Computed tomography measure of lung injury and future interstitial features: The CARDIA Lung Study

Gabrielle Y. Liu, Laura A. Colangelo, Samuel Y. Ash, Raul San Jose Estepar, David R. Jacobs, , Bharat Thyagarajan, J Michael Wells, Rachel K. Putman, Bina Choi, Christopher S. Stevenson, Mercedes Carnethon, George R. Washko, Ravi Kalhan


This manuscript has recently been accepted for publication in the ERJ Open Research. It is published here in its accepted form prior to copyediting and typesetting by our production team. After these production processes are complete and the authors have approved the resulting proofs, the article will move to the latest issue of the ERJOR online.

Copyright ©The authors 2023. This version is distributed under the terms of the Creative Commons Attribution Non-Commercial Licence 4.0. For commercial reproduction rights and permissions contact permissions@ersnet.org
Title: “Computed tomography measure of lung injury and future interstitial features: The CARDIA Lung Study”

AUTHORS: Gabrielle Y. Liu¹, Laura A. Colangelo², Samuel Y. Ash³,⁴, Raul San Jose Estepar³,⁵, David R. Jacobs, Jr⁶, Bharat Thyagarajan⁷, J Michael Wells⁸, Rachel K. Putman⁶, Bina Choi³,⁴, Christopher S. Stevenson⁹, Mercedes Carnethon², George R. Washko³,⁵, Ravi Kalhan¹,²

AFFILIATIONS:
¹Division of Pulmonary and Critical Care Medicine, Northwestern University Feinberg School of Medicine, Chicago, IL; ²Department of Preventive Medicine, Northwestern University Feinberg School of Medicine, Chicago, IL; ³Applied Chest Imaging Laboratory, Brigham and Women’s Hospital, Boston, MA; ⁴Division of Pulmonary and Critical Care Medicine, Brigham and Women’s Hospital, Boston, MA; ⁵Department of Radiology, Brigham and Women’s Hospital, Boston, MA; ⁶Division of Epidemiology and Community Health, University of Minnesota, Minneapolis, MN; ⁷Department of Laboratory Medicine and Pathology, University of Minnesota, Minneapolis, MN; ⁸Division of Pulmonary, Allergy, and Critical Care Medicine, University of Alabama at Birmingham, Birmingham, AL; ⁹Lung Cancer Initiative at Johnson & Johnson, London, UK

Corresponding Author:
Gabrielle Y. Liu, MD, 240 E. Huron St., McGaw 2-410, Chicago, Illinois 60611
gabrielle.liu@northwestern.edu

Take-home message: “CT lung injury” is an objective computed tomography feature that is associated with future interstitial features and restrictive spirometry
ABSTRACT:

**Introduction:** Visually normal areas of the lung with high attenuation on computed tomography (CT) imaging, termed CT lung injury, may represent injured but not yet remodeled lung parenchyma. This prospective cohort study examined if CT lung injury is associated with future interstitial features on CT and restrictive spirometry abnormality among participants from the Coronary Artery Risk Development in Young Adults (CARDIA) study.

**Methods:** CARDIA is a population-based cohort study. CT scans obtained at two time points were assessed objectively for amount of lung tissue characterized as CT lung injury and interstitial features. Restrictive spirometry was defined as having a forced vital capacity (FVC) less than 80% predicted with forced expiratory volume in one second/FVC ratio greater than 70%.

**Results:** Among 2,213 participants, the median percentage of lung tissue characterized as CT lung injury at mean age 40 was 3.4% (IQR 0.8% - 18.0%). After adjustment for covariates, a 10% higher amount of CT lung injury at mean age 40 was associated with a 4.37% (95% CI 3.99 – 4.74%) higher amount of lung tissue characterized as interstitial features at mean age 50. Compared to those with the lowest quartile of CT lung injury at mean age 40, there were higher odds of incident restrictive spirometry at mean age 55 in quartile 2 (OR 2.05, 95% CI 1.20–3.48), quartile 3 (OR 2.80, 95% CI 1.66–4.72), and quartile 4 (OR 3.77, 95% CI 2.24–6.33).

**Conclusions:** CT lung injury is an early objective measure that indicates risk of future lung impairment.
INTRODUCTION:
Investigations into the development of idiopathic interstitial lung disease (ILD) have been limited by incomplete understanding of the mechanisms and timing of transitions from ideal lung health to chronic idiopathic ILD. Therefore, recent investigations have centered on identifying imaging markers of impaired respiratory health which may precede clinically overt ILD. Interstitial lung abnormalities (ILA) are specific radiologic patterns on visual assessment of computed tomography (CT) scans that may represent early ILD. ILA are associated with accelerated decline in lung function and increased all-cause mortality.

Localized inflammation appears to be one of the earliest structural manifestations of lung injury and may begin decades prior to the development of pulmonary fibrosis. Inflammatory markers, C-reactive protein (CRP) and intercellular adhesion molecule (ICAM)-1, have been associated with future development of interstitial lung abnormalities (ILA) in population-based studies. Recent studies have also identified a novel tissue class on CT imaging which corresponds to visually normal appearing areas of lung with high attenuation that we have termed “CT lung injury.” It is hypothesized that this may represent injured but not yet remodeled lung tissue that precedes the development of ILA. CT lung injury, even in those without ILA, has been associated in cross-section with increased CRP and ICAM-1, decreased lung function and 6-minute walk distance, as well as increased future mortality.

Using longitudinal data from the CARDIA (Coronary Artery Risk Development in Young Adults) study, we examined whether CT lung injury in early middle age precedes the development of future interstitial changes and restrictive spirometry pattern. Additionally, we examined whether markers of systemic inflammation, CRP and ICAM-1, are associated with future CT lung injury.

METHODS:
Study Design and Participants:

CARDIA is a prospective cohort study with Black and White participants aged 18-30 who were recruited from 1985 to 1986 from four field centers (Birmingham, AL; Chicago, IL; Minneapolis, MN; and Oakland, CA). Recruitment achieved nearly equal numbers based on race, sex, education (more than high school or high school or less), and age (18-24 yr or 25-30 yr). Reexamination occurred after 2, 5, 7, 10, 15, 20, 25, and 30 years, with 71% of the surviving cohort returning for the year 30 exam. The detailed methods, instruments and quality control procedures for the CARDIA study have been previously described.11,12 The CARDIA study is reviewed annually by the internal review boards at each participating institution and participants sign a new informed consent form at every examination.

Study Design:

This is a prospective cohort study examining the association between CT lung injury at mean age 40 (year 15 exam) and interstitial features at mean age 50 (year 25 exam) and restrictive spirometry pattern at mean age 55 (year 30 exam).

CT Acquisition and Analysis:

CT scans of the chest were obtained at mean age 40 and mean age 50. All CT scans were obtained at end-inspiration using a single breath hold. Details on CT acquisition at both time points are presented in the supplementary appendix. For this study, the primary predictor was the percentage of total lung tissue characterized as CT lung injury at mean age 40 (year 15 exam). The primary outcome was the percentage of total lung tissue characterized as interstitial features at mean age 50 (year 25 exam). The detailed methods used to objectively detect and quantify the volume of radiologic feature subtypes have been previously described.10,13–15 In brief, the CT analysis tool was trained by two experts placing a total of 57,647 reference markers in CT scans from the CARDIA cohort on specific radiologic features to create a library
of training points. The radiologic features included normal parenchyma, interstitial feature subtypes (reticular or ground-glass), and emphysema (centrilobular or paraseptal). The tool then uses the properties of the local tissue and distance from the pleural surface to create a tissue classification vector for every portion of the lung. That vector is then evaluated using a k-nearest neighbors approach to determine its similarity to tissue classification vectors extracted from the library of training points previously placed. This process results in the classification of each area of the lung as being characterized as one of the following features: normal, CT lung injury, interstitial features or emphysema. To account for differences in CT acquisition protocol and technology between exams at mean age 40 (year 15: 2000-2001) and mean age 50 (year 25: 2010-2011), this tool was trained separately for the mean age 40 and mean age 50 CT scans. The CT lung injury feature was defined as visually normal-appearing regions with high attenuation. High attenuation was defined as attenuation greater than the 95th percentile for visually normal parenchyma among training cases of never-smoking individuals with normal lung function.

Spirometry:

Spirometry was measured at years 0, 2, 5, 10, 20, and 30 and followed standard procedures as recommended by the American Thoracic Society at all examinations. Incident restrictive spirometry was defined as having a prebronchodilator forced vital capacity (FVC) less than 80% predicted at year 30 (mean age 55), with forced expiratory volume in one second/FVC ratio greater than 70%, but an FVC ≥80% at the time of their peak lung function.

Inflammatory markers:

CRP and ICAM-1 were selected as biomarkers of inflammation based on their availability and prior associations with ILA. CRP was measured at mean age 32, 40, 45, and 50 (years 7, 15, 20, and 25 exams). ICAM-1 was measured at mean age 32, 40, and 50. CRP and ICAM-1
measured at mean age 32 and year 40 were used for this study. CRP was measured using a BNII nephelometer (Dade Behring, Deerfield, IL), with intra- and inter-assay coefficients of variation ranging from 2.3% to 4.4%, and 2.1% to 5.7%. ICAM-1 levels were measured using serum samples diluted 1:400 fold and an ELISA assay (R&D Systems; Cat No DY720). The limit of sensitivity of the ICAM-1 assay was 15 pg/ml respectively with a coefficient of variation of 9.4%.

Statistical Analysis:
Summary statistics including means and standard deviations were calculated as appropriate. Multivariable linear regression was used to assess the association between the percentage of lung characterized as CT lung injury at mean age 40 and percentage of lung characterized as interstitial features at mean age 50. Multivariable logistic regression was used to determine the association between quartile of CT lung injury at mean age 40 and odds of incident restrictive spirometry at mean age 55. Quartile of CT lung injury was used to account for the fact that CT lung injury was not normally distributed and there were several zero values. Secondary analyses included multivariable linear regression models to assess the associations between: CRP at mean age 40 and percentage of lung characterized as CT lung injury at mean age 50; CRP at mean age 40 and percentage of lung characterized as interstitial features at mean age 50; ICAM-1 at mean age 40 and CT lung injury at mean age 50; and ICAM-1 at mean age 40 and interstitial features at mean age 50. Multivariable logistic regression models were used to assess the association between CRP at mean age 40 and odds of incident restrictive spirometry at mean age 55, and the association between ICAM-1 at mean age 40 and odds of incident restrictive spirometry at mean age 55. There was no adjustment for multiple comparisons given that the secondary analyses were considered exploratory. CRP, ICAM-1, and the percentage of lung tissue characterized as CT lung injury and interstitial features were log-transformed prior to being used in all regression models. Sensitivity analyses were also performed which examined
the association between CT lung injury, CRP, and ICAM-1 at mean age 40 and only reticular-type interstitial features at mean age 50. For these sensitivity analyses, ground glass abnormalities were excluded from the outcome.

For CT lung injury and interstitial features values equal to zero, the datum was reset equal to half the minimum value of CT lung injury or interstitial features prior to log transformation. This resetting of the data points was done for one CT lung injury datum point and 14 interstitial features data points. All models were adjusted for field center, age, sex, self-reported race, and years of education. Given that Black race and lower socioeconomic status have both been previously associated with elevated markers of systemic inflammation\textsuperscript{21–23} and could therefore be potential confounders, we included self-reported race and years of education as covariates. Each model was also adjusted for: BMI at the year the predictor variable was measured; change in BMI from the year of predictor variable to the year of outcome measure; smoking status and cigarettes smoked per day at the year the predictor variable was measured; and smoking pack years at the year the outcome was measured.

RESULTS:

Study Participants:

Of the 5,115 initial participants, the analytic sample size for the association between CT lung injury at mean age 40 and interstitial features at mean age 50 was 2,213 and was 1,925 for its association with incident restrictive spirometry at mean age 55 (Supplementary Figure S1). Missing covariates in each model account for the varying number of participants included in the different models. The median and interquartile range for amount of lung tissue characterized as CT lung injury at mean age 40 was 3.4\% (0.8\% - 18.0\%). The demographic characteristics of participants by quartile of CT lung injury at mean age 40 are included in Table 1. Compared to
those in the lowest quartiles of CT lung injury, those in the highest quartiles were more likely to be female, self-identify as Black, have a high school education or less, and be a current smoker.

**CT lung injury:**

**Interstitial features:**

After adjustment for age, sex, race, field center, BMI, smoking measures, and years of education, a 10% higher percentage of lung tissue characterized as CT lung injury at mean age 40 was associated with a 4.37% (95% CI 3.99–4.74, p<0.001) higher percentage of lung tissue characterized as interstitial features at mean age 50. **Figure 1** shows representative CT scan images from a participant with a high percentage of lung tissue characterized as CT lung injury at age 39 who develops a high percentage of lung tissue characterized as interstitial features at age 49. The unadjusted association between CT lung injury and interstitial features is depicted graphically in **Figure 2**.

**Incident restrictive spirometry:**

There were 3,163 participants who had pulmonary function tests performed at mean age 55. Of these participants, 42 (1.3%) had an abnormal restrictive pattern of lung function (FVC<80% with FEV1/FVC>0.7) at the time of their peak lung function (when their FVC was highest). Of the 3,121 participants without a restrictive pattern at their peak lung function, 396 (12.7%) developed an abnormal restrictive pattern of lung function by mean age 55. Analyses included 1,925 participants who had complete data for spirometry and CT features at mean age 40.

To examine the association between CT lung injury at mean age 40 and incident restriction at mean age 55, we first divided the cohort by quartiles of percentage of lung characterized as CT lung injury. Quartile of CT lung injury was used due to the non-normal distribution of CT lung injury. We found that compared to those with the lowest quartile of CT lung injury, there were
higher odds of incident restriction in quartile 2 (OR 2.05, 95% CI 1.20–3.48), quartile 3 (OR 2.80, 95% CI 1.66–4.72), and quartile 4 (OR 3.77, 95% CI 2.24–6.33) (Table 2).

**Inflammatory markers at mean age 40:**

**CT features:**

To better understand the pathway from lung injury and inflammation to interstitial features, we performed exploratory secondary analyses examining the association between markers of systemic inflammation, CRP and ICAM-1, and future CT lung injury and interstitial features. At mean age 40, the mean value of CRP was 3.12 µg/mL (SD 4.99 µg/mL) and the mean value of ICAM-1 was 153.4 ng/L (SD 43.2 ng/L). After adjustment for covariates, higher levels of both CRP and ICAM-1 at mean age 40 were associated with greater CT lung injury at mean age 50. For a 10% higher CRP, there was an associated 1.07% (95% CI 0.34–1.80%, p=0.004) higher percentage of lung characterized as CT lung injury (Table 3). For a 10% higher ICAM-1, there was an associated 6.28% (95% CI 2.34–10.4%, p=0.002) higher percentage of lung characterized as CT lung injury.

We also examined the association between CRP and ICAM-1 at mean age 40 and interstitial features at mean age 50. We found that for a 10% higher CRP, there was an associated 0.87% (95% CI 0.28–1.46, p=0.004) higher percentage of lung characterized as interstitial features (Table 2). For a 10% higher ICAM-1, there was an associated 5.00% (95% CI 1.85–8.24, p=0.002) higher percentage of lung characterized as interstitial features. Distribution of CT lung features across quartiles of CRP and ICAM-1 at mean age 40 are seen in Figure 3 and Table 3. Similar analyses were run to examine the associations between CRP and ICAM-1 at an even earlier time point, mean age 32, and CT features at mean age 50. However, after adjustment for covariates, there were no statistically significant associations between CRP or ICAM-1 at mean age 32 and CT lung injury or interstitial features at mean age 50 (Supplementary Table 1).
Incident restrictive spirometry:

We evaluated the relationship between systemic inflammation in early middle age and incident restriction 15 years later. CRP at mean age 40 was associated with incident restriction at mean age 55 (OR per standard deviation log-CRP: 1.37, 95% CI: 1.19–1.59) as was ICAM-1 (OR per standard deviation log-ICAM-1: 1.23, 95% CI: 1.07–1.42).

Sensitivity Analyses

Given that reticular markings are an imaging pattern subtype of ILA that are highly associated with increased risk of ILA progression and mortality,24 we performed sensitivity analyses to examine whether CT lung injury, CRP, and ICAM-1 were associated specifically with reticular-type interstitial features. After adjustment for covariates, we found that there remained a significant association such that a 10% higher amount of lung tissue characterized as CT lung injury at mean age 40 was associated with a 4.35% (95% CI: 3.98–4.73%) higher amount of reticular-only interstitial features at mean age 50. Similarly, we found that a 10% higher CRP at mean age 40 was associated with a 0.86% (95% CI: 0.28–1.45%) higher amount of reticular-only interstitial features at mean age 50, and a 10% higher ICAM-1 was associated with a 4.93% (95% CI: 1.79–8.16%) higher amount of reticular-only interstitial features.

DISCUSSION:

In this study, we demonstrated that CT lung injury, an imaging classifier suggestive of injured but not yet remodeled lung parenchyma, in early middle age was associated with future interstitial features and restrictive spirometry. We also found that markers of systemic inflammation (CRP, ICAM-1) were positively associated with future CT lung injury, interstitial features on CT, and restrictive spirometry; however, these secondary analyses were exploratory and not adjusted for multiple comparisons. These associations were independent of field center,
age, race, education, smoking status, cigarettes smoked per day, smoking pack-years, and BMI at multiple time points. These findings suggest that identifying CT lung injury may be a way to detect some of the earliest changes which lead to interstitial features. Additionally, our study supports that elevations in markers of systemic inflammation may precede the development of lung injury and interstitial features by a decade.

The mechanisms involved in the pathogenesis of pulmonary fibrosis are complex and incompletely understood. One well-described pathway starts with repetitive microinjuries to the alveolar epithelium which lead to several maladaptive responses that promote the proliferation of myofibroblasts and excessive deposition of extracellular matrix proteins that lead to scar formation.\textsuperscript{25} Inflammation and immune cells, particularly monocyte-derived macrophages, play an important role in promoting fibrogenesis.\textsuperscript{26,27} An earlier cross-sectional study demonstrated that participants with more lung tissue characterized by CT lung injury had higher CRP and ICAM-1 levels, demonstrating its utility as a potential imaging biomarker for lung inflammation and injury.\textsuperscript{10} CRP is a nonspecific acute phase reactant and is widely used as a clinical marker of inflammation.\textsuperscript{28} ICAM-1 mediates trafficking of leukocytes into the alveolar space as part of the acute and chronic inflammatory response to lung injury.\textsuperscript{29,30} CRP and ICAM-1 have been associated with incident ILA in prior studies.\textsuperscript{8,9} Our research complements these studies by demonstrating similar findings in a younger cohort. This has important implications for the study of subclinical ILD in demonstrating that an inflammatory phenotype may precede the development of ILD by a decade and begin in early middle-age. The findings from our study also support that an inflammatory phenotype exists independent of smoking and obesity. Notably, a Mendelian randomization study by Sunyer et al found that specific polymorphisms in the CRP gene were associated with decreased CRP levels and higher FEV1 and FVC, suggesting heritability of lung function was at least partly controlled by the CRP gene.\textsuperscript{31}
We also found that higher levels of CT lung injury, CRP and ICAM-1 were all associated with increased odds of developing a restrictive pattern of abnormal lung function by age 55. At the time of this study, there has been conflicting data with regard to whether CRP is associated with lung function decline. Given that ground-glass abnormalities are included in the definition of ILA put forth by the Fleischner Society, they were also included as part of the primary outcome of interstitial features in this study. However, in sensitivity analyses we also found that CT lung injury, CRP and ICAM-1 were also significantly associated with reticular-only interstitial features, when ground-glass abnormalities were excluded. This is notable given that the presence of reticular markings are associated with higher risk of ILA progression and increased mortality. Our finding that CT lung injury is associated with both future interstitial features and incident restriction strengthen the argument that CT lung injury may be an important marker of vulnerable lung tissue which is injured but not yet fibrotic or remodeled. Areas for future research include examining whether interstitial features can be identified in the same locations where there were previously foci of CT lung injury.

Strengths of our study include that CARDIA is a large, multiracial cohort of healthy young adults who were followed for three decades. Additionally, repeated CT chest imaging, measures of inflammatory markers, and spirometry allow for the longitudinal assessment of the relationship between systemic inflammation and markers of subclinical ILD in an initially healthy population. We also have robust data on important covariates such as obesity and smoking, which allow for improved understanding of the relationship between systemic inflammation and future CT abnormalities.

There were limitations to our study. First, this study does not use specimens or biomarkers specific to lung inflammation, and therefore we are unable to definitively show that systemic inflammation leads to lung inflammation. Since CRP and ICAM-1 are non-specific markers of
inflammation, we cannot rule out that elevations in these markers are associated with other disease states. Second, we do not have data on clinical diagnoses of ILD or clinical events such as ILD hospitalizations or death related to ILD, which limits our analyses to measures of subclinical ILD. Third, although we adjusted for BMI and smoking variables, there may be residual confounding due to more refined measures of obesity, such as visceral fat measures. Finally, because CARDIA is a longitudinal study that utilizes evolving technology, there are differences in CT acquisition and quality from the exams at mean age 40 (conducted in year 2000-2001) to mean age 50 (conducted in year 2010-2011) which may have affected the results of this study. However, because the CT analysis tool was trained separately for each exam year, it is unlikely that this would have led to an incorrect direction of association between higher amount of CT lung injury at mean age 40 and higher amount of interstitial features at mean age 50. Additionally, we do not currently have data on visually-defined ILA in the CARDIA cohort; however, a prior study has shown that interstitial features characterized using the image processing tool from this study correlate with ILA.\textsuperscript{14}

In conclusion, this study demonstrates the utility of CT lung injury as an objective and quantitative CT measure which can indicate risk of future ILD at the earliest stages, before significant remodeling has occurred. Additionally, this study reinforces that elevations in systemic inflammatory markers may precede the earliest imaging findings of parenchymal lung injury and damage. There remains a need for further research into how objective imaging processing tools can be used to further characterize phenotypes of impaired respiratory health and early disease.

**Acknowledgments:**

**Funding/Support:**
GYL is supported by NIH grant F32-HL162318. SYA is supported by NIH K08HL145118. RK is supported in part by NHLBI grant R01 HL122477(CARDIA Lung Study). The Coronary Artery Risk Development in Young Adults Study (CARDIA) is conducted and supported by the National Heart, Lung, and Blood Institute (NHLBI) in collaboration with the University of Alabama at Birmingham (HHSN268201800005I & HHSN268201800007I), Northwestern University (HHSN268201800003I), University of Minnesota (HHSN268201800006I), and Kaiser Foundation Research Institute (HHSN268201800004I). Additional funding from NHLBI R01 HL122477 (CARDIA Lung Study), F32 HL162318 (GYL). This work was partially funded by Janssen Research & Development, LLC. This manuscript has been reviewed by CARDIA for scientific content.

Disclosures:
Gabrielle Y. Liu has no conflicts of interest or financial disclosures.
Laura A. Colangelo has no conflicts of interest or financial disclosures.
Samuel A. Ash reports equity/dividends from Quantitative Imaging Solutions, unrelated to the current work.
Raul San Jose Estepar is co-founder and stockholder of Quantitative Imaging Solutions, unrelated to the current work.
David R. Jacobs Jr. has no conflicts of interest or financial disclosures.
Bharat Thyagarajan has no conflicts of interest or financial disclosures.
J. Michael Wells has no conflicts of interest or financial disclosures.
Rachel K. Putman has no conflicts of interest or financial disclosures.
Bina Choi receives consulting fees from Quantitative Imaging Solutions, unrelated to the current work.
Christopher S. Stevenson is employed by Johnson & Johnson, Inc.
Mercedes R. Carnethon has no conflicts of interest or financial disclosures.
George R. Washko reports grants from Boehringer Ingelheim, BTG Interventional Medicine and Janssen Pharmaceuticals. Dr. Washko reports consultancies/advisory board participation for Boehringer Ingelheim, Janssen Pharmaceuticals, Pulmonx, Novartis, Philips, CSL Behring and Vertex, all outside the submitted work. Dr. Washko is a co-founder and equity share holder of Quantitative Imaging Solutions, unrelated to this work. Dr. Washko's wife works for Biogen.

Ravi Kalhan receives grants and personal fees from AstraZeneca, personal fees from CVS Caremark, personal fees from Aptus Health, grants and personal fees from GlaxoSmithKline, personal fees from Boston Scientific, personal fees from Boston Consulting Group, all outside the submitted work.

Tables and Figures:

Table 1: Characteristics of participants included in this analysis at mean age 40 (year 15 visit) by quartile of CT lung injury*

<table>
<thead>
<tr>
<th>Quartile of CT lung injury</th>
<th>Q1</th>
<th>Q2</th>
<th>Q3</th>
<th>Q4</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>553</td>
<td>554</td>
<td>552</td>
<td>554</td>
</tr>
<tr>
<td>Median % CT lung injury</td>
<td>0.4% (0.3%)</td>
<td>1.6% (1.2%)</td>
<td>7.7% (6.8%)</td>
<td>36.2% (24.1%)</td>
</tr>
<tr>
<td>(IQR)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age (SD)</td>
<td>40.5 (3.5)</td>
<td>40.6 (3.5)</td>
<td>40.6 (3.5)</td>
<td>40.1 (3.7)</td>
</tr>
<tr>
<td>Female</td>
<td>46%</td>
<td>54%</td>
<td>57%</td>
<td>63%</td>
</tr>
<tr>
<td>Race</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Black</td>
<td>23%</td>
<td>41%</td>
<td>50%</td>
<td>58%</td>
</tr>
<tr>
<td>White</td>
<td>77%</td>
<td>59%</td>
<td>50%</td>
<td>42%</td>
</tr>
<tr>
<td>Education</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HS education or less</td>
<td>14%</td>
<td>19%</td>
<td>21%</td>
<td>26%</td>
</tr>
<tr>
<td>More than HS</td>
<td>86%</td>
<td>81%</td>
<td>79%</td>
<td>74%</td>
</tr>
<tr>
<td>Smoking Status</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Never smoker</td>
<td>65%</td>
<td>63%</td>
<td>64%</td>
<td>55%</td>
</tr>
<tr>
<td>Former smoker</td>
<td>21%</td>
<td>21%</td>
<td>16%</td>
<td>16%</td>
</tr>
<tr>
<td>Current smoker</td>
<td>14%</td>
<td>16%</td>
<td>20%</td>
<td>30%</td>
</tr>
<tr>
<td>Cigarettes per day (SD)</td>
<td>1.4 (4.4)</td>
<td>2.3 (6.7)</td>
<td>2.4 (6.0)</td>
<td>4.1 (8.3)</td>
</tr>
<tr>
<td>Pack-years</td>
<td>3.0 (6.9)</td>
<td>4.7 (9.0)</td>
<td>4.3 (7.9)</td>
<td>5.7 (9.3)</td>
</tr>
<tr>
<td>BMI (kg/m²) (SD)</td>
<td>25.7 (4.3)</td>
<td>28.6 (5.7)</td>
<td>29.2 (6.4)</td>
<td>31.1 (7.2)</td>
</tr>
<tr>
<td>Peak FEV1% predicted (SD)</td>
<td>103% (13%)</td>
<td>101% (12%)</td>
<td>101% (13%)</td>
<td>101% (12%)</td>
</tr>
<tr>
<td>Peak FVC% predicted (SD)</td>
<td>107% (11%)</td>
<td>104% (12%)</td>
<td>104% (12%)</td>
<td>103% (12%)</td>
</tr>
</tbody>
</table>

*Quartile of percentage of lung characterized as CT lung injury, defined as visually normal appearing regions with attenuation >90th percentile for normal lung tissue. IQR=interquartile range. SD=standard deviation. HS=high school. BMI=body mass index. FEV1=forced expiratory volume in one second. FVC=forced vital capacity.
Table 2. Association between CT lung injury at mean age 40 (year 15 exam) and incident restrictive spirometry at mean age 55 (year 30 exam) (N=1925).

<table>
<thead>
<tr>
<th>Quartile of CT lung injury* at mean age 40</th>
<th>Q1</th>
<th>Q2</th>
<th>Q3</th>
<th>Q4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Incident Restriction† OR (95% CI)</td>
<td>Ref (1.0)</td>
<td>2.05 (1.20 – 3.48)</td>
<td>2.80 (1.66 – 4.72)</td>
<td>3.77 (2.24 – 6.33)</td>
</tr>
</tbody>
</table>

*Quartile of percentage of lung characterized as CT lung injury, defined as visually normal-appearing regions with attenuation >90th percentile for normal lung tissue
†Incident restriction was defined as having FVC < 80% with FEV1/FVC ≥ 0.7 at mean age 55, but FVC ≥ 80% with FEV1/FVC ≥ 0.7 at peak lung function
Models adjusted for year 15 field center, age, race, sex, years of education, smoking status, cigarettes smoked per day, BMI, year 30-year15 BMI change, and year 30 smoking pack-years.
OR=odds ratio. CI=confidence interval. CRP=C-reactive protein. ICAM=intercellular adhesion molecule.

Table 3. Association between inflammatory markers at mean age 40 (year 15 exam) and CT features at mean age 50 (year 25 exam)

<table>
<thead>
<tr>
<th>Median (IQR) percentage of lung characterized as CT feature, by quartile of inflammatory marker*</th>
<th>Quartile of CRP at mean age 40</th>
<th>Associated percent increase (95% CI) in lung characterized as each CT feature, per 10% increase in inflammatory marker†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean (SD) CRP in µg/mL</td>
<td>Q1</td>
<td>Q2</td>
</tr>
<tr>
<td>CT lung injury‡</td>
<td>0.56%</td>
<td>1.12%</td>
</tr>
<tr>
<td>N=2570</td>
<td>(0.10-2.90%)</td>
<td>(0.17-6.52%)</td>
</tr>
<tr>
<td>Interstitial features§</td>
<td>0.44%</td>
<td>0.72%</td>
</tr>
<tr>
<td>N=2570</td>
<td>(0.16-1.26%)</td>
<td>(0.23-2.35%)</td>
</tr>
<tr>
<td>Mean (SD) ICAM-1 in ng/L</td>
<td>Q1</td>
<td>Q2</td>
</tr>
<tr>
<td>CT lung injury‡</td>
<td>0.56%</td>
<td>0.91%</td>
</tr>
<tr>
<td>N=2200</td>
<td>(0.09-3.30%)</td>
<td>(0.16-5.03%)</td>
</tr>
<tr>
<td>Interstitial features§</td>
<td>0.53%</td>
<td>0.62%</td>
</tr>
<tr>
<td>N=2200</td>
<td>(0.16-1.61%)</td>
<td>(0.22-2.28%)</td>
</tr>
</tbody>
</table>

*Unadjusted
†Based on multivariable linear regression, after log-transformation of inflammatory marker and log-transformation of CT feature.
Models adjusted for year 0 field center, age, race, and sex, and year 15 years of education, smoking status, cigarettes smoked per day, BMI, year 25-year15 BMI change, and year 25 smoking pack-years.
‡Median (IQR) percentage of lung tissue characterized as CT lung injury, defined as visually normal-appearing regions with attenuation >90th percentile for normal lung tissue.
§Median (IQR) percentage of lung tissue characterized as interstitial features, defined as reticular, centrilobular nodule, linear scar, nodular, subpleural line, ground glass, and honeycombing.
CT=computed tomography. IQR=interquartile range. CRP=C-reactive protein. ICAM=intercellular adhesion molecule.
Figure 1. Representative CT scan images from a participant with progression from high percentage of lung tissue characterized as CT lung injury to high percentage of lung tissue characterized as interstitial features. (A) At age 39 (year 15 exam), this participant had 33.9% of lung tissue characterized as CT lung injury (86th percentile for the cohort) and 1.1% of lung tissue characterized as interstitial features (76th percentile for the cohort). (B) At age 49 (year 25 exam), this participant had 55.9% of lung tissue characterized as CT lung injury (98th percentile for the cohort) and 26.6% of lung tissue characterized as interstitial features (99th percentile for the cohort).

Figure 2. Scatterplot with locally weighted smoothing regression line depicting the association between the percent of lung tissue characterized as CT lung injury at mean age 40 and the percent of lung tissue characterized as interstitial features at mean age 50. Both variables were log-transformed due to non-normal distributions.

Figure 3. Differences in the log percentage of lung characterized as CT lung injury and interstitial features at mean age 50 by quartile of CRP and ICAM-1 at mean age 40. CT lung injury was defined as visually normal-appearing regions with attenuation >90th percentile for normal lung tissue. Interstitial features were defined as reticular, centrilobular nodule, linear scar, nodular, subpleural line, ground glass, and honeycombing. The mean value of CRP in μg/ml from lowest to highest quartile was 0.33, 0.92, 2.27, 9.04. The mean value of ICAM-1 in ng/L from lowest to highest quartile was 112, 136, 158, 209. CT=computed tomography. CRP = C-reactive protein. ICAM = intercellular adhesion molecule.

References:


Figure 2. Association between CT lung injury and future interstitial features
Figure 3. Association between inflammatory markers and future CT features
Computed tomography measure of lung injury and future interstitial features: The CARDIA Lung Study

AUTHORS: Gabrielle Y. Liu, Laura A. Colangelo, Samuel Y. Ash, Raul San Jose Estepar, David R. Jacobs, Jr, Bharat Thyagarajan, J Michael Wells, Rachel K. Putman, Bina Choi, Christopher S. Stevenson, Mercedes Carnethon, George R. Washko, Ravi Kalhan

ONLINE DATA SUPPLEMENT

Supplemental Methods:

CT Acquisition

Year 15 (mean age 40): Computed tomography scans were obtained at the year 15 CARDIA examination using the following scanners at each field center: GE Lightspeed QX/I (Birmingham), Imatron C-150 (Chicago, Oakland), Siemens S4+Volume Zoom (Minneapolis). All scans were performed with a single breath hold with images taken at end-inspiration. The following image acquisition protocols were used: 130 kVp, 630 mA, 100 msec scan time, 3mm collimation, sharp reconstruction filter with 35 cm field of view and sharp reconstruction kernel (Imatron C-150); 120 kVp, 200 mA, 800 ms scan, 4x2.5mm collimation, sequential axial scans, segmented reconstruction, standard filter with a 35 cm field of view and standard kernel reconstruction (GE Lightspeed QX/I); 140 kVp, 100 mA, 500 ms scan, 4 x2.5mm collimation, sequential axial scans with 35 cm field of view and standard filter reconstruction (Siemens S4+ Volume Zoom).

Year 25 (mean age 50): Computed tomography scans were obtained at the year 25 CARDIA examination using the following scanners at each field center: GE Discovery CT750 (Birmingham), Siemens Sensation 64 (Chicago, Minneapolis), GE Lightspeed VCT 64 (Oakland). All were 64+ channel multi-detector computed tomography scanners. All scans utilized a single breath hold with images taken at end-inspiration with thorax scanned from
posterior lung recesses to the lung apex. The following image acquisition protocols were used: 100 kVp, 130 mAs, prospective ECG gating at 75%, 0.625 mm x 64 slices (64i mode GE, or equivalent on Siemens, Philips or Toshiba), 0.33 second gantry, CINE, snapshot pulse/prospective triggering. Standard reconstruction: 35 cm DFOV, 2.5-3 mm slice thickness. High Resolutions reconstruction: 25 cm DFOV, 0.5-0.6 mm slice thickness. Third reconstruction: 50 cm DFOV, 0.5-0.6 mm.

**Supplemental Figures and Tables:**

**Supplementary Figure S1.** Numbers of participants included in the primary analyses and Table 3 based on the availability of Year 7 (mean age 32), Year 15 (mean age 40), Year 25 (mean age 50), Year 30 (mean age 55) clinical data, laboratory data, CT scans, and spirometry. *Figure created with BioRender.com.*
**Supplementary Table 1.** Association between inflammatory markers at mean age 32 (year 7) and CT features at mean age 50 (year 25)

<table>
<thead>
<tr>
<th>Median (IQR) percentage of lung characterized as CT feature, by quartile of inflammatory marker*</th>
<th>Quartile of Year 7 CRP</th>
<th>Quartile of Year 7 ICAM-1</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Q1</td>
<td>Q2</td>
</tr>
<tr>
<td><strong>CRP mean (SD) in µg/mL</strong></td>
<td>0.29 (0.11)</td>
<td>0.75 (0.17)</td>
</tr>
<tr>
<td><strong>CT lung injury‡</strong> (N=2484)</td>
<td>0.59% (0.10-3.55%)</td>
<td>0.95% (0.15-5.75%)</td>
</tr>
<tr>
<td><strong>Interstitial features§</strong> (N=2484)</td>
<td>0.50% (0.15-1.61%)</td>
<td>0.66% (0.23-2.34%)</td>
</tr>
<tr>
<td><strong>ICAM-1 mean (SD) in ng/L</strong></td>
<td>105.9 (10.2)</td>
<td>127.6 (4.9)</td>
</tr>
<tr>
<td><strong>CT lung injury‡</strong> (N=1649)</td>
<td>0.52% (0.09-3.27%)</td>
<td>0.78% (0.15-5.35%)</td>
</tr>
<tr>
<td><strong>Interstitial features§</strong> (N=1649)</td>
<td>0.43% (0.14-1.55%)</td>
<td>0.67% (0.20-2.10%)</td>
</tr>
</tbody>
</table>

*Unadjusted

†Based on multivariable linear regression, after log-transformation of inflammatory marker and log-transformation of CT feature. Covariates: center, age, race, sex, educational attainment, BMI, smoking status, cigarettes smoked per day, smoking pack-years.

Models adjusted for year 0 field center, age, race, and sex, and year 7 years of education, smoking status, cigarettes smoked per day, BMI, year 25-year 7 BMI change, and year 25 smoking pack-years.

‡CT lung injury feature defined as visually normal-appearing regions with attenuation >90th percentile for normal lung tissue.

§Interstitial features defined as reticular, centrilobular nodule, linear scar, nodular, subpleural line, ground glass, and honeycombing.

CT=computed tomography. IQR=interquartile range. CRP=C-reactive protein. ICAM=intercellular adhesion molecule. SD=standard deviation.