Background: Chest imaging is performed for a variety of reasons in HIV-infected adults. There are limited data on the prevalence of incidental findings, progression of these findings over time and the relationship with inflammation in antiretroviral therapy (ART)-treated HIV-infected adults.

Methods: This study utilized data from a randomized clinical trial of rosuvastatin in HIV-infected adults on ART. Incidental findings were reported from chest computed tomography (CT) scans obtained for coronary artery calcium score at entry, week 48 and 96. Markers of immune activation and inflammation were measured concurrently. Poisson regression and generalized estimating equations were used.

Results: A total of 147 participants were enrolled. Median age was 46 years, 78% were male, 68% African American and 63% current smokers. At baseline, 57% of participants had at least one incidental lung finding (ILF) and four additional participants had at least one ILF by week 96. At baseline, older age, current smoking, lower nadir CD4+ T-cell count and low-density lipoprotein and higher lipoprotein-associated phospholipase A2 (Lp-PLA2) were independently associated with having a greater number of ILFs. In the longitudinal analyses, older age, lower nadir CD4+ T-cell count and higher baseline soluble tumour necrosis factor α-receptor I (sTNF-RI) were independently associated with having a greater number of ILFs over 96 weeks.

Conclusions: Over half of participants had at least one incidental finding on chest CT. Beyond traditional factors of older age and smoking, lower nadir CD4+ T-cell count and higher markers of inflammation were associated with having a greater number of ILFs in HIV-infected adults on ART.

Introduction

Pulmonary disease is common in HIV-infected individuals. Prior to widespread availability and use of antiretroviral therapy (ART), pulmonary infections, such as Pneumocystis jirovecii pneumonia, tuberculosis and community-acquired pneumonia, predominated the burden of pulmonary disease [1,2]. With ART, mortality associated with these infections and all AIDS-related causes of death have decreased dramatically [3,4]. In turn, the spectrum of pulmonary diseases has shifted, such that now chronic pulmonary diseases such as chronic obstructive pulmonary disease (COPD) and pulmonary hypertension have emerged [5]. Additionally, with HIV infection, lung cancer risk is higher than in the general population; a risk that appears to be beyond that explained by the high prevalence of smoking alone [6,7]. The causes of pulmonary diseases in HIV-infected individuals are varied and include traditional risk factors, namely smoking, immunosuppression and perhaps heightened systemic and pulmonary inflammation and HIV-related chronic immune activation [8,9].

Increased usage of advanced imaging techniques such as computed tomography (CT) has led to the discovery of an increasing number of incidental findings, that is, findings not related to the indication for the study [10]. Although directly comparative studies are lacking, one study showed HIV-infected individuals to have a high number of incidental findings, mostly involving the lung, on screening cardiac CT [11]. While the importance to the patient of incidental findings is unclear, in this study, 33% of the incidental findings were deemed
clinically significant by the need for further work-up including imaging or medical referral [11].

We have previously shown that rosuvastatin decreases monocyte and T-cell activation, and vascular inflammation [12,13]. The aims of this current analysis are to describe the incidental findings noted on cardiac CT completed as part of a randomized clinical trial of rosuvastatin in HIV-infected adults on ART, to examine the relationships between markers of inflammation and immune activation, and the effect of rosuvastatin on the number of incidental lung findings identified over time. In doing this, our overarching goals were twofold. Our first goal is to increase awareness of the numbers and types of incidental findings identified in HIV-infected adults on ART as imaging studies such as chest CT scans are being utilized more and more. Our second goal is to add to the literature regarding possible mechanisms underlying the heightened risk of lung disease in the ageing HIV-infected population.

Methods

Study design

The Stopping Atherosclerosis and Treating Unhealthy Bone With Rosuvastatin in HIV (SATURN-HIV) trial is a 96-week, randomized, double-blind, placebo-controlled trial designed to measure the effect of rosuvastatin on markers of cardiovascular risk, skeletal health and immune activation in adults with HIV-infection. Data related to changes in these outcomes were previously reported [12–14]. Full eligibility criteria for SATURN-HIV may be found on ClinicalTrials.gov (NCT01218802). In brief, all participants were ≥18 years old with HIV-1 infection on stable ART for at least 3 months with HIV-1 RNA <1,000 copies/ml and fasting low-density lipoprotein (LDL) ≤130 mg/dl. Additional entry criteria included proportion of CD8+ T-cells that express CD38 and HLA-DR. Monocyte phenotype was determined by flow cytometry as previously described [16]. CD4+ and CD8+ T-cells that express CD38 and HLA-DR. Monocyte phenotype was determined by flow cytometry as previously described [16]. CD4+ and CD8+ T-cells that express CD38 and HLA-DR. Monocyte phenotype was determined by flow cytometry as previously described [16]. CD4+ and CD8+ T-cells that express CD38 and HLA-DR. Monocyte phenotype was determined by flow cytometry as previously described [16]. CD4+ and CD8+ T-cells that express CD38 and HLA-DR. Monocyte phenotype was determined by flow cytometry as previously described [16]. CD4+ and CD8+ T-cells that express CD38 and HLA-DR. Monocyte phenotype was determined by flow cytometry as previously described [16].

Markers of immune activation and inflammation

Blood was drawn after a 12-h fast at 0, 24, 48 and 96 weeks for measurement of soluble and cellular markers of immune activation and soluble markers of systemic and vascular inflammation and coagulation. Soluble CD14 (sCD14), soluble CD163 (sCD163), interleukin-6 (IL-6), soluble vascular cell adhesion molecule-1 (sVCAM-1), soluble intercellular adhesion molecule 1 (sICAM-1), interferon γ-inducible protein (IP-10), soluble tumour necrosis factor α receptors I and II (sTNF-RI and RII) and lipoprotein-associated phospholipase A2 (Lp-PLA2) levels were measured by ELISA (R&D Systems, Minneapolis, MN, USA for all except diaDexus, South San Francisco, CA, USA for Lp-PLA2). High sensitivity C-reactive protein (hsCRP), fibrinogen and cystatin C levels were measured by particle-enhanced immunonephelometric assay on BNII nephelometer (Siemens, Munich, Germany). D-dimer levels were measured by immuno-turbidometric assay on a STA-R coagulation analyzer (DiagnosticaStago, Parsippany-Trou Hills, NJ, USA).

Monocyte and T-cells were phenotyped by flow cytometry as previously described [16]. CD4+ and CD8+ T-cell activation was defined as co-expression of CD38 and HLA-DR. Monocyte phenotype was determined by the relative expression of CD14, CD16 and surface tissue factor (TF).

Statistical methods

Baseline demographics and HIV-related factors are described overall by median and IQR for continuous variables and frequency and percent for categorical variables. Frequency analysis was performed for...
each variable in the study to check data distribution and quality of the data. Necessary transformation and recoding were performed on some of the variables. For example, several inflammation markers were rescaled by dividing the variable’s standard deviation to allow for better interpretation of the variable’s coefficient in the regression analysis. T-tests or Wilcoxon rank sum tests for continuous variables and χ² tests or Fisher’s Exact tests for categorical variables were used to compare baseline variables between groups as appropriate. All incidental findings reported on baseline CTs are described overall by frequency and percent.

Incidental findings involving the lung parenchyma and airways were aggregated and a variable capturing the number of incidental lung findings (ILFs; 0, 1, 2, 3, 4 or ≥5) at each time point (0, 48 and 96 weeks) was created. This variable was used as the dependent or outcome variable for the regression analyses presented. Generalized linear models with Poisson distribution and log link function were used for assessing the association between variables of interest and the number of ILFs at baseline. Stepwise selection was used to build a multivariable model. All variables with P-value <0.1 in univariable analysis were considered for inclusion in the multivariable model. For measuring associations between variables of interest and the number of ILFs over 96 weeks, generalized estimating equations with Poisson distribution and log link function were utilized. Unstructured correlations among the repeated measurements with robust standard errors of the estimates were used in statistical inference. A multivariable model was built by including all variables with P-value <0.1 in single predictor models, that is, models with a single variable and time only. Additional models were created to assess the effect of rosuvastatin on the number of ILFs over time. The first model included randomization group, time and a randomization group by time interaction term. The second model included the same plus clinically relevant confounders, that is, age, sex, race and smoking status. Participants were analyzed in the group to which they were randomized. All statistical tests were two-sided and considered significant if P<0.05. Analyses were performed using Stata 13 software (StataCorp. 2013; Stata Statistical Software: Release 13. College Station, TX, USA: StataCorp LP).

Results

In total, 147 adults met entry criteria and were randomized (72 to rosuvastatin, 75 to placebo). By 96 weeks, 28 participants (9 on rosuvastatin, 19 on placebo) were lost to follow-up or withdrew from the study for reasons unrelated to the study medication as previously described [17]. Additionally, none of the 28 participants withdrew or were lost to follow-up due to lung cancer.

Baseline characteristics

Demographics and HIV-related characteristics were balanced between groups at baseline. Overall, 78% were men, 68% were African American and the median age was 46 (IQR 40–53) years. Over half (63%) were current smokers (median 0.5 [0.25–1] packs per day) and an additional 16% were past smokers. The median current and nadir CD4⁺ T-cell counts were 613 (425–853) and 182 (89–300) cells/mm³, respectively. All participants were on ART by design with a median cumulative duration of ART of 5.5 (3.2–9.9) years and known duration of HIV infection of 11.6 (6.2–17.8) years. 78% of participants had HIV-1 RNA <50 copies/ml (range <20–600 copies/ml). Information on prior opportunistic infections was available for 120 participants. Of these, 12 had a history of Pneumocystis jirovecii pneumonia (PJP), 4 had cryptococcal meningitis, 4 had cytomegalovirus disease (retinitis and/or colitis), 2 had disseminated mycobacterium avium complex and 1 had latent tuberculosis infection (LTBI).

Incidental findings on chest computed tomography at baseline

Table 1 describes the 169 incidental findings reported on the 147 chest CTs performed at baseline. Of these, ILFs, that is, findings involving the lung parenchyma or airways, comprised 73% (124/169). Further, over half of participants (57% or 84/147) had at least one ILF at baseline. Findings occurring most frequently included: parenchymal scar or atelectasis and pulmonary nodules. Of those participants that had a prior history of PJP or LTBI (n=13), 11 had an ILF on the baseline chest CT.

Factors associated with having a higher number of ILFs at baseline in univariable analyses included: older age (P<0.01), current smoking (P=0.03), lower nadir CD4⁺ T-cell count (P=0.01), and higher cystatin C (P<0.05) and Lp-PLA2 (P<0.01). Associations with lower creatinine clearance and LDL, and higher peripheral white blood cell count, sTNF-RI, Framingham risk score and CAC score trended towards significance (P<0.1 for all). Selecting from these variables, factors independently associated with having a greater number of ILFs at baseline included: older age (P<0.01), current smoking (P=0.02), lower nadir CD4⁺ T-cell count (P=0.01) and LDL (P=0.03) and higher Lp-PLA2 (P<0.01).

Incidental lung findings over 96 weeks

Compared to baseline, 7 and 4 additional participants had at least one ILF found on chest CT at weeks 48 and 96, respectively. With regard to the pulmonary nodules incidentally found on baseline CT, most improved over the course of the study (Figure 1). Between the baseline and week 96 CT scans, two participants’ pulmonary nodules worsened. The first participant had a solitary nodule on the baseline CT that was unchanged on the
week 96 CT; however, this participant developed a new nodule not apparent on the initial CT. The second participant had multiple pulmonary nodules on the baseline CT, two of which were decreased in size on the week 48 scan, but one of the baseline nodules increased in size on the week 96 CT. Additionally, there was one participant that developed presumed lung cancer while on study. On the baseline CT, this participant had several non-specific pulmonary nodules measuring up to 5 mm. At week 48, there was a change in the appearance of the lung parenchyma with more discrete, spiculated nodules. A CT chest was performed a week later that showed a large solid mass that was concerning for malignancy. The patient was diagnosed and treated at an outside institution, and while he did complete the week 96 visit, he did not have a week 96 cardiac CT performed.

In the longitudinal analyses (Table 2), older age, being a current smoker at baseline, lower creatinine clearance and nadir CD4+ T-cell count, and higher HIV-1 RNA level, cystatin C, sTNF-RI, Lp-PLA2 and CAC score were each associated with a higher number of ILFs over 96 weeks in individual models adjusting for time only ($P<0.05$ for all). Lower body mass index and current CD4+ T-cell count trended towards significance ($P<0.1$ for all). In multivariable modelling, older age, being a current smoker at baseline, lower nadir CD4+ T-cell count and higher baseline sTNF-RI remained independently associated with the outcome.

**Effect of rosuvastatin**
Randomization group was not associated with the number of ILFs over 96 weeks in a model adjusting for time ($P=0.45$ for randomization group and $P=0.71$ for randomization group by time interaction) or with adjustment for time and clinically relevant variables including age, sex, race and smoking status ($P=0.39$ for randomization group and 0.95 for randomization group by time interaction).

**Discussion**
In this clinically relevant sample of HIV-infected adults on ART, incidental findings on chest CT were highly
prevalent and this prevalence increased over time. Further, our study shows that higher markers of systemic (sTNF-RI) and vascular (Lp-PLA2) inflammation in addition to and independent of HIV-related factors (lower nadir CD4\(^+\) T-cell count) and traditional factors associated with lung disease (older age and smoking) are associated with a greater number of ILFs at baseline and longitudinally. These novel findings add to the literature on the potential role of inflammation in pulmonary disease among HIV-infected individuals.

In our study, 57% of participants had at least one ILF at baseline. In a systematic review of studies from the general population, of chest CTs performed for coronary artery disease or lung cancer screening, 34% (range 8–58.1%) and 65.2% (43.8–73.3%) had at least one incidental finding, respectively [18]. As these studies did not separate pulmonary and extrapulmonary findings, it appears that ILFs may be more common in our study population despite the median age of participants in our study being only 46 years compared with a pooled mean age of 56.7 years in the systematic review. The association of greater number of ILFs at baseline with current smoking, which is a highly prevalent risk factor for lung disease among HIV-infected individuals [7], and also lower nadir CD4\(^+\) T-cell count suggesting a role for immunodeficiency-related infectious complications, are two possible reasons for the high prevalence in our study compared with the general population. Among HIV-infected populations there are limited data. However, in a study of 215 HIV-infected men undergoing CT to screen for CAC, 43% had incidental findings noted [11]. Again, it appears that the prevalence of ILFs in our study is higher and differences in participant characteristics likely explains some of the difference. Importantly, in our study, more participants were current smokers (63% versus 17%) and a greater proportion were African-American (68% versus 22%).

Further, eligibility criteria for our study necessitated heightened T-cell activation or systemic inflammation. In actuality, half of study participants had hsCRP levels >2 mg/l and the other half had high CD8\(^+\) activation markers. Nonetheless, if the hypothesis that heightened inflammation increases pulmonary disease in HIV is correct, our study sample would be more likely to have incidental findings.

To our knowledge, this is the first study to evaluate the association of inflammation and ILFs longitudinally. In the cross-sectional study by Crum-Cianflone et al. [11], HIV-infected men with incidental findings did have higher erythrocyte sedimentation rates, a general measure of inflammation, but, this factor was not associated with the presence of incidental findings in multivariable modelling [11]. There are several studies, however, that do support the role of inflammation in the pathogenesis of lung disease in HIV. In vitro, HIV virus can enter bronchial epithelial cells and alter function by impairing cell adhesion and increasing inflammatory mediator expression locally [19]. Further, in SIV-infected rhesus macaques, increased monocyte turnover correlates with severity of damage to lung tissue and higher IL-6 expression in tissue exposed to lipopolysaccharide (LPS), suggesting that monocyte activation contributes to chronic pulmonary inflammation with HIV infection [20]. Additionally, Twigg et al. [21] have recently shown that individuals with advanced HIV infection may have alterations in the lung microbiome including increased abundance of organisms associated with lung inflammation; changes that do not completely reverse with 3 years of ART. In our study, number of ILFs were not associated with soluble or cellular measures of monocyte or lymphocyte activation. However, higher Lp-PLA2, a marker of vascular inflammation,
was independently associated with greater number of ILFs at baseline and higher baseline sTNF-RI, a soluble marker of tumour necrosis factor α activity and systemic inflammation, was associated with a greater number of ILFs over time.

Importantly, the HIV-related factor, lower nadir CD4+ T-cell count, was associated with a greater number of ILFs in this study. This was not shown in the study by Crum-Cianflone et al. [11] where participants with incidental findings did have lower current CD4+ T-cell counts, but this factor was not associated with incidental findings in multivariable analysis [11]. However, data from the Examinations of HIV Associated Lung Emphysema (EXHALE) cohort, show that HIV-infected individuals with CD4+ T-cell counts <200 cells/mm³ were more likely to have incidental findings than those with CD4+ T-cell counts ≥200 cells/mm³ [22]. This and results from our study have important implications for screening imaging studies in HIV-infected patients with a history of advanced immunosuppression as incidental findings are more likely to be encountered.

In addition to an effect on systemic inflammation, HMG CoA reductase inhibitors or statins may affect pulmonary inflammatory cells, endothelial, epithelial and smooth muscle cells [23–26] – any modulatory effects of these could be beneficial in the lung. In the HIV-uninfected COPD population, trials of statins have produced conflicting results depending on the population studied, the primary end point and the statin used [27–29]. A recent trial found no effect of statins on acute pulmonary exacerbation rate, but there was a trend towards slower decline in FEV1 [27]. As such, the effect of statins in HIV-uninfected patients is unclear. Further, in HIV-infected patients the effect is really unknown. Although we have shown that rosuvastatin reduces markers of systemic and vascular inflammation including sTNF-RII, IP-10, cystatin C and Lp-PLA2, immune activation including sCD14, proportion of tissue factor expressing patrolling monocytes and proportion of activated CD4+ and CD8+ T-cells and oxidized low density lipoprotein [12,13,16,30,31] in HIV-infected adults on ART, being on rosuvastatin does not appear to be associated with the number of ILFs over time. The lack of effect of rosuvastatin may be because the damage done to the lungs related to inflammation may require a longer period of time to improve or be irreversible.

Our study has several strengths, including a randomized, placebo-controlled trial design with a large number of HIV-infected individuals having undergone extensive evaluation of markers of inflammation, immune activation and cardiovascular disease risk over 96 weeks. However, findings may not be generalizable to all HIV-infected individuals as we have only included those on ART and with heightened inflammation. Further, we do not know the significance of the incidental findings noted beyond 96 weeks. The outcome of the study, ILFs, is a composite of several findings; some of which have unknown clinical significance, for example, scarring. All these findings were included to improve statistical power. Larger studies are needed to evaluate predictors of individual incidental lung findings. In addition, the CTs were cardiac CTs; thus, ILFs in the upper lobes of the lungs may have been missed.

In conclusion, higher markers of systemic and vascular inflammation in addition to and independent of HIV-related factors and traditional factors associated with lung disease are associated with a greater number of ILFs at baseline and longitudinally in adults with treated HIV infection. Additional longitudinal studies are warranted to evaluate the effect of inflammation and HIV-related immune activation on the incidence of pulmonary disease in HIV-infected individuals as well as the long-term clinical significance of incidental findings on screening chest imaging over time in this patient group.

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Disclosure statement

COH has served on a medical advisory board for Gilead Sciences. GAM has received research grants from BMS, Gilead Sciences, AstraZeneca, Merck and GSK and has served as a consultant to BMS, Viiv/GSK, ICON and Gilead. MSP, RG and AS have no competing interests.

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