients are also highly susceptible to chronic obstructive pulmonary disease (COPD), which may manifest two decades earlier in life than in non-HIV-infected individuals.[7-9] Consequently, this phenomenon has recently led clinicians and researchers to investigate on the causes of this early onset COPD in HIV. Yet, even with the expanding literature in this field, vital questions are still inadequately answered: could accelerated cellular aging play a role in HIV-associated COPD, does it play any role in reduced health status of HIV patients with and without COPD, and above all, what are variables that are most influential on the quality of life in patients with HIV-associated COPD? This thesis presents our team’s research which has partially provided answers to these questions.

COPD is the fourth leading cause of death worldwide and is a heterogeneous disease with two major phenotypes: emphysema, which is characterized by destruction of lung parenchyma, and small airways disease,[10] both of which are characterized by airflow limitation, hyperinflation, gas trapping, and gas exchange abnormalities, and give rise to symptoms such as cough, breathlessness, cor pulmonale.[11] Commonly accepted hypotheses for pathogenesis of COPD include protease-antiprotease imbalance, inflammation-induced impaired repair processes, and accelerated cellular aging.[12-15] Although cigarette smoking is the dominant risk factor,[14] COPD does not develop in all smokers, suggesting that other factors are involved and pathogenesis is multifaceted.[16, 17] The story of how COPD develops becomes increasingly convoluted with chronic HIV infection.
Is it possible that chronic HIV infection is accelerating cellular aging in a similar manner as inflammaging? Apart from COPD, HIV-infected individuals increasingly experience diseases of old age including cancers, cardiovascular diseases, artherosclerosis, and metabolic syndromes. Indeed, recent literature provides evidence that accelerated aging might be the link between HIV infection and COPD [18, 19]. In HIV-infected individuals, two processes of accelerated cellular aging have been shown to take place: a shift in immune system to an aged phenotype and advanced shortening of telomere length.[20, 21]

Remarkably, several immunological changes are similar in natural old age (age 70+) and HIV-infection. In both cases, the immune system undergoes a similar shift towards a more robust innate immunity.[22, 23] For example, macrophages have been shown to be chronically activated and anti-apoptotic and result in a persistent low-grade inflammation. Moreover, pro-inflammatory cytokines such as TNF-alpha, IL-1B, and IL-6 are upregulated in senescent macrophages and have been shown to be elevated in both aged and HIV-infected individuals.[24] On the other hand, changes to the adaptive immune system are more profound and include reduced thymus size and function, reduced response to vaccines, low CD4+ to CD8+ lymphocyte count, and dramatic drop in CD28 co-stimulatory receptor expression on T cells. Chronic antigen exposure such as with chronic viral infection is associated with progressive expansion of CD8+CD28- cell T-cell population.[20] These cells have been shown to be pro-inflammatory by their increased secretion of TNF-alpha and IFN-gamma.[24]

Telomeres in mammalian cell are tandem repeats of TTAGGG/CCCTAA DNA sequences, which cap the ends of chromosomes to protect the genetic structures from enzymatic degradation. Inevitably in normal cells, telomere length shortens due to repeated rounds of cell division.[25, 26] This phenomenon is commonly known as the Hayflick Limit and leads the cell to replicative senescence (permanent cell cycle arrest) or apoptosis.[27] Oxidative stress that is often caused by
inflammation also increases the rate of telomere attrition.[28, 29] Thus, telomere length provides a good depiction the cellular aging process.

With recent advances in quantitative polymerase chain reaction (QPCR) techniques, telomere lengths can be readily and economically measured.[30, 31] Historically, assessment of telomere length relied primarily on Terminal Restriction Fragment (TRF) assay, which utilizes the fact that telomeric regions, in contrast to extra-telomeric DNA, lack restriction digest sites and are preserved after digestion with restriction enzymes. Subsequent to digestion, telomere lengths are visualized and measured through southern blot and hybridization with radioactive probes.[32] Although TRF assay has been widely used, the process requires a substantial amount of labour and sample DNA to complete and may not be feasible in large studies. A more recent method for telomere assessment is the flow cytometry- fluorescent in situ hybridization (Flow-FISH) technique, which incorporates flow cytometry to quantify telomere length through fluorescent probes hybridized to telomeric repeats in individual cells.[33] Even though this method is well regarded, its extensive use is impeded by high cost and the requirement for fresh samples. The need for a high-throughput and cost-effective method for telomere length measurement, which requires minimal amount of sample, was fulfilled with the development of a QPCR technique by Cawthon[31] and further modified by O'Callaghan.[34] These methods employ PCR to specifically amplify telomeric repeats, quantify amplification product through SYBR green, and determines the telomere length as a ratio of the amount of genetic material in the sample. O'Callaghan's modification, which incorporates a standard curve with known quantity of telomeric repeats for comparision, allows for relatively inexpensive, streamlined, and accurate determination of absolute telomere length through QPCR.

In the context of HIV, shortened telomeres were found in lymphocytes of HIV-infected subjects compared to uninfected subjects.[35] The telomere lengths measured from the HIV-infected subjects were comparable to those of healthy centenarians. In non-HIV COPD, telomere
shortening has been demonstrated in both peripheral leukocytes and lung fibroblasts [36, 37]. Intriguingly, COPD patients have increased risk of extra-pulmonary diseases which are associated with old age such as atherosclerosis, cancer, cardiovascular disease, osteoporosis, and wasting similarly to HIV-infected population.[38-41] Also, comparable inflammatory profile and decrease in CD28+ T-cell population can be found in COPD lungs.[42] However, the link between shorter telomere length in peripheral leukocytes of HIV infection and COPD, as measured by emphysema severity, remains unclear.

In the literature of HIV-associated COPD, numerous reports have described HIV-infection in relationship to spirometry measurements and computed tomography assessment of COPD.[43, 44] However, arguably the most important question remains nebulous: What are the determinants of quality of life with HIV-infection? The degree to which health-related quality of life is affected by respiratory impairment can be measured by tools such as the St. George’s Respiratory Questionnaire (SGRQ).[45] Developed and validated for patients with obstructive lung disease, the SGRQ assesses the severity and frequency of respiratory symptoms, their subsequent effect on a patient’s ability to perform daily activities and the impact they have on a patient’s self-perceived social and psychological status. In HIV-specific cohorts, the SGRQ score is known to worsen with increasing airflow limitation,[46] but the predominant risk factors for poor respiratory status in this population remain unknown.

This thesis will explore the effects of accelerated cellular aging in HIV-associated COPD and discuss the determinants of reduced health-related quality of life with HIV infection. More specifically, we will first compare the peripheral leukocyte telomere length between HIV-infected individuals with and without COPD and further compare these individuals to an uninfected control group. Additionally, we will describe the risk factors ranging from biomarkers to spirometry data that are associated with reduced health-related quality of life as measured by SGRQ scores.
II. **Shorter Telomere Length in Patients with HIV-Associated COPD**

In recent years, evidence has suggested that disease models of accelerated cellular senescence apply well to both HIV [9, 18, 19] and COPD [47]. In HIV-infected individuals, advanced aging is reflected in two processes: the loss of the cell surface co-receptor CD28 on T cells [21] and the progressive shortening of leukocyte telomere length, the result of repeated cell division [35, 48]. Telomeres are structures found at the ends of chromosomes, made up of a repetitive TTAGGG DNA sequence and responsible for protecting genomic integrity. While shortening of the telomere inevitably occurs with each cell cycle, once a critical length is reached cell cycle arrest or apoptosis ensues [27]. In COPD, telomere shortening has been demonstrated in both peripheral leukocytes and lung fibroblasts [36, 37]. Greater severity of COPD, as measured by decreased forced expiratory volume in 1 second (FEV1), is associated with reduced telomere lengths [37]. In HIV, the chronic immune activation caused by the virus may be driving the particular senescence pattern seen in this disease; whereas in “garden variety” COPD, the oxidative and inflammatory stress induced by long-term cigarette smoke exposure may be to blame. In HIV-infected patients who smoke, these effects may be additive and could contribute to the advanced emphysema seen in this population [49, 50].

In this study, we hypothesized that HIV-infected patients with COPD demonstrate shortened peripheral leukocyte telomere lengths when compared to those without COPD, as well as when compared to HIV-negative patients. Such a finding would support the notion that an accelerated aging mechanism may contribute to the susceptibility of HIV-infected patients to COPD.
METHODS

Study Population. Study participants were derived from a cohort of patients with documented HIV-1 infection at St. Paul’s Hospital, an acute care hospital serving downtown Vancouver, British Columbia. The cohort was assembled as part of a prospective observational study of lung disease in the setting of HIV infection. Although follow-up data were obtained over 3 years in this cohort, only the cross-sectional data derived from the participant’s first study visit were included in this analysis. Patients were consecutively recruited based on interest in their respiratory health. All cohort participants had to meet the following entry criteria: serologically documented HIV-1 infection and age 19 years or older. Patients with acute respiratory symptoms were not eligible for enrollment. Following informed consent, study participants completed a structured questionnaire for the collection of demographic data and clinical history. Pertinent elements of this questionnaire included age, sex, BMI, smoking status (cigarette and marijuana), and history of any respiratory infection, including Pneumocystis jirovecii pneumonia (PJP). Information was obtained through an in-person interview that was complemented by data abstraction from clinic charts. HIV-related clinical information, including laboratory data and cART history, were obtained through a linkage with the British Columbia Centre for Excellence in HIV/AIDS Drug Treatment Program database. Baseline plasma HIV RNA levels and nadir CD4 cell counts were measured at the time of HIV diagnosis.

A control population was derived from the Canadian Cohort of Obstructive Lung Disease (CanCOLD) study, a population-based multisite prospective longitudinal cohort study designed to follow both COPD and non-COPD subjects [51]. Demographic data including age, sex, and smoking status and duration were available for this cohort, as were spirometry measurements including FEV1 and FEV1 percent predicted (FEV1 %Pred). For analysis, data from female subjects were excluded in order to better match the HIV-infected group.
**Spirometry.** Spirometry was performed using a Jaeger Flow Screen spirometer according to the American Thoracic Society (ATS)/European Respiratory Society (ERS) recommendations [52]. Individual FEV1 measurements were expressed as a percentage of predicted (FEV1 %Pred) using the NHANES III reference equation [53].

**Computed Tomography (CT) Scans.** Patients underwent chest CT scanning with images acquired using a 64 detector CT scanner (VCT XT and Discovery HD 750 GE Healthcare, Waukasha, WI) under a modified low effective dose protocol. Images were acquired using 1.0-mm slice thickness, 120 kVp, 215 mA, 0.6-second rotation time and pitch of 1.5 and reconstructed using both high and intermediate spatial frequency reconstruction kernels. Two highly experienced radiologists (JL and RR), blinded to pulmonary function testing and laboratory data, interpreted the CT images together to achieve consensus. Emphysema severity was quantified based on a modified method of Kazerooni et al [54], also employed in the COPDGene study [55]. Individual emphysema scores for each of the 5 lobes plus the lingula of the lung were assessed as a percentage of total lung volume with a score of 0-4 assigned, defined as follows: 0=absence of emphysema, 1=1-25% emphysema, 2=26-50% emphysema, 3=51-75% emphysema, and 4=76-100% emphysema. A total score was obtained by the summation of the scores of the 5 lobes and lingula.

**Measurement of Absolute Telomere Length in Peripheral Leukocytes.** Genomic DNA from peripheral blood buffy coat in HIV-infected participants and whole blood from CanCOLD participants was harvested using the Qiagen DNeasy Blood & Tissue Kit (Qiagen, Venlo, the Netherlands). Absolute telomere length was measured by quantitative polymerase chain reaction (PCR) consistent with methods outlined by O’Callaghan and Fenech [34]. In brief, standard curves for telomere DNA (TEL) and for a single copy gene (36B4) were generated. The housekeeping gene 36B4 encodes for the acidic ribosomal subunit, is ubiquitously present in leukocytes, and thus
provides a good indicator of the amount of genetic material present in each DNA sample. Telomere length was then assessed based on the ratio of telomere DNA length to 36B4 DNA length as obtained from their respective standard curves. Intra-plate controls were provided by a short telomere length cell line (HEK293) and a long telomere length cell line (K562) [56]. Samples were run in triplicate using the ABI ViiA 7 Real Time PCR System (Applied Biosystems, Foster City, CA). If the coefficient of variation was less than 5% between the triplicates, then the average telomere length between the replicates were used for analysis.

**Measurement of Inflammatory Biomarkers.** Blood samples were collected after an overnight fast using a standard venipuncture method, separated into their various components and assayed for CD4 cell count and plasma HIV-1 RNA level (Roche Amplicor Ultrasensitive Assay and Roche Taqman Ultrasensitive Assay, Laval, Quebec, Canada) in the laboratory facilities of St. Paul’s Hospital. Plasma samples which had been stored at -80°C were thawed once for the measurements of C-reactive protein (CRP), interleukin-6 (IL-6), surfactant protein-D (SP-D), lipopolysaccharide (LPS), soluble cluster of differentiation 14 (sCD14), granzyme B (GzmB), and alpha-1-antitrypsin (A1AT). These plasma assays were chosen because they have been previously implicated as potential biomarkers of COPD and/or inflammatory biomarkers of HIV [57-64]. CRP, IL-6, and sCD14 were measured using commercially available high-sensitivity enzyme linked immunoassay (ELISA) kits (R&D Systems, Minneapolis, MN), and had lower detection limits of 0.01ng/mL, 0.039pg/mL, and 0.125ng/mL with coefficients of variation per assay of 4.1%, 3.9%, and 2.8%, respectively. SP-D (BioVendor, Brno, Czech Republic) and GzmB (eBioscience, San Diego, CA) plasma levels were measured with ELISA kits with a lower limit of detection of 0.01ng/mL and 0.8pg/mL, and coefficients of variation of 3.0% and 4.5%, respectively. LPS levels were measured using a commercially available kinetic chromogenic Limulus amebocyte lysate (LAL) assay kit (Lonza Walkersville, Walkersville, MD), following plasma dilution and heat inactivation pre-treatment, with the lower limit of detection of 0.5pg/mL. All biomarker assays were measured in
duplicates and the average between the duplicates for each sample were used for statistical analysis. A1AT levels were determined by St. Paul’s Hospital Laboratory (Department of Pathology & Laboratory Medicine, Vancouver, BC) using serum samples that were stored at -80°C prior to the assay. Patients who were found to have A1AT levels <1.3g/L went on to have A1AT genotyping performed. In brief, SERPINA1 status for the E366K and E288V mutations associated with the A1AT deficiency Z and S phenotypes, respectively, were determined via a PCR reaction with melting curve analyses on the Roche (Basel) LightCycler™ instrument.

**Statistical Analysis.** To evaluate the relationships of peripheral leukocyte telomere length with the clinical characteristics of HIV-infected patients and with plasma biomarkers, we first divided the cohort into quartiles of telomere length. The baseline characteristics across the quartiles were compared using Jonckheere-Terpstra trend test for continuous variables and Cochran-Armitage test for dichotomous variables [65, 66]. Collinearity, normality, heteroscedascity, and autocorrelation were assessed to validate the linear regression model. Biomarkers were log-transformed to achieve normality.

A backward stepwise procedure was employed to build the multivariable model. The selection of variables was based on two criteria: Akaike Information Criterion (AIC) and Type III p-values. These two criteria balance the model choice on finding the best explanatory model (Type III p-values: lower p-values indicate more significance) and at the same time, a model with the best goodness-of-fit statistics (AIC: lower values indicate better fit). At each step during backward stepwise multivariate analysis, the AIC value and the Type III p-value of each variable were recorded and the variable with the highest Type III p-value was dropped until we obtained the lowest AIC [67]. All analyses were performed using JMP statistical software (version 10.0; SAS Institute, Cary, NC) and two-sided p-values <0.05 were considered significant.
Ethics and Informed Consent. This study received approval from the UBC-Providence Health Care ethics review committee (No.H11-02713) and all subjects provided written informed consent to participate in the study.
RESULTS

Subjects. Of the 267 patients invited to participate in the study since June 2009, 42 patients declined. Of the 225 consenting participants, 17 did not have appropriate buffy coat to extract DNA. Since the clinic sees overwhelmingly more males than females, only male patient data were used for analysis (thus 21 females were excluded). Thus, a total of 187 males formed the study cohort and contributed data to all statistical analyses.

Demographic and spirometry data and telomere lengths for the study cohort are shown in Table 2.1. The mean telomere length (±standard deviation [SD]) of the entire cohort was 132±31 kbp/genome, the mean age was 49±10 years, and the mean body mass index (BMI) was 26±5 kg/m^2. The cohort was segregated into quartiles based on telomere length. In the univariate analyses, a significant trend was observed for age across the telomere quartiles (p=0.016) while BMI approached statistical significance (p=0.053). About half (53%) of study participants were smokers who had smoked on average for 28±19 pack-years; however, smoking did not show a significant association with telomere length. On the other hand, prior marijuana smoking was associated with shorter telomere length (p=0.028). Of the 87 (49%) participants with a history of prior respiratory infection, 23 (12%) had a prior PJP infection, which was more prevalent in the shorter telomere length quartiles (p=0.022). Co-morbidities included 33 (18%) participants with hepatitis C and 39 (22%) with asthma, neither of which showed a significant association across telomere quartiles. In terms of the spirometry data, both FEV1 %Pred and FEV1/FVC showed a significant trend with telomere length (p=0.036 and p=0.027, respectively), while FVC %Pred did not

HIV-Specific Clinical Variables. Associations between absolute telomere length and HIV-specific variables are shown in Table 2.2. Subjects were known to be HIV-infected, on average, for 12 years (145±90 months) prior to enrolment in this study. Almost all study participants (99%) were on
cART at the time of study enrolment. There was no difference in the baseline (pre-cART) plasma viral load across the telomere quartiles. Interestingly, pre-cART CD4 cell counts at the time of HIV diagnosis showed a positive trend with telomere length (p=0.027). Current CD4 count, however, did not differ across the quartiles.

**Blood Biomarker Measurements.** As shown in Table 2.3, sCD14 showed a significant negative association with telomere length (p-trend=0.017). None of the other biomarkers measured were significantly associated with telomere length. However, A1AT, SP-D, and GzmB were also included in the multivariate model, given that their p-values were below the pre-established threshold for inclusion (p = 0.15). A1AT genotyping for the 16 individuals with A1AT levels <1.3g/L showed that 11 had the MM genotype, 2 the MZ genotype, 1 the SZ genotype, and 2 the MS genotype.

**Emphysema Severity and Telomere Length.** Figure 2.1 shows the relationship between telomere length and total emphysema score as measured by CT. Emphysema scores were segregated into four ordinal groups: 0, 1 to 2, 3 to 4, and 5 and above. The telomere lengths (mean±standard error [SEM]) of each group were 131±4, 142±6, 133±9, and 110±5 kbp/genome, respectively. Telomere length was significantly negatively related to emphysema total scores (p=0.041), indicating that more severe emphysema was associated with shorter telomeres.
Figure 2.1. Absolute leukocyte telomere length as a continuous variable is plotted against the total emphysema score as assessed by CT imaging. Higher CT emphysema scores reflect greater severity of disease. Significantly shorter absolute telomere lengths are seen with worse emphysema scores.
**Multivariate Analysis.** In a multivariate linear regression analysis that included age, BMI, smoking, FEV1 %Pred, ever marijuana use, prior PJP, SP-D, nadir CD4 cell count, current viral load, A1AT, sCD14, and GzmB, the final model showed that only age (p<0.0001), FEV1 %Pred (p=0.037) and sCD14 (p=0.006) were persistently and significantly associated with telomere length (see Table 2.4). All other variables dropped out of the model while following the statistical procedure described in methods section. As shown in Figure 2.2, when telomere length was plotted against FEV1 %Pred while adjusting for age, BMI, and smoking, a significant linear association remained (p=0.013).

| Table 2.4. Multivariable Analysis (Telomere Length as Continuous Y-Variable) |
|-----------------|-----------------|-----------------|-----------------|-----------------|
|                 | β (SE)          | Standardized β  | VIF          | P-value        |
| Age (years)     | -0.765 (0.218)  | -0.250          | 1.080        | <0.0001        |
| FEV1 %Pred      | 0.203 (0.097)   | 0.148           | 1.053        | 0.037          |
| sCD14 (µL/mL)†  | -18.435 (6.672) | -0.197          | 1.079        | 0.006          |
| GzmB (pg/mL)†   | 3.201 (1.891)   | 0.117           | 1.019        | 0.092          |
| Prior Marijuana Smoking | -3.289 (2.166) | -0.106          | 1.029        | 0.131          |

† Logarithmic scale.

Abbreviation definition: SE = standard error; VIF = variance inflation factor; FEV1 %Pred = forced expiratory volume in 1 second percent predicted; sCD14 = soluble cluster of differentiation 14; GzmB = granzyme B.
Figure 2.2. A multiple regression of absolute telomere length against FEV1 %Pred after adjusting for age, BMI, and smoking is shown for patients with HIV. More severe airflow obstruction as measured by FEV1 %Pred is significantly associated with shorter telomere lengths. The solid line represents the regression line with dashed lines representing the 95% confidence interval for the slope of the regression line.
**Telomere Length in a Non-HIV Cohort.** Additionally, telomere lengths were measured in 345 HIV-uninfected male individuals from the CanCOLD cohort and compared to those measured in HIV-infected participants in our study. After adjusting for age, BMI, smoking, and FEV1 %Pred, there was a significant negative correlation between telomere length and age, in both the CanCOLD and HIV groups (R²=0.035, p=0.001 and R²=0.076, p<0.001, respectively) (see Figure 2.3). The y-intercepts of the HIV and CanCOLD lines (174 kbp/genome and 208 kbp/genome, respectively) differed significantly (p<0.001). However, the slopes of decline in telomere length were similar between the two groups (p=0.721) with both groups sharing a common slope of -0.935±0.197 kbp/genome/year (p<0.001). Figure 2.4 further illustrates the difference in telomere length between males in the CanCOLD and HIV cohorts (mean±SEM of 150±3 and 123±4 kbp/genome, respectively) with the HIV group showing significantly shorter telomere length (p<0.0001), after adjusting for age, BMI, smoking, and FEV1 %Pred.
**Figure 2.3.** Telomere length vs. age linear regression comparison between HIV and CanCOLD. When HIV-infected males are compared to non-HIV-infected males (CanCOLD cohort), significant differences in telomere length are seen. For any given age, the absolute leukocyte telomere length in HIV-infected males is approximately 30 kbp/genome shorter than that seen in non-HIV-infected males. Alternatively, HIV-infected males appear to demonstrate telomere lengths equivalent to non-HIV-infected males approximately 40 years older. Although the respective y-intercepts differ significantly (p<0.001), the respective rates of telomere attrition do not differ significantly (p=0.721). Solid lines represent the regression line; dashed lines represent the 95% confidence interval.
Figure 2.4. Mean telomere length comparison between HIV and CanCOLD. Absolute leukocyte telomere lengths are compared between HIV-infected patients and non-HIV-infected patients (CanCOLD cohort) who were age- and sex-matched to the HIV-infected cohort. Even after adjustment for BMI, smoking pack-years, and FEV1 %Pred, telomere lengths appear to be significantly shorter in HIV patients.
DISCUSSION

In this study, we demonstrate that HIV infection is associated with shortened telomeres. Furthermore, within HIV-infected individuals, COPD (as measured by both spirometry and CT imaging) is associated with the greatest reduction in telomere length. These findings support the notion that HIV infection results in an accelerated aging process that can be associated with further deleterious clinical consequences in the lung. Telomere attrition in HIV is widely believed to be the product of rapid immune cell turnover driven not only by ongoing viral replication, but also potentially by microbial translocation and comorbid chronic infections such as hepatitis C and cytomegalovirus [24]. In addition to telomere shortening, the loss of the CD28 cell surface receptor on T cells and the resulting dysfunction of these T cells help to drive an inflammatory cascade marked by increased levels of IL-1, IL-6, and TNF-α. This process, known as “inflamm-aging” [68], could potentially serve as the driving force behind emphysema, triggering apoptotic pathways that lead to emphysematous destruction of lung tissue. The toxic and inflammatory byproducts of such destruction could go on to serve as self-antigens and perpetuate a vicious “inflamm-aging” cycle [69]. Our finding that shorter telomere lengths are seen in HIV-infected patients with a history of PJP and in those with the lowest nadir CD4 cell counts suggests that infection-related inflammation may contribute to the aging process in HIV-infected patients.

One of the key assets of this study is the determination of absolute telomere length through QPCR. This method allows for cost-efficient and fluent measurement of telomere length when compared to traditional methods involving TRF and FISH. [70] Each of the currently available methods for telomere length measurement offers advantages and disadvantages. Firstly, the inter-experimental coefficient of variation (CV) for both TRF and FISH is low. It is reported that the mean CV for TRF and FISH are 1.7% and 1.6%, respectively, while QPCR based assays range between 5 to 7%.[34, 70, 71] Secondly, both TRF and FISH-based assays directly measure each strand of
telomeric repeat and provide true absolute telomere length. In TRF techniques, genomic DNA content is digested through restriction enzymes, leaving undigested individual telomeric regions to be analyzed by southern blotting.[71, 72] In FISH-based methods, fluorescent probes bind directly to telomere repeats and allow for telomere length determination through imaging devices or a flow-cytometer. In contrast, QPCR impartially amplify all telomere sequences in the sample of interest and only allows determination of the relative telomere length compared to a reference. These inherent differences prohibit direct comparison of telomere length results between the techniques. The disadvantages of TRF and FISH based techniques are that they are labour-intensive, costly, and demanding on both quantity and quality of samples; these factors make them unsuitable for high-throughput analysis, unlike QPCR-based assays. Recently, O’Callaghan developed a system in which telomere length can be determined quantitatively by including standard curves with known amounts of telomeric repeats for each assay. The standard curves not only act as experimental control, but also as a gauge for absolute telomere length of the sample expressed in number of nucleobases.[30] This version of the QPCR-based assay has recently grown in popularity and appeared in various publications. [73-75] To our knowledge, this study is the first to present the distribution of telomere lengths across ages in both HIV-infected males and a large cohort of uninfected males and may provide a telomere length reference for future studies using this novel technique.

Although we measured a number of inflammatory markers, only one (sCD14) maintained a significant association with reduced telomere length, in contrast to previous studies that have associated higher IL-6 levels with telomere shortening in COPD patients [76]. sCD14 is a monocyte-expressed LPS receptor secreted by the liver, thought to reflect microbial translocation [77]. Significantly higher levels of sCD14 are seen in HIV-infected individuals compared to uninfected controls [78]. Moreover, elevated sCD14 levels strongly predict mortality in HIV (those with the highest levels have a 6-fold higher rate of death), even after adjustment for baseline HIV viral loads.
and nadir CD4 cell counts [79]. Ongoing damage to the intestinal mucosal barrier, despite adequate viral suppression with cART, may contribute to increased plasma sCD14 levels and propagate systemic inflammation as a result. What role sCD14 has in further inducing lung damage has yet to be fully elucidated; however, it is known that sCD14 levels in bronchoalveolar lavage (BAL) fluid and cells are higher in smokers compared to non-smokers [80]. Murine models deficient in SP-D, a known biomarker whose absence in the lung is associated with COPD [81], have been shown to have increased sCD14 in their BAL fluid through interaction with matrix metalloproteinase-12 (MMP-12) [82]. MMP-12, in turn, has been shown to induce elastin degradation and subsequent emphysema [83], with minor allele single nucleotide polymorphisms in the MMP12 gene associated with a higher risk of COPD [84]. While the exact mechanisms leading to the development of emphysema need to be explored further, our data suggest plausible ties between sCD14, telomere length, and COPD.

Our findings support the known associations between shortened telomere length and the severity of airflow obstruction, demonstrated previously in peripheral leukocytes [37, 76] and lung tissue [36]. A link between mortality and short telomere length has further been established in patients with COPD [85]. Ongoing cell proliferation within emphysematous lungs [86] may be the primary trigger for telomere attrition, with the resulting apoptosis leading to emphysematous damage. However, we propose based on our data that telomere attrition is even more profound when HIV is present in conjunction with emphysema, a possible dual hit to telomeres that results in profound shortening. Indeed, when age- and sex-matched patients without HIV are compared to our cohort and statistical adjustments made for cigarette smoking, BMI, and FEV1, the differences in telomere length are striking.

Interestingly, when compared to an HIV-uninfected cohort, participants in our study demonstrated a “telomere age” roughly 40 years older than their biologic age, yet their respective
slopes of decline with age remained parallel. These results suggest that telomere length may have acutely shortened prior to enrollment in the study, perhaps at the time of acute HIV infection or during periods of the severe immunosuppression. Supporting this notion is the fact that nadir (but not current) CD4 cell counts and a history of PJP were significantly associated with reduced telomere lengths. However, since the vast majority of our patients were subsequently initiated on cART with adequate viral suppression, telomere length may have stabilized with the rate of attrition then mirroring that of the general population. Such a protective effect of cART stands in contradiction to *in vitro* studies demonstrating decreased telomerase activity (and thus accelerated telomere shortening) in peripheral blood monocytes treated with nucleoside and nucleotide reverse transcriptase inhibitors [87]. While previous smaller studies have implicated cART in the accelerated aging process [88], more recent data suggest no greater telomere attrition with increased exposure to cART [89]. In fact, the findings of our study support the early initiation of cART in order to abrogate the accelerated aging process and potentially avert the adverse consequences of accelerated aging such as emphysema.

Our study has several limitations, including the fact that due to the demographics of our HIV clinic population, only men were included in the final analysis. The validity of generalizing our findings to HIV-infected women is not known at this time. Secondly, we measured telomere length in a population of leukocytes for which further differentiation was not performed. It is possible that telomere attrition is more prominent in certain leukocyte subsets, for instance CD8+ T cells that are known to proliferate rapidly in HIV-infected patients and specifically CD28-CD8+ T cells that are known to exhibit a senescent phenotype [24]. Our measurements are likely to overestimate telomere lengths of our HIV cohort as a result. Furthermore, telomere length can only be measured in intact cells, raising the possibility that cellular environments prone to rapid apoptosis and destruction may not necessarily be reflected in the measurements obtained. Thirdly, the measurement of telomere length in peripheral blood cells is only presumed but not proven to...
mimic the lung. Recent work has demonstrated excellent correlation between peripheral leukocyte and lung tissue telomere length in patients with A1AT deficiency (A. Saferali, personal communication), but whether or not such a relationship holds in HIV-associated COPD needs to be assessed with further experiments in lung-specific tissue. Finally, while statistical adjustments were made to account for the demographic differences between the HIV and CanCOLD cohorts, a true age- and sex-matched control population would strengthen the argument that significant differences in cellular senescence exist between HIV-infected and HIV-uninfected individuals.
III. THE DETERMINANTS OF POOR RESPIRATORY HEALTH STATUS IN ADULTS LIVING WITH HUMAN IMMUNODEFICIENCY VIRUS INFECTION

The St. George’s Respiratory Questionnaire (SGRQ) is a tool which allows for the measurement of the influence of respiratory symptoms on a patient’s self-perceived health, social, and psychological status.[45] In non-HIV-infected cohorts, the SGRQ score has been shown to correlate well with functional parameters such as the 6 minute walk test and general health status scores such as the Short Form (36) Health Survey (SF-36).[90] In HIV-specific cohorts, the SGRQ score is known to worsen with increasing airflow limitation,[46] but the predominant risk factors for poor respiratory status in this population remain unknown. A thorough investigation of the relevant clinical and biochemical variables that contribute to overall respiratory health status has yet to be performed in HIV-infected patients. Identification of these risk factors may shed light on why HIV-infected patients are more prone to developing obstructive lung disease. In this study, a prospective cohort of HIV-infected men was evaluated to identify clinical, respiratory, and laboratory factors potentially affecting the risk for poor respiratory-related health status as measured by the SGRQ.
METHODS

Study Population. Study participants were derived from a cohort of patients with documented HIV-1 infection attending an HIV outpatient clinic at St. Paul’s Hospital in Vancouver, British Columbia. Ongoing since 2009, the cohort was assembled as part of a prospective observational study on HIV lung disease with cross-sectional data included in this analysis derived from the participant’s first visit in the study. Patients were referred to the study and consecutively recruited based on interest in their lung health and did not necessarily have lung disease. Cohort participants met the following entry criteria: serologically documented HIV-1 infection, at least 18 months of cART exposure, and age 19 years or older. Patients with acute respiratory symptoms were excluded. Following informed consent, study participants completed a structured questionnaire for the collection of demographic data and clinical history. Pertinent elements of this questionnaire included age, sex, cigarette and marijuana smoking status, the duration and amount of smoking, and history of any respiratory infection, including Pneumocystis jirovecii pneumonia (PJP). Information was obtained through an in-person interview that was complemented by data abstraction from clinic charts and electronic medical records. Complete HIV medical records, including clinical and laboratory data as well as cART exposure history, were obtained through a linkage with the British Columbia Centre for Excellence in HIV/AIDS Drug Treatment Program database. Baseline HIV RNA levels and nadir CD4 counts were considered to be those measured at the time of diagnosis.

SGRQ Protocol. The SGRQ was self-administered by all study participants following the guidelines set at St. George’s, University of London.[45] Scores were calculated in the three domains of the SGRQ (Symptom, Activity, and Impact). The complete survey consists of 50 items. The Symptom domain is allotted 8 items with that assess the frequency of respiratory symptoms over the past year. The 16 items allotted to Activity domain identifies physical activities which cause breathlessness or is affected by breathlessness. Finally, the 26 items in the Impact domain broadly
assess the impact of respiratory diseases on social and emotional function.[90] From these domain scores, a Total score (SGRQ-T) was calculated. Possible SGRQ scores range from 0 to 100, with higher scores reflecting worse respiratory-related health status. As only the SGRQ-T was designed to assess global respiratory health status,[45] this was used as the main outcome variable in the analyses.

**Spirometry.** Spirometry was performed using a Jaeger Flow Screen spirometer according to the American Thoracic Society/European Respiratory Society (ATS/ERS) recommendations.[52] Individual measurements of forced expiratory volume in 1 second (FEV1) were expressed as a percentage of predicted (FEV1 %Pred) using the NHANES III reference equation.[53] Some subjects had spirometry measured in the clinical laboratory at St. Paul’s Hospital, BC, using procedures and equipment consistent with the ATS/ERS guidelines.[91]

**Computed Tomography (CT) Scans.** Patients underwent chest CT scanning with images acquired using a 64 detector CT scanner (VCT XT and Discovery HD 750 GE Healthcare, Waukasha, WI) under a modified low effective dose protocol. Images were acquired using 1.0-mm detector aperture, 120 kVp, 215 mA, 0.6-second rotation time and pitch of 1.5 and reconstructed using both high and intermediate spatial frequency reconstruction kernels. CT scans were analyzed using the Apollo image analysis system (VIDA Diagnostics Inc., Coralville, IA). Two highly experienced radiologists independently interpreted the CT images. Emphysema severity was quantified based on a modified method of Kazerooni et al.[54] Individual emphysema scores for each of the 5 lobes of the lung plus the lingula were assessed as a percentage of total lung volume with a score of 0-4 assigned, defined as follows: 0=absence of emphysema, 1=1-25% emphysema, 2=26-50% emphysema, 3=51-75% emphysema, and 4=76-100% emphysema. A total score was obtained by the summation of the 6 individual “lobe” scores (5 lobes and lingula).
**Laboratory Studies.** Blood samples were collected after an overnight fast using a standard venipuncture method, separated into their various components, then assayed for CD4 T-lymphocyte count and plasma HIV-1 RNA (Roche Amplicor Ultrasensitive Assay and Roche Taqman Ultrasensitive Assay, Laval, Quebec, Canada) in the laboratory facilities of St. Paul's Hospital. Plasma samples which had been stored at -80°C were thawed once for the measurements of C-reactive protein (CRP), interleukin-6 (IL-6), surfactant protein-D (SP-D), soluble cluster of differentiation-14 (sCD14), granzyme B (GzmB), and alpha-1-antitrypsin (A1AT). These plasma assays were chosen because they have been previously implicated as potential biomarkers of chronic obstructive pulmonary disease (COPD) and/or HIV/AIDS-related inflammation.[57-60, 62-64] CRP, IL-6, and sCD14 were measured using commercially available high-sensitivity enzyme linked immunoassay (ELISA) kits (R&D Systems, Minneapolis, MN), and had lower detection limits of 0.01 ng/mL, 0.039 pg/mL, and 0.125 ng/mL with coefficients of variation per assay of 4.1%, 3.9%, and 2.8%, respectively. SP-D (BioVendor, Brno, Czech Republic) and GzmB (eBioscience, San Diego, CA) plasma levels were measured with ELISA kits with a lower limit of detection of 0.01ng/mL and 0.8pg/mL, and coefficients of variation of 3.0% and 4.5%, respectively. All biomarker assays were measured in duplicate and the average of the duplicates for each sample measurement was used for statistical analysis. A1AT levels were determined by St. Paul’s Hospital Laboratory (Department of Pathology & Laboratory Medicine, Vancouver, BC) using serum samples which were stored at -80°C prior to the assay. Patients who were found to have A1AT levels <1.3 g/L went on to have A1AT genotyping performed. In brief, SERPINA1 status for the E366K and E288V mutations associated with the A1AT deficiency Z and S phenotypes, respectively, was determined via a PCR reaction with melting curve analyses on the Roche (Basel) LightCycler™ instrument.
**Measurement of Absolute Telomere Length in Peripheral Leukocytes.** Genomic DNA from peripheral blood buffy coat in HIV-infected participants and whole blood from CanCOLD participants was harvested using the Qiagen DNeasy Blood & Tissue Kit (Qiagen, Venlo, the Netherlands). Absolute telomere length was measured by quantitative polymerase chain reaction (PCR) consistent with methods outlined by O’Callaghan and Fenech [34]. In brief, standard curves for telomere DNA (TEL) and for a single copy gene (36B4) were generated. The housekeeping gene 36B4 encodes for the acidic ribosomal subunit, is ubiquitously present in leukocytes, and thus provides a good indicator of the amount of genetic material present in each DNA sample. Telomere length was then assessed based on the ratio of telomere DNA length to 36B4 DNA length as obtained from their respective standard curves. Intra-plate controls were provided by a short telomere length cell line (HEK293) and a long telomere length cell line (K562) [56]. Samples were run in triplicate using the ABI ViiA 7 Real Time PCR System (Applied Biosystems, Foster City, CA). If the coefficient of variation was less than 5% between the triplicates, then the average telomere length between the replicates were used for analysis.

**Statistical Analysis.** To evaluate the relationship of SGRQ-T to clinical, respiratory, and laboratory parameters, we first divided the cohort into quartiles of SGRQ-T. The baseline characteristics across the quartiles were compared using the Jonckheere-Terpstra trend test for continuous variables and the Cochran-Armitage test for dichotomous variables.[65, 66] To determine the robustness of the analysis, we repeated the linear regression analysis using SGRQ-T. Collinearity, normality, heterocedascity, and auto-correlation were assessed to validate the linear regression model. Biomarkers were log-transformed to achieve normality.

A backward stepwise procedure was employed to build the multivariable model. The selection of variables was based on two criteria: Akaike Information Criterion (AIC) and Type III p-
values. These two criteria balanced the model choice on finding the best explanatory model (Type III p-values: lower p-values indicate more significance) and at the same time, a model with the best goodness-of-fit statistics (AIC: lower values indicate better fit). At each step in the backwards stepwise multivariable analysis, the AIC value and the Type III p-value of each variable were recorded and the variables with a Type III p-value >0.2 were dropped until we obtained the lowest AIC.[67] All analyses were performed using JMP statistical software (version 10.0; SAS Institute, Cary, NC) and two-sided p-values <0.05 were considered significant.

**Ethics and Informed Consent.** This study received approval from the UBC-Providence Health Care ethics review committee (No.H11-02713) and all subjects provided written informed consent to participate in the study.
RESULTS

Study Population. Of the 269 patients invited to participate in the study, 42 declined. Of the 227 consenting participants, 7 did not have technically acceptable spirometry and were excluded from analysis. Since our clinic sees overwhelmingly more men than women, only male patients were included in the final analysis (21 females were removed from the study cohort). Thus, a total of 199 males formed the study cohort. Demographic characteristics of the cohort are listed in Table 3.1. The mean age (± standard deviation [SD]) of the study cohort was 49 ± 10 years. 104 patients (52%) were current smokers and the mean amount of cigarette smoking (±SD) for current and former smokers was 28 ± 19 pack-years.

Study participants were divided into quartiles based on their SGRQ-T scores (Table 3.1). The upper limits of these quartiles were as follows: 13.8 for Quartile 1, 31.9 for Quartile 2, and 46.8 for Quartile 3; Quartile 4 included scores greater than 46.8. In the univariate analysis, there were no differences in age, body mass index (BMI), current smoking status, history of marijuana use, history of PJP infection, hepatitis C, or asthma across the SGRQ-T score quartiles. Higher SGRQ-T score quartiles, indicating worse respiratory-related health status, were significantly associated with a greater amount of smoking (p=0.028), prior history of respiratory infection (p=0.002), and worse emphysema severity on CT scanning (p=0.017), as well as with spirometric measures of airflow limitation, including reduced FEV1 (p<0.001), forced vital capacity (FVC) (p=0.012), FEV1 %Pred (p<0.001), FVC %Pred (p=0.003), and FEV1/FVC (p=0.001). SGRQ-T scores as a function of FEV1 %Pred are graphed in Figure 3.1. While SGRQ-T scores were abnormal for the majority of patients with FEV1 %Pred <80%, a striking number of patients also had abnormal SGRQ-T scores (>8.4[92]) despite relative preservation of airflow. In fact, the majority of patients with normal FEV1 %Pred (>80%) had abnormal SGRQ-T scores, indicating significant impairments in respiratory-related health status.
Figure 3.1 SGRQ-T scores are plotted against FEV1 %Pred for all study participants. Dashed lines are drawn for FEV1 %Pred of 80% and for the SGRQ-T score (8.4) below which is considered to be the normal range.[92] This figure demonstrates that even among patients who have preserved FEV1 %Pred (>80%), the majority have abnormal SGRQ-T scores (shaded). Regression line (not shown) $R^2=0.11$, $p<0.001$
SGRQ-T Scores and HIV-Specific Variables. Univariate analyses of associations between SGRQ-T scores and HIV-specific variables are shown in Table 3.2. Nearly all (99%) of the study cohort were receiving cART at the time of enrollment, and the majority (74%) had good current virologic control (HIV RNA <40 copies/mL). There were no significant differences in the classes of cART medications across the quartiles (p=0.214 for protease inhibitors, p=0.186 for non-nucleoside reverse transcriptase inhibitors, and p=0.217 for nucleoside reverse transcriptase inhibitors). However, the severity of HIV infection in the past, as assessed by higher plasma viral load (VL) (VL>100,000 copies/mL) and lower nadir CD4 T cell count were significantly associated with higher SGRQ-T score quartiles indicating worse respiratory-related health status (p=0.043 for baseline plasma VL >100,000 HIV RNA copies/mL and p=0.040 for nadir CD4). Current CD4 count ≤350 cell/μL was also associated with worse SGRQ-T score quartiles (p=0.005), although current VL was not. 

SGRQ-T Scores and Blood Biomarkers. Univariate analyses for associations between SGRQ-T scores and blood biomarkers are shown in Table 3.3. Higher IL-6 (p=0.002) and A1AT (p=0.005) levels were seen in the highest SGRQ-T score quartiles indicating worse respiratory-related health status. None of the other biomarkers, however, including CRP, SP-D, sCD14, and GzmB, were significantly different across the SGRQ-T score quartiles. Of note, for individuals with A1AT levels <1.3 g/L, A1AT genotyping was performed (11 with the MM genotype, 2 with the MZ genotype, 1 with the SZ genotype, and 2 with the MS genotype).

SGRQ Scores and Telomere Length. In table 3.3, telomere length did not show an association with SGRQ-T scores in univariate analysis. However, when we compared SGRQ Activity score with telomere length in a multiple regression model (figure 3.2), the two variables showed significant correlation (p = 0.037), while adjusting for Age, BMI, and smoking pack years. Other domains of SGRQ did not show the same relationship with telomere length.
Figure 3.2 SGRQ-Activity scores plotted against telomere length for all study participants after adjusting for age, BMI, and smoking pack years. The solid red line represents the linear regression while the dotted line represents the 95% confidence interval for the slope of regression line. The dotted blue line represents the mean of response for SGRQ Activity in the model (43.7). There is a significant linear relationship between SGRQ Activity score and telomere length (p=0.037, R²=0.077)
**Multiple Linear Regression Analysis.** In a multiple linear regression model (Table 3.4) that included age, BMI, smoking pack-years, FEV1 %Pred, prior respiratory infection, history of asthma, use of non-nucleoside reverse transcriptase inhibitors, baseline plasma VL >100,00 HIV RNA copies/mL, nadir CD4 count, current CD4 count ≤350 cells/μL, A1AT, IL-6, and CT emphysema score, only FEV1 %Pred (β-coefficient = -0.287, p=<0.001), current CD4 count ≤350 cells/μL (β-coefficient = 3.830, p=0.002), and IL-6 (β-coefficient = 1.362, p=0.018) were found to be significant predictors of SGRQ-T scores when SGRQ-T scores were treated as a continuous variable (Table 3.4).

**Table 3.4: Multiple Linear Regression for SGRQ Total Score**

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>R² = 0.1707</th>
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<tr>
<td></td>
<td>β (Standard Error)</td>
</tr>
<tr>
<td>FEV1 %Pred</td>
<td>-0.287 (0.066)</td>
</tr>
<tr>
<td>Current CD4 ≤350 cells/μL</td>
<td>3.830 (1.663)</td>
</tr>
<tr>
<td>IL-6 (pg/mL)</td>
<td>1.362 (0.057)</td>
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**DISCUSSION**

To our knowledge, this is the first study to identify specific risk factors associated with reduced respiratory health status in an HIV-infected population. Most significantly, our data reveal the considerable respiratory limitations encountered by HIV-infected patients despite relatively preserved airflow as measured by spirometry. In fact, in mildly impaired to normal FEV1 %Pred ranges, patients in our study cohort reported worse health status compared with COPD patient populations, which have average SGRQ-T scores of 18.2 as described in the literature.[93, 94] This is even more impressive considering that our patient cohort was approximately 20 years younger than the average COPD patient and was not recruited based on presence of lung disease. Whether other HIV-infected patients outside of our cohort demonstrate similar impairments in respiratory-related health status is difficult to ascertain, as only one other study has ever evaluated SGRQ scores in a similar population.[46] The scores collected in our study appear to be worse in comparison to this cohort (mean score 32.0 vs. 11.6), although the higher mean age (49.3 vs. 44.8 years) and greater smoking pack-year history (27.6 vs. 9.6 pack-years) of our patient population might account for this discrepancy. When other measures of respiratory dysfunction are considered, however, the results from our study are highly consistent with those of other HIV-infected cohorts. Drummond *et al.*, for instance, found worse Medical Research Council dyspnea scores in patients with HIV, particularly in those patients with both HIV and COPD.[95]

Unlike the general population, in which individuals within the range of normal FEV1 %Pred demonstrate normal SGRQ scores,[96] HIV-infected patients appear to shoulder respiratory limitations even in the absence of airflow obstruction as detected by spirometry. The disproportionate severity of SGRQ-T scores when compared to FEV1 measurements raises the possibility that respiratory health status is not simply determined by airflow obstruction in the HIV-infected population. Indeed, HIV-specific risk factors such as low CD4 T cell counts also appear
to influence patient-perceived respiratory limitations. One plausible mechanism by which this could occur is through the development of opportunistic pulmonary infections during times of the greatest immunosuppression. Indeed, having any history of a respiratory infection (PJP) was associated with worse SGRQ-T scores in our cohort.

Alternatively, the link between HIV-associated risk factors and respiratory status could be attributed to key inflammatory pathways related to HIV infection. In particular, the significant association we detected between IL-6 levels and SGRQ-T scores is consistent with previously known studies implicating the same pathway in both HIV and COPD. IL-6 is a well-characterized inflammatory cytokine,[97] elevations in which appear to strongly predict mortality in HIV-infected patients and COPD patients.[58, 98] Fluctuations in HIV viral loads and CD4 T cell counts also appear to be consistently mirrored by IL-6 levels, with worse HIV control associated with the highest IL-6 levels.[99] That circulating IL-6 may be associated with reduced physical function in HIV-infected patients has been suggested by Erlandson et al. who showed that high IL-6 levels were associated with frailty and low functional status.[75]

How IL-6 may specifically induce lung disease in HIV-infected patients has yet to be fully elucidated, but animal models suggest a possible role for this cytokine pathway in the development of COPD. Evidence supporting this mechanistic pathway comes from gp130 knockout murine models whose high IL-6 expression is associated with a predisposition to apoptosis and emphysema.[100] Not surprisingly, single nucleotide polymorphisms in the human *IL6* gene have been shown to modify the genetic risk of COPD.[101, 102] Our finding of a close association between respiratory-related health status and plasma IL-6 levels lends credence to the notion that inflammatory pathways may play a role in the development of HIV-associated emphysema, a notion that has long been proposed as an etiologic mechanism for COPD itself.[103]
The discordance between airflow limitation on spirometry and poor respiratory health status in our cohort suggest that the degree of respiratory-related disease may ultimately be underestimated in the HIV-infected population if only FEV1 measurements are considered. While reduced FEV1 %Pred measurements are certainly associated with poor respiratory health status, spirometry alone may not achieve the desired sensitivity for identifying those HIV patients with significant respiratory symptoms. FEV1, historically the measurement by which airflow obstruction is defined, is nonetheless a weak predictor of disease outcomes such as symptoms, exacerbations, and death in COPD,[104] possibly due to its static rather than dynamic reflection of airflow. Exercise-induced dynamic hyperinflation has thus been shown to be a better predictor of physical activity levels in COPD than FEV1 measurements.[105] Moreover, airflow as measured by spirometry likely better reflects the larger, more central airways and may subsequently fail to detect emphysematous changes in the small airways, the site of early disease. Impaired diffusion capacity, on the other hand, could potentially identify more peripheral disease[106] and in fact has been shown to be much more prevalent amongst HIV-infected patients compared to non-HIV-infected patients.[60] Alternatively, our study identified a significant association between health status and CT emphysema scoring, suggesting another possible diagnostic method. Future evaluation of respiratory disease in HIV patients could potentially employ these methods for improved diagnostic sensitivity.

The current literature relating SGRQ to markers of aging is scarce. Our study included telomere length measured from peripheral leukocytes as a marker for aging. While we did not see a correlation between telomere length and SGRQ-T score, the SGRQ Activity domain suggests an association after adjusting for age, BMI, and cigarette smoking (Figure 3.2). Although a direct link between SGRQ and telomere length has yet to be established, there is evidence that the two variables could be related. Firstly, immune cells which have reached replicative senescence have shown to have shorter telomere length and be pro-inflammatory. T cells which have reached
replicative senescence often lack the expression of CD28 surface marker; the increase in proportion of CD28- T cells have been associated with suboptimal reconstitution of CD4 cell count with cART as well as non-AIDS comorbidities. Moreover, senescent immune cells increase secretion of pro-inflammatory cytokines such as IL-1, TNF-alpha, and IL-6 which have been associated with comorbidities such as atherosclerosis, Alzheimer's disease, osteoporosis, and diabetes, all of which have significant impact on patients’ health quality. Also, as described in the previous chapter, telomere attrition with HIV infection has been linked to increased morbidity and mortality. Our current finding which shows an association between telomere length and the activities domain of the SGRQ, rather than total score, suggest that SGRQ is specific for respiratory health status and may not reflect extra-pulmonary comorbidities. Since SGRQ-T is our main outcome, we present SGRQ-Activity not as a major finding, but rather as a suggestion for future studies to address the potential link between telomere lengths and the ability for HIV-infected individuals to perform daily activities.

Our analysis of respiratory health status in HIV-infected individuals has several limitations. Firstly, the lack of women in our cohort may limit the generalizability of our results beyond male HIV patients. The one other published analysis of SGRQ-T scores in HIV also assessed HIV-infected men so additional studies incorporating HIV-infected women will be a welcome addition to the field. Also, while respiratory infections such as community acquired pneumonia were quite common amongst our patients, other pulmonary infections such as tuberculosis were rare (data not shown). As such, these data may not apply well to HIV populations where such infections are more common. Secondly, the SGRQ was primarily developed to assess respiratory health status in patients with obstructive airway disease. While validation has subsequently occurred in diseases such as bronchiectasis[107] and interstitial lung disease,[108, 109] the performance of the questionnaire in an HIV-infected population still needs to be fully characterized. In addition, it is possible that certain symptoms that are captured by the SGRQ such as dyspnea and exercise limitation could
reflect not just respiratory disease, but also diseases like cardiomyopathy or pulmonary hypertension that can be more prevalent among HIV-infected individuals. The majority of our study cohort, however, did not have a documented history of heart disease. Despite the uncertainty of the SGRQ, we feel that this tool still offers a useful general assessment of respiratory-related health status. Further refinement of respiratory tools to meet the specific needs of the HIV population will be required in future.
IV. CONCLUSION

With modern advancement in treatment and patient care, the HIV-positive population is living longer and better but are increasingly at risk for diseases of old age such as COPD. Although more attention and resources have recently been address to this field and literature for HIV-associated COPD is rapidly expanding, holes in our understanding of this disease still remain. Our study adds to this field and provides evidence that firstly, telomere length is shorter in our HIV-infected patient cohort and is associated with worse emphysema, and secondly, worse quality of life as measured by SGRQ is associated with lower CD4 count.

First of all, we demonstrate that HIV infection is associated with shortened telomeres. When compared to non-HIV controls, our HIV cohort has on average 27 kbp/genome shorter telomere length \((p < 0.0001; \text{mean±SEM of 150±3 and 123±4 kbp/genome, respectively})\). Furthermore, within HIV-infected individuals, COPD is associated with shorter telomeres. Specifically, Telomere length was significantly negatively related to emphysema total scores \((p=0.041)\), indicating that more severe emphysema was associated with shorter telomeres. Lastly, our multivariable model shows that lower FEV1 and higher sCD14 are independent risk factors for shorter telomere length \((p = 0.025 \text{ and } p = 0.001, \text{ respectively})\). While the exact mechanisms and implications of this observation need to be explored further, our data suggest plausible ties between sCD14, telomere length, and COPD. In essence, our first study supports an accelerated aging mechanism contributing to the development of COPD in HIV disease.

Our second find shows that even in the presence of effective cART, HIV-infected men report significantly worse respiratory-related health status compared with non-HIV-infected individuals who have similar degrees of airflow limitation. In part, their perceived respiratory limitations may be related to HIV-specific risk factors such as low CD4 T cell count which may drive particular inflammatory pathways associated with the development of emphysema. This is supported by our
data which shows current CD4 count ≤350 cells/μL (p = 0.002), and IL-6 (p = 0.018) were significant predictors of SGRQ total scores.

While we did not see a correlation between telomere length and SGRQ-T score, our data showed evidence for a relationship between SGRQ Activity domain and telomere length after adjusting for age, BMI, and cigarette smoking. This suggests that although leukocytes are shorter in HIV, accelerated cellular aging does not significantly affect global respiratory health status of HIV subjects. But rather, it is possible that accelerated aging negatively impacts the patients’ ability to perform daily activities due to breathlessness. Further studies are needed to elucidate the mechanism behind this observation.

This thesis demonstrated the potential role of advanced aging and discussed the determinants of poorer health related quality of life with HIV-associated COPD. However, further studies are required to evaluate potential therapies to normalize the rate of telomere attrition in this setting. Moreover, given the relative insensitivity of common spirometry measurements and need for robust biomarkers in detecting significant respiratory symptom burdens, greater awareness of respiratory disease on the part of clinicians and researchers will be needed to optimize the quality of life in this patient population.
REFERENCES


Table 2.1: Baseline Characteristics of Study Participants, For All Subjects and By Telomere Length Quartiles.

<table>
<thead>
<tr>
<th>Group</th>
<th>All</th>
<th>1st (&lt;107.9)</th>
<th>2nd (107.9-132.3)</th>
<th>3rd (132.4-157.5)</th>
<th>4th (≥157.6)</th>
<th>P-trend</th>
</tr>
</thead>
<tbody>
<tr>
<td>Telomere Length (kbp/genome)</td>
<td>132 ± 31</td>
<td>92 ± 12</td>
<td>120 ± 7</td>
<td>144 ± 7</td>
<td>170 ± 10</td>
<td></td>
</tr>
<tr>
<td>No. of Patients (all males, % of total)</td>
<td>187 (100%)</td>
<td>47 (25%)</td>
<td>46 (25%)</td>
<td>47 (25%)</td>
<td>47 (25%)</td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td>49 ± 10</td>
<td>52 ± 9</td>
<td>51 ± 9</td>
<td>49 ± 11</td>
<td>47 ± 10</td>
<td>0.016</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>26 ± 5</td>
<td>25 ± 4</td>
<td>26 ± 5</td>
<td>26 ± 4</td>
<td>27 ± 4</td>
<td>0.053</td>
</tr>
<tr>
<td>Ever Smoker</td>
<td>99 (53%)</td>
<td>22 (47%)</td>
<td>25 (54%)</td>
<td>29 (62%)</td>
<td>23 (49%)</td>
<td>0.672</td>
</tr>
<tr>
<td>Smoking, pack-years†</td>
<td>28 ± 19</td>
<td>29 ± 19</td>
<td>32 ± 22</td>
<td>25 ± 21</td>
<td>26 ± 15</td>
<td>0.321</td>
</tr>
<tr>
<td>Ever Marijuana Smoker</td>
<td>116 (62%)</td>
<td>34 (72%)</td>
<td>31 (67%)</td>
<td>26 (55%)</td>
<td>25 (53%)</td>
<td>0.028</td>
</tr>
<tr>
<td>Ever IVDU</td>
<td>123 (66%)</td>
<td>31 (66%)</td>
<td>27 (59%)</td>
<td>31 (66%)</td>
<td>34 (72%)</td>
<td>0.396</td>
</tr>
<tr>
<td>FEV1 (Liters)</td>
<td>3.2 ± 0.9</td>
<td>2.9 ± 0.9</td>
<td>3.2 ± 1.0</td>
<td>3.3 ± 0.9</td>
<td>3.5 ± 0.8</td>
<td>0.004</td>
</tr>
<tr>
<td>FEV1 %Pred</td>
<td>84 ± 22</td>
<td>78 ± 23</td>
<td>86 ± 26</td>
<td>85 ± 20</td>
<td>90 ± 17</td>
<td>0.036</td>
</tr>
<tr>
<td>FVC (Liters)</td>
<td>4.5 ± 0.9</td>
<td>4.3 ± 0.9</td>
<td>4.4 ± 1.1</td>
<td>4.5 ± 0.8</td>
<td>4.7 ± 1.0</td>
<td>0.045</td>
</tr>
<tr>
<td>FVC %Pred</td>
<td>91.3 ± 16.7</td>
<td>89.5 ± 17.4</td>
<td>92.1 ± 20.3</td>
<td>90.3 ± 15.1</td>
<td>95.0 ± 14.2</td>
<td>0.149</td>
</tr>
<tr>
<td>FEV1/FVC (%)</td>
<td>72 ± 13</td>
<td>67 ± 16</td>
<td>71 ± 13</td>
<td>73 ± 11</td>
<td>74 ± 10</td>
<td>0.027</td>
</tr>
<tr>
<td>Prior Respiratory Infection</td>
<td>87 (49%)</td>
<td>25 (56%)</td>
<td>23 (55%)</td>
<td>23 (53%)</td>
<td>16 (36%)</td>
<td>0.057</td>
</tr>
<tr>
<td>PJP Infection</td>
<td>23 (12%)</td>
<td>8 (17%)</td>
<td>8 (17%)</td>
<td>6 (13%)</td>
<td>1 (2%)</td>
<td>0.022</td>
</tr>
<tr>
<td>Co-morbidity</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hepatitis C</td>
<td>33 (18%)</td>
<td>7 (15%)</td>
<td>8 (17%)</td>
<td>9 (19%)</td>
<td>9 (19%)</td>
<td>0.559</td>
</tr>
<tr>
<td>Asthma</td>
<td>39 (22%)</td>
<td>9 (20%)</td>
<td>6 (14%)</td>
<td>12 (27%)</td>
<td>12 (26%)</td>
<td>0.265</td>
</tr>
</tbody>
</table>

Mean ± standard deviation (SD) values are given for normally distributed variables, while dichotomous data are given as counts (% of total).

Abbreviation definition: FEV1 = forced expiratory volume in 1 second; FEV1 %Pred = forced expiratory volume in 1 second percent predicted; FVC = forced vital capacity; FVC %Pred = forced vital capacity percent predicted; IVDU = intravenous drug use; PJP = Pneumocystis jirovecii pneumonia.

P-trend values were obtained through Jonckheere-Terpstra trend test for continuous variables and Cochran-Armitage test for dichotomous data.

† for current and ex-smokers.
Table 2.2: HIV-Related Clinical and Laboratory Data, For All Subjects and By Telomere Length Quartiles

<table>
<thead>
<tr>
<th>Group</th>
<th>All</th>
<th>1&lt;sup&gt;st&lt;/sup&gt; (&lt;107.9)</th>
<th>2&lt;sup&gt;nd&lt;/sup&gt; (107.9-132.3)</th>
<th>3&lt;sup&gt;rd&lt;/sup&gt; (132.4-157.5)</th>
<th>4&lt;sup&gt;th&lt;/sup&gt; (≥157.6)</th>
<th>P-trend</th>
</tr>
</thead>
<tbody>
<tr>
<td>Telomere Length (kbp/genome)</td>
<td>132 ± 31</td>
<td>92 ± 12</td>
<td>120 ± 7</td>
<td>144 ± 7</td>
<td>170 ± 10</td>
<td></td>
</tr>
<tr>
<td>No. of Patients (all males, % of total)</td>
<td>187 (100%)</td>
<td>47 (25%)</td>
<td>46 (25%)</td>
<td>47 (25%)</td>
<td>47 (25%)</td>
<td></td>
</tr>
<tr>
<td>Time from HIV diagnosis to enrollment (months)</td>
<td>145 ± 90</td>
<td>158 ± 88</td>
<td>178 ± 93</td>
<td>149 ± 90</td>
<td>104 ± 78</td>
<td>0.002</td>
</tr>
<tr>
<td>Current cART use</td>
<td>185 (99%)</td>
<td>47 (100%)</td>
<td>46 (100%)</td>
<td>47 (100%)</td>
<td>45 (96%)</td>
<td></td>
</tr>
<tr>
<td>PI</td>
<td>140 (75%)</td>
<td>37 (79%)</td>
<td>32 (74%)</td>
<td>34 (72%)</td>
<td>37 (79%)</td>
<td>0.954</td>
</tr>
<tr>
<td>NNRTI</td>
<td>31 (17%)</td>
<td>9 (19%)</td>
<td>6 (13%)</td>
<td>10 (21%)</td>
<td>6 (13%)</td>
<td>0.650</td>
</tr>
<tr>
<td>NRTI</td>
<td>167 (89%)</td>
<td>42 (89%)</td>
<td>42 (91%)</td>
<td>44 (94%)</td>
<td>39 (83%)</td>
<td>0.405</td>
</tr>
<tr>
<td>At time of first encounter</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plasma viral load &gt;100,000 HIV RNA copies/mL</td>
<td>116 (64%)</td>
<td>31 (68%)</td>
<td>26 (62%)</td>
<td>31 (68%)</td>
<td>28 (60%)</td>
<td>0.589</td>
</tr>
<tr>
<td>Nadir CD4 (cells/µL)</td>
<td>193 ± 164</td>
<td>180 ± 177</td>
<td>170 ± 151</td>
<td>204 ± 175</td>
<td>239 ± 159</td>
<td>0.027</td>
</tr>
<tr>
<td>At study enrollment</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plasma viral load &lt;40 HIV RNA copies/mL†</td>
<td>139 (74%)</td>
<td>36 (77%)</td>
<td>38 (83%)</td>
<td>36 (77%)</td>
<td>29 (62%)</td>
<td>0.076</td>
</tr>
<tr>
<td>CD4 (cells/µL)</td>
<td>531 ± 247</td>
<td>540 ± 278</td>
<td>495 ± 233</td>
<td>526 ± 195</td>
<td>567 ± 258</td>
<td>0.331</td>
</tr>
<tr>
<td>CD4 ≤350 cells/µL</td>
<td>44 (24%)</td>
<td>12 (26%)</td>
<td>11 (24%)</td>
<td>10 (21%)</td>
<td>11 (23%)</td>
<td>0.745</td>
</tr>
</tbody>
</table>

†Cut-off for successful viral suppression adopted from AIDS Clinical Trials Group [110]
Abbreviation definition: cART = combination antiretroviral therapy; PI = protease inhibitor; NNRTI = non-nucleoside reverse transcriptase inhibitor; NRTI = nucleoside reverse transcriptase inhibitor.
P-trend values were obtained through the Jonckheere-Terpstra trend test for continuous variables and the Cochran-Armitage test for dichotomous data.
## Table 2.3: Blood Biomarkers of Study Participants, For All Subjects and By Telomere Length Quartiles

<table>
<thead>
<tr>
<th>Group</th>
<th>All</th>
<th>1&lt;sub&gt;st&lt;/sub&gt; (&lt;107.9)</th>
<th>2&lt;sub&gt;nd&lt;/sub&gt; (107.9-132.3)</th>
<th>3&lt;sub&gt;rd&lt;/sub&gt; (132.4-157.5)</th>
<th>4&lt;sub&gt;th&lt;/sub&gt; (≥157.6)</th>
<th>P-trend</th>
</tr>
</thead>
<tbody>
<tr>
<td>Telomere Length (kbp/genome)</td>
<td>132 ± 30.5</td>
<td>91.7 ± 11.7</td>
<td>120 ± 6.50</td>
<td>144 ± 7.24</td>
<td>170 ± 10.4</td>
<td>0.822</td>
</tr>
<tr>
<td>No. of Patients (all males, % of total)</td>
<td>187 (100%)</td>
<td>47 (25%)</td>
<td>46 (25%)</td>
<td>47 (25%)</td>
<td>47 (25%)</td>
<td></td>
</tr>
<tr>
<td>CRP (mg/L)</td>
<td>2.6 ± 2.8</td>
<td>2.7 ± 2.8</td>
<td>2.0 ± 2.1</td>
<td>2.4 ± 2.8</td>
<td>3.0 ± 3.0</td>
<td>0.118</td>
</tr>
<tr>
<td>A1AT (g/L)</td>
<td>1.6 ± 0.3</td>
<td>1.6 ± 0.2</td>
<td>1.6 ± 0.2</td>
<td>1.5 ± 0.3</td>
<td>1.5 ± 0.3</td>
<td>0.549</td>
</tr>
<tr>
<td>IL-6 (pg/mL)</td>
<td>2.5 ± 2.5</td>
<td>2.7 ± 2.4</td>
<td>2.3 ± 2.4</td>
<td>2.2 ± 2.4</td>
<td>2.8 ± 2.7</td>
<td>0.148</td>
</tr>
<tr>
<td>SP-D (ng/mL)</td>
<td>59 ± 42</td>
<td>68 ± 58</td>
<td>63 ± 40</td>
<td>55 ± 39</td>
<td>53 ± 30</td>
<td>0.284</td>
</tr>
<tr>
<td>LPS (µg/mL)</td>
<td>0.4 ± 0.1</td>
<td>0.5 ± 0.1</td>
<td>0.4 ± 0.1</td>
<td>0.4 ± 0.1</td>
<td>0.4 ± 0.1</td>
<td>0.017</td>
</tr>
<tr>
<td>sCD14 (µl/mL)</td>
<td>1.6 ± 0.53</td>
<td>1.8 ± 0.6</td>
<td>1.6 ± 0.5</td>
<td>1.5 ± 0.5</td>
<td>1.5 ± 0.4</td>
<td>0.017</td>
</tr>
<tr>
<td>GzmB (pg/mL)</td>
<td>37 ± 53</td>
<td>30 ± 35</td>
<td>36 ± 43</td>
<td>45 ± 75</td>
<td>42 ± 54</td>
<td>0.105</td>
</tr>
</tbody>
</table>

Mean ± standard deviation (SD) values are given.

Abbreviation definition: CRP = C-reactive protein; IL-6 = interleukin-6; LPS = lipopolysaccharide; GzmB = granzyme B; SP-D = surfactant protein-D; sCD14 = soluble cluster of differentiation 14; A1AT = alpha-1-antitrypsin.

P-trend values were obtained through Jonckheere-Terpstra trend.
Table 3.1: Baseline Characteristics of Study Participants, For All Subjects and By SGRQ Total Score Quartiles.

<table>
<thead>
<tr>
<th>Group</th>
<th>All</th>
<th>1(^{st}) (&lt;13.8)</th>
<th>2(^{nd}) (13.8 – 31.9)</th>
<th>3(^{rd}) (32.0 – 46.8)</th>
<th>4(^{th}) (&gt;46.8)</th>
<th>P-trend</th>
</tr>
</thead>
<tbody>
<tr>
<td>SGRQ Total Score</td>
<td>32.0 ± 20.6</td>
<td>6.8 ± 3.8</td>
<td>21.9 ± 4.8</td>
<td>39.0 ± 4.0</td>
<td>59.8 ± 9.3</td>
<td></td>
</tr>
<tr>
<td>No. of Patients</td>
<td>199 (100%)</td>
<td>49 (25%)</td>
<td>50 (25%)</td>
<td>50 (25%)</td>
<td>50 (25%)</td>
<td>0.346</td>
</tr>
<tr>
<td>(all males, % of total)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td>49 ± 10</td>
<td>48 ± 12</td>
<td>50 ± 10</td>
<td>49 ± 10</td>
<td>51 ± 9</td>
<td>0.792</td>
</tr>
<tr>
<td>Body mass index (kg/m(^2))</td>
<td>26 ± 5</td>
<td>25 ± 4</td>
<td>27 ± 4</td>
<td>26 ± 5</td>
<td>26 ± 5</td>
<td>0.028</td>
</tr>
<tr>
<td>Current Smoker</td>
<td>104 (52%)</td>
<td>24 (51%)</td>
<td>24 (48%)</td>
<td>24 (48%)</td>
<td>31 (62%)</td>
<td>0.296</td>
</tr>
<tr>
<td>Smoking, pack-years(†)</td>
<td>28 ± 19</td>
<td>23 ± 19</td>
<td>26 ± 16</td>
<td>31 ± 19</td>
<td>30 ± 20</td>
<td></td>
</tr>
<tr>
<td>Ever Marijuana Smoker</td>
<td>121 (61%)</td>
<td>33 (67%)</td>
<td>26 (52%)</td>
<td>31 (62%)</td>
<td>31 (62%)</td>
<td>0.824</td>
</tr>
<tr>
<td>FEV1 (Liters)</td>
<td>3.2 ± 0.9</td>
<td>3.5 ± 0.7</td>
<td>3.3 ± 0.8</td>
<td>3.2 ± 0.8</td>
<td>2.8 ± 1.1</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>FEV1 %Pred</td>
<td>85 ± 22</td>
<td>93 ± 17</td>
<td>87 ± 16</td>
<td>84 ± 20</td>
<td>73 ± 27</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>FVC (Liters)</td>
<td>4.5 ± 0.9</td>
<td>4.7 ± 0.8</td>
<td>4.5 ± 0.9</td>
<td>4.4 ± 0.9</td>
<td>4.2 ± 1.0</td>
<td>0.012</td>
</tr>
<tr>
<td>FVC %Pred</td>
<td>91 ± 17</td>
<td>97 ± 15</td>
<td>92 ± 16</td>
<td>89 ± 15</td>
<td>87 ± 19</td>
<td>0.003</td>
</tr>
<tr>
<td>FEV1/FVC (%)</td>
<td>72 ± 13</td>
<td>75 ± 9</td>
<td>75 ± 11</td>
<td>73 ± 11</td>
<td>64 ± 16</td>
<td>0.001</td>
</tr>
<tr>
<td>CT Emphysema Score (total)(§)</td>
<td>2.0 ± 1.2</td>
<td>1.8 ± 1.1</td>
<td>1.7 ± 1.0</td>
<td>2.0 ± 1.3</td>
<td>2.5 ± 1.3</td>
<td>0.017</td>
</tr>
<tr>
<td>Prior Respiratory Infection</td>
<td>94 (50%)</td>
<td>14 (30%)</td>
<td>21 (47%)</td>
<td>32 (64%)</td>
<td>27 (57%)</td>
<td>0.002</td>
</tr>
<tr>
<td>PJP Infection</td>
<td>24 (12%)</td>
<td>3 (6%)</td>
<td>7 (14%)</td>
<td>9 (18%)</td>
<td>5 (10%)</td>
<td>0.456</td>
</tr>
<tr>
<td>Co-morbidities</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hepatitis C</td>
<td>36 (18%)</td>
<td>8 (16%)</td>
<td>5 (10%)</td>
<td>11 (22%)</td>
<td>12 (24%)</td>
<td>0.150</td>
</tr>
<tr>
<td>Asthma</td>
<td>42 (22%)</td>
<td>7 (15%)</td>
<td>9 (20%)</td>
<td>12 (24%)</td>
<td>14 (30%)</td>
<td>0.073</td>
</tr>
</tbody>
</table>

Mean ± standard deviation (SD) values are given for normally distributed variables, while dichotomous data are given as counts (% of total).

Abbreviation definition: COPD = chronic obstructive pulmonary disease; PJP = Pneumocystis jiroveci pneumonia.

\(†\)for current and ex-smokers

\(§\)CT scans were available for review for 107 of 199 total patients. Scores for each of the 6 lobes of the lung were defined as follows: 0=absence of emphysema, 1=1-25% emphysema, 2=26-50% emphysema, 3=51-75% emphysema, and 4=76-100% emphysema. The total score is presented here as the sum of the individual lobe scores.
Table 3.2: HIV-Related Clinical and Laboratory Data, For All Subjects and By SGRQ Total Score Quartiles

<table>
<thead>
<tr>
<th>Group</th>
<th>All</th>
<th>1st (&lt;13.8)</th>
<th>2nd (13.8 – 31.9)</th>
<th>3rd (32.0 – 46.8)</th>
<th>4th (&gt;46.8)</th>
<th>P-trend</th>
</tr>
</thead>
<tbody>
<tr>
<td>SGRQ Total Score</td>
<td>32.0 ± 20.6</td>
<td>6.8 ± 3.8</td>
<td>21.9 ± 4.8</td>
<td>39.0 ± 4.0</td>
<td>59.8 ± 9.3</td>
<td></td>
</tr>
<tr>
<td>No. of Patients (all males, % of total)</td>
<td>199 (100%)</td>
<td>49 (25%)</td>
<td>50 (25%)</td>
<td>50 (25%)</td>
<td>50 (25%)</td>
<td></td>
</tr>
<tr>
<td>Duration of HIV Infection (months)</td>
<td>145 ± 90</td>
<td>141 ± 96</td>
<td>145 ± 82</td>
<td>143 ± 92</td>
<td>150 ± 89</td>
<td>0.717</td>
</tr>
<tr>
<td>Current cART use</td>
<td>197 (99%)</td>
<td>49 (100%)</td>
<td>49 (98%)</td>
<td>49 (98%)</td>
<td>50 (100%)</td>
<td></td>
</tr>
<tr>
<td>PI</td>
<td>151 (76%)</td>
<td>37 (76%)</td>
<td>32 (64%)</td>
<td>42 (84%)</td>
<td>40 (80%)</td>
<td>0.214</td>
</tr>
<tr>
<td>NNRTI</td>
<td>33 (17%)</td>
<td>10 (20%)</td>
<td>8 (16%)</td>
<td>11 (22%)</td>
<td>4 (8%)</td>
<td>0.186</td>
</tr>
<tr>
<td>NRTI</td>
<td>179 (90%)</td>
<td>45 (92%)</td>
<td>48 (96%)</td>
<td>42 (84%)</td>
<td>44 (88%)</td>
<td>0.217</td>
</tr>
<tr>
<td>At time of first encounter</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plasma viral load &gt;100,000 HIV RNA copies/mL</td>
<td>127 (66%)</td>
<td>28 (57%)</td>
<td>29 (63%)</td>
<td>33 (69%)</td>
<td>38 (76%)</td>
<td>0.043</td>
</tr>
<tr>
<td>Nadir CD4 (cells/µL)</td>
<td>193 ± 164</td>
<td>224 ± 150</td>
<td>178 ± 150</td>
<td>199 ± 193</td>
<td>168 ± 156</td>
<td>0.040</td>
</tr>
<tr>
<td>At study enrollment</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plasma viral load &gt;40 HIV RNA copies/mL</td>
<td>52 (26%)</td>
<td>13 (7%)</td>
<td>12 (6%)</td>
<td>11 (6%)</td>
<td>16 (8%)</td>
<td>0.602</td>
</tr>
<tr>
<td>CD4 ≤350 cells/µL</td>
<td>49 (25%)</td>
<td>8 (16%)</td>
<td>6 (12%)</td>
<td>18 (36%)</td>
<td>17 (34%)</td>
<td>0.005</td>
</tr>
</tbody>
</table>

Mean ± standard deviation (SD) values are given for normally distributed variables.
Abbreviation definition: cART = combination antiretroviral therapy; PI = protease inhibitor; NNRTI = non-nucleoside reverse transcriptase inhibitor; NRTI = nucleoside reverse transcriptase inhibitor.
Table 3.3: Blood Biomarkers of Study Participants, For All Subjects and By SGRQ Total Score Quartiles

<table>
<thead>
<tr>
<th>Group</th>
<th>All</th>
<th>1st (&lt;13.8)</th>
<th>2nd (13.8 – 31.9)</th>
<th>3rd (32.0 – 46.8)</th>
<th>4th (&gt;46.8)</th>
<th>P-trend</th>
</tr>
</thead>
<tbody>
<tr>
<td>SGRQ Total Score</td>
<td>32.0 ± 20.6</td>
<td>6.8 ± 3.8</td>
<td>21.9 ± 4.8</td>
<td>39.0 ± 4.0</td>
<td>59.8 ± 9.3</td>
<td></td>
</tr>
<tr>
<td>CRP (mg/L)</td>
<td>1.36 (0.78 - 3.72)</td>
<td>1.10 (0.78 - 2.28)</td>
<td>1.54 (0.81 - 3.42)</td>
<td>1.22 (0.66 - 3.51)</td>
<td>1.74 (0.75 - 5.16)</td>
<td>0.210</td>
</tr>
<tr>
<td>A1AT (g/L)</td>
<td>1.55 (1.40 - 1.72)</td>
<td>1.47 (1.37 - 1.67)</td>
<td>1.53 (1.41 - 1.68)</td>
<td>1.56 (1.36 - 1.76)</td>
<td>1.63 (1.52 - 1.82)</td>
<td>0.005</td>
</tr>
<tr>
<td>IL-6 (pg/mL)</td>
<td>1.46 (0.92 - 2.95)</td>
<td>1.24 (0.78 - 1.90)</td>
<td>1.48 (0.94 - 2.74)</td>
<td>1.35 (0.85 - 3.16)</td>
<td>2.72 (1.13 - 4.61)</td>
<td>0.002</td>
</tr>
<tr>
<td>SP-D (ng/mL)</td>
<td>47.9 (33.9 - 75.2)</td>
<td>48.6 (32.7 - 78.0)</td>
<td>45.0 (31.8 - 59.7)</td>
<td>49.9 (28.5 - 86.3)</td>
<td>50.2 (37.2 - 64.7)</td>
<td>0.307</td>
</tr>
<tr>
<td>sCD14 (μL/mL)</td>
<td>1.54 (1.24 - 1.90)</td>
<td>1.56 (1.20 - 1.95)</td>
<td>1.45 (1.21 - 1.76)</td>
<td>1.54 (1.29 - 1.95)</td>
<td>1.54 (1.15 - 1.90)</td>
<td>0.769</td>
</tr>
<tr>
<td>GzmB (pg/mL)</td>
<td>21.4 (11.8 - 40.1)</td>
<td>17.8 (9.70 - 44.7)</td>
<td>19.6 (10.4 - 38.3)</td>
<td>21.0 (10.8 - 44.6)</td>
<td>25.9 (13.4 - 37.5)</td>
<td>0.314</td>
</tr>
<tr>
<td>Telomere Length</td>
<td>131 (126 – 135)</td>
<td>130 (121 – 139)</td>
<td>138 (130 – 147)</td>
<td>127 (118 – 136)</td>
<td>126 (117 – 135)</td>
<td>0.272</td>
</tr>
</tbody>
</table>

Median with 25th – 75th percentile values are given for all blood biomarker data. 
Abbreviation definition: CRP = C-reactive protein; IL-6 = interleukin-6; GzmB = granzyme B; SP-D = surfactant protein-D; sCD14 = soluble cluster of differentiation 14; A1AT = alpha-1-antitrypsin.