CHEST™

Serum Bilirubin and Disease Progression in Mild COPD

Scott Apperley, MD; Hye Yun Park, MD; Daniel T. Holmes, MD; S. F. Paul Man, MD; Donald Tashkin, MD; Robert A. Wise, MD; John E. Connett, PhD; and Don D. Sin, MD, FCCP

BACKGROUND: COPD is a chronic inflammatory disorder associated with oxidative stress. Serum bilirubin has potent antioxidant actions, and higher concentrations have been shown to protect against oxidative stress. The relation between serum bilirubin and COPD progression is unknown.

METHODS: Serum bilirubin was measured in 4,680 smokers aged 35 to 60 years old with mild to moderate airflow limitation. The relationship of serum bilirubin to postbronchodilator FEV_1 and rate of FEV_1 decline over 3 to 9 years was determined using regression modeling. Total and disease-specific mortality were also ascertained.

RESULTS: Serum bilirubin was positively related to FEV_1 ($P < .001$). Serum bilirubin was also negatively related to the annual decline in FEV_1 when adjusted for baseline demographics, pack-years smoked, and baseline measures of lung function ($P = .01$). Additionally, serum bilirubin was negatively associated with risk of death from coronary heart disease ($P = .03$); however, the relationships between bilirubin and other mortality end points were not statistically significant ($P > .05$).

CONCLUSIONS: Bilirubin is inversely related to COPD disease severity and progression. Higher serum bilirubin concentration was associated with a higher FEV_1 and less annual decline in FEV_1. Bilirubin was also associated with less coronary heart disease mortality. These data support the hypothesis that bilirubin has a protective effect on COPD disease progression, possibly through its antioxidant actions. Bilirubin may prove useful as an easily accessible and readily available blood-based COPD biomarker.

CHEST 2015; 148(1):169-175

Manuscript received August 30, 2014; revision accepted November 19, 2014; originally published Online First December 24, 2014.

ABBREVIATIONS: CHD = coronary heart disease; CVD = cardiovascular disease; HO-1 = heme oxygenase-1; LHS = Lung Health Study; LMCR = logarithm of methacholine reactivity

AFFILIATIONS: From the Department of Medicine, Pulmonary Division (Drs Apperley, Man, and Sin), and the Department of Pathology (Dr Holmes), University of British Columbia, Vancouver, BC, Canada; the UBC James Hogg Research Centre and the Institute for Heart and Lung Health (Drs Park, Man, and Sin), St. Paul's Hospital, Vancouver, BC, Canada; the Division of Pulmonary and Critical Care Medicine (Dr Park), Department of Medicine, Samsung Medical Center, Seoul, South Korea; the Division of Pulmonary and Critical Care Medicine (Dr Tashkin), David Geffen School of Medicine at UCLA, Los Angeles, CA; the Division of Pulmonary and Critical Care Medicine (Dr Wise), Johns Hopkins University School of Medicine, Baltimore, MD; and the Division of Biostatistics (Dr Connett), School of Public Health, University of Minnesota, Minneapolis, MN.

FUNDING/SUPPORT: The LHS Biomarker Study was in part funded by the Canadian Respiratory Research Network, which is supported by grants from the Canadian Institutes of Health Research-Institute of Circulatory and Respiratory Health; Canadian Lung Association/Canadian Thoracic Society; British Columbia Lung Association; and industry partners Boehringer-Ingelheim Canada Ltd, AstraZeneca Canada Inc, and Novartis Canada Ltd. The original LHS was funded by the US National Heart, Lung, and Blood Institute. Dr Sin is a Canada Research Chair in COPD.

CORRESPONDENCE TO: Don D. Sin, MD, FCCP, St. Paul's Hospital, 1081 Burrard St, Vancouver, BC, V6Z 1Y6, Canada; e-mail: don.sin@hli.ubc.ca

DOI: 10.1378/chest.14-2150

COPD is a global health concern with an estimated worldwide prevalence of 300 to 600 million adults.[1] COPD results in nearly 3 million annual deaths[2] and is estimated to be the third leading cause of death worldwide.[1] Despite this, there are no proven pharmacologic therapies that have been shown unequivocally to reduce disease progression, defined as rate of lung function decline, or mortality in COPD. The current management focuses largely on symptomatic palliation and prevention of exacerbations, mostly through bronchodilator use with or without inhaled corticosteroids. There are very few promising nonbronchodilator therapies in late-phase drug development. Most new compounds (aside from bronchodilators) are abandoned early owing to the enormous cost (and risk) associated with large phase 3 trials powered on rate of lung function decline or mortality. As a possible solution to this dilemma, some have advocated the use of simple, inexpensive, and readily accessible biomarkers (for lung function decline).[3] Regrettably, to date, no such biomarkers exist. Similar to drug development, biomarker discovery has been extremely challenging, and it may be years before a novel blood test that is sensitive and specific for COPD makes it to the market. One possible solution is to extend the use of existing blood tests that are currently available for clinical care as biomarkers of COPD, analogous to the use of serum C-reactive protein for cardiac risk stratification.

One potential candidate is serum bilirubin. Oxidative stress plays a significant role in COPD disease progression.[4] In vitro studies have demonstrated bilirubin to be an efficient scavenger of free radicals.[5] Additionally, a large epidemiologic study found bilirubin concentrations to be negatively associated with COPD incidence.[6] A more recent study in a general adult population found bilirubin to be significantly related to FEV_1 in a subset of ever smokers but did not specifically examine this association in patients with COPD.[7] Using data from the Lung Health Study (LHS), we investigated the relationship between serum bilirubin and COPD severity, progression, and clinical outcomes in smokers with COPD.

Materials and Methods

Participants

Data from the LHS cohort were used. The details of this cohort have been reported previously.[8] The original LHS enrolled active smokers aged 35 to 60 years with mild to moderate airflow limitation on spirometry defined as FEV_1 between 55% and 90% predicted and FEV_1 to FVC ratio < 0.70. Participants were excluded if they had comorbidities that could have influenced follow-up, including myocardial infarction (in the past 2 years), angina, heart failure, stroke (in the past 2 years), renal failure, insulin-dependent diabetes, liver disease, pulmonary embolism, cancer (excluding carcinoma in situ or basal cell carcinoma of the skin), or CNS disorders. Smoking status was recorded by self-report and confirmed by either expiratory carbon monoxide or salivary cotinine levels. Sustained quitters were defined as those with self-reported tobacco abstinence at each annual visit that was biochemically confirmed by either salivary cotinine or expired carbon monoxide. Continued smokers were defined as those who reported smoking at each annual visit. Intermittent quitters were defined as participants whose smoking status varied across the study period. The biomarker protocol was approved by UBC/Providence Health Care Research Ethics Committee (No. H08-01864).

Serum Bilirubin Measurement

At year 5 of the study, venous blood samples were collected from 89% of eligible participants who originally consented. Samples were centrifuged, aliquoted, frozen, transferred to the LHS coordinating center, and stored at −70°C until analysis. Serum samples were thawed, and total bilirubin concentrations were measured on a Siemens Advia 1800 high-volume clinical chemistry analyzer using a vanadate oxidase method. Bilirubin concentrations were measured to the nearest 0.06 mg/dL (1 μmol/L). Total bilirubin concentrations > 1.75 mg/dL for women and > 2.34 mg/dL for men were excluded from analysis. These limits represent concentrations 1 SD above the mean serum total bilirubin associated with the most common variant of Gilbert syndrome, a benign hereditary cause of indirect hyperbilirubinemia,[6] and were chosen to include those with Gilbert syndrome. All subsequent bilirubin concentrations refer to total serum bilirubin.

Lung Function Measurements

Postbronchodilator spirometry was measured at the time of recruitment, annually to year 5,[9] and at year 11.[10] The original LHS randomized participants to different interventions, including usual care or smoking cessation therapy with or without an inhaled bronchodilator. Owing to these interventions, some participants experienced a significant increase in FEV_1 during the first 2 years of the study. In subsequent years, study participants (on average) demonstrated a linear rate of decline in FEV_1.[10] To remove the confounding effects of smoking cessation and the bronchodilator intervention, we defined baseline FEV_1 as that measured in year 2. Lung function decline was measured over two time intervals: year 2 (defined as baseline) to year 5 (time of blood draw) and year 2 to year 11, representing 3 years and 9 years of follow-up, respectively. Two methods were used to measure lung function decline over each interval. First, lung function decline was calculated as the difference in FEV_1 measurements between the first and last measurement in each time interval divided by the number of elapsed years between measurements (method one). Lung function decline was also measured by accounting for all measured spirometric data over each time interval for each participant using a repeated measures model with PROC Mixed in SAS (method two). As the results of the more complex models (method two) were similar to the simple models, the primary analyses used method one. The response to methacholine challenge was measured at study enrollment, and the details of this have been described previously.[11] Airway reactivity was quantified by the O'Connor two-point slope method. A constant (3.105) was added to the slope to avoid negative values, and the natural logarithm transformation was applied to the data to obtain the logarithm of methacholine reactivity (LMCR). Thus, higher LMCR values indicated increased bronchial reactivity.

Mortality

The vital status of each participant was investigated by an independent morbidity and mortality committee, which ascertained the vital status of 98.3% of the LHS participants and assigned the cause of death for each deceased participant based on patient records, death certificates, autopsy reports, and interviews with physicians and eyewitnesses. In the United States, the National Death Index also provided the cause and date of death of American participants up to 2001. Mortality end points

recorded at year 15 were total mortality, coronary heart disease (CHD) death, cardiovascular disease (CVD) death (including CHD death), cancer death, lung cancer death, respiratory death (excluding lung cancer), other cause of death, or unknown cause of death.

Statistical Analysis

The relationships between bilirubin and (1) smoking status, (2) post-bronchodilator FEV_1 at year 5, (3) annual average decline in FEV_1, and (4) mortality end points were analyzed. Participants were divided into quintiles of bilirubin, and baseline characteristics were compared using analysis of variance for continuous variables and a χ^2 test for dichotomous variables. To evaluate the robustness of analysis, we repeated the analysis using linear regression and bilirubin as a continuous variable rather than in quintiles. Linear regression with and without statistical adjustment for age, sex, BMI, race, FEV_1, LMCR, and pack-years smoked was used to examine the relationship between bilirubin and both FEV_1 and annual decline in FEV_1. Using bilirubin quintile 5 as the reference, logistic regression modeling was used to assess the relationship between each bilirubin quintile and mortality end points with statistical adjustments for age, sex, BMI, race, and pack-years smoked. Logistic regression modeling was also used with statistical adjustments for age, sex, BMI, race, and smoking status. All analyses were performed using IBM SPSS Statistics 21.0 (IBM) and SAS, version 9.2 (SAS Institute Inc). Statistical significance was set at $\alpha = 0.05$ using two-sided *P* values.

Results

Participants

Serum total bilirubin was measured in 4,680 LHS participants; two participants were excluded because bilirubin concentrations were above the cutoff threshold (women > 1.75 mg/dL, men > 2.34 mg/dL). Demographic and clinical characteristics across bilirubin quintiles are presented in Table 1. FEV_1 at baseline (year 2), time of blood sampling (year 5), and year 11 were positively related to serum bilirubin (Table 1). The mean (SD) serum bilirubin of all participants was 0.43 (0.19) mg/dL. As shown in Figure 1, continued smokers had the lowest serum bilirubin concentrations (0.41 [0.17] mg/dL, $P < .001$ vs sustained quitters), whereas sustained quitters had the highest concentrations (0.46 [0.20] mg/dL). Bilirubin in

TABLE 1] Baseline and Lung Function Characteristics of Participants Divided Into Quintiles of Serum Bilirubin at Year 5

Characteristic	Quintile 1	Quintile 2	Quintile 3	Quintile 4	Quintile 5	*P* for Trend
No.	678	1,500	685	927	888	…
Bilirubin, mg/dL	0.12-0.23	0.29-0.35	0.41	0.47-0.53	0.58-2.10	…
Age, y	52.9 (6.6)	53.5 (6.6)	54.2 (6.8)[a]	53.5 (6.9)	53.3 (7.0)[b]	.33
Men, No. (%)	277 (40.8)	885 (59.0)[a]	447 (65.2)[a]	652 (70.3)[a,c]	695 (78.3)[a,b,c,d]	<.001
White race, No. (%)	650 (95.9)	1,441 (96.1)	662 (96.6)	896 (96.6)	861 (97.0)	.16
BMI, kg/m^2	25.4 (4.3)	25.6 (3.9)	25.7 (3.9)	25.5 (3.8)	25.6 (3.7)	.20
Smoking status, No. (%)						<.001
Sustained quitters	86 (12.7)	253 (16.9)	104 (15.2)	181 (19.5)	207 (23.3)	
Intermittent quitters	188 (27.7)	391 (26.1)	187 (27.3)	280 (30.2)	271 (30.5)	
Continued smokers	404 (59.6)	856 (57.1)	394 (57.5)	466 (50.3)	410 (46.2)	
Pack-y smoking	38.3 (17.2)	40.7 (18.6)[a]	41.6 (18.4)[a]	40.5 (19.7)	39.2 (18.8)	.41
Lung function						
FEV_1 at y 2, L	2.50 (0.64)	2.69 (0.64)[a]	2.72 (0.64)[a]	2.78 (0.65)[a,c]	2.92 (0.63)[a,b,c,d]	<.001
FEV_1 at y 5, L	2.30 (0.64)	2.51 (0.64)[a]	2.54 (0.65)[a]	2.63 (0.67)[a,b,c]	2.75 (0.64)[a,b,c,d]	<.001
FEV_1, y 5, % predicted	73.4 (12.1)	75.1 (11.7)[a]	74.2 (12.3)	75.4 (12.4)[a]	76.5 (12.1)[a,b,c]	<.001
FEV_1, at y 11, L	1.99 (0.66)	2.21 (0.68)[a]	2.22 (0.68)[a]	2.30 (0.70)[a]	2.42 (0.69)[a,b,c,d]	<.001
FEV_1 decline y 2-5, mL/y	66.3 (64.8)	60.9 (66.0)	61.3 (69.7)	54.1 (67.6)[a]	57.0 (70.7)	.001
FEV_1 decline y 2-11, mL/y	56.6 (34.7)	53.4 (34.5)	54.8 (37.1)	54.0 (34.8)	55.2 (36.6)	.64
LMCR	2.35 (0.87)	2.28 (0.85)	2.32 (0.89)	2.28 (0.90)	2.20 (0.85)[a]	.002

Data presented as mean (SD) unless otherwise noted. Natural logarithm transformation applied to BMI for statistical analysis. LMCR = logarithm of methacholine responsiveness.
[a]*P* < .05 vs quintile 1.
[b]*P* < .05 vs quintile 2.
[c]*P* < .05 vs quintile 3.
[d]*P* < .05 vs quintile 4.

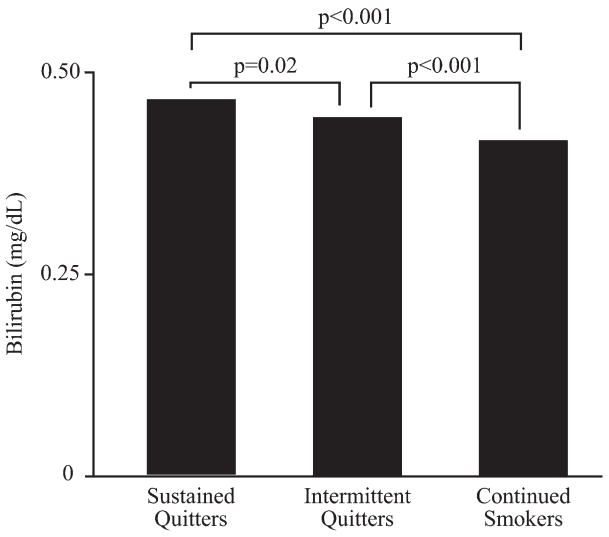

Figure 1 – *Serum bilirubin (mg/dL) of participants at y 5 for each smoking status.*

intermittent quitters was intermediate to these other smoking groups (0.44 [0.20] mg/dL, $P = .02$ vs sustained quitters).

Bilirubin and Lung Function

There was a significant positive relationship between bilirubin quintile and FEV_1 at year 5 in univariate analysis ($P < .001$) (Table 1) and when adjusted for age, sex, race, BMI, and number of pack-years smoked ($P < .001$). This relationship was present regardless of smoking status (Table 2, left columns), when each sex was analyzed separately ($P < .001$ for women, $P < .001$ for men) and when bilirubin was analyzed as a continuous variable ($P < .001$). Stratifying by smoking status and adjusting for age, sex, race, and BMI yielded positive relationships in each smoking group (sustained quitters, $P = .03$; intermittent quitters, $P = .001$; continued smokers, $P = .048$) (Table 2, right columns).

Bilirubin was also inversely related to the rate of FEV_1 decline (mL/y) between years 2 and 5 using bilirubin in quintiles (Table 1) (P = .001 for linear trend) and as a continuous variable (Fig 2A) ($P = .004$). Higher bilirubin concentration was associated with a reduced rate of decline in FEV_1. This relationship persisted following adjustment for age, sex, BMI, race, baseline FEV_1, LMCR, and number of pack-years smoked for both bilirubin in quintiles ($P < .001$ for linear trend) and as a continuous variable (Fig 2B) ($P < .001$). Lung function decline over the entire 9-year study period was inversely related to bilirubin following adjustment for age, sex, BMI, race, baseline FEV_1, LMCR, and number of pack-years smoked ($P = .01$) (e-Fig 1). There were also significant relationships between lung function decline and bilirubin stratified by sex (e-Fig 2) and smoking status (e-Fig 3), which are presented in the supplemental analysis.

Bilirubin and Total Mortality and Disease-Specific Causes of Mortality

Of the 4,678 included participants, there were 449 deaths during follow-up, including 61 CHD deaths (13.6%), 110 CVD deaths (24.5%), 229 cancer deaths (51.0%), 135 lung cancer deaths (30.1%), and 43 respiratory deaths (9.6%). Bilirubin was inversely correlated with CHD mortality after adjustment for age, sex, BMI, race, and number of pack-years smoked ($P = .03$) (Fig 3) and achieved borderline significance after adjustment for age, sex, BMI, race, and smoking status ($P = .05$). Total mortality and other disease-specific causes of mortality were not significantly related to serum bilirubin (Table 3).

Discussion

We found that serum bilirubin was positively related to lung function and rate of progression of airflow limitation in smokers with mild to moderate COPD independent of cigarette smoking and other risk factors. These data are consistent with those of Horsfall et al,[6] who showed that reduced serum bilirubin concentrations

TABLE 2] Relationship Between Serum Bilirubin and Postbronchodilator FEV_1 at Year 5 According to Smoking Status

	Bilirubin (Quintiles)						Bilirubin (Continuous)	
Smoking Status	Quintile 1	Quintile 2	Quintile 3	Quintile 4	Quintile 5	*P* for Trend	β ± SE	*P* Value[a]
Sustained quitters	2.42 (0.66)	2.67 (0.66)	2.74 (0.67)	2.69 (0.63)	2.93 (0.59)	<.001	0.16 ± 0.07	.03
Intermittent quitters	2.34 (0.66)	2.49 (0.67)	2.54 (0.67)	2.65 (0.65)	2.81 (0.64)	<.001	0. 21 ± 0.06	.001
Continued smokers	2.22 (0.60)	2.44 (0.62)	2.44 (0.67)	2.57 (0.71)	2.62 (0.64)	<.001	0.10 ± 0.05	.048

Data presented as mean (SD). Bilirubin was analyzed across quintiles and as a continuous variable. FEV_1 data presented in liters.
[a]*P* value for linear trend analysis adjusted for age, sex, race, and BMI.

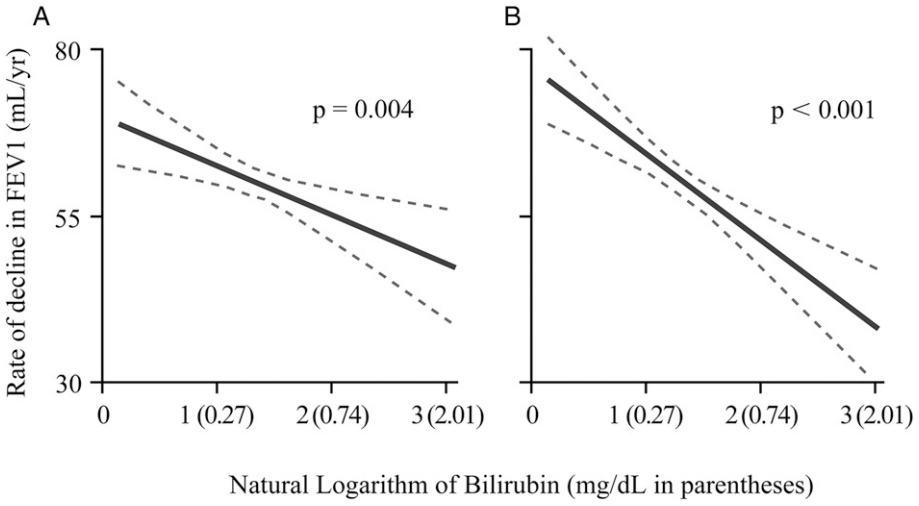

Figure 2 – *A, B, Average annual lung function decline in postbronchodilator FEV_1 (mL/y) between y 2 and 5 relative to the natural logarithm of serum bilirubin. Bilirubin is expressed in mg/dL in parentheses. The univariate relationship (A), and the relationship statistically adjusted for age, sex, BMI, race, postbronchodilator FEV_1 at baseline, logarithm of methacholine reactivity (ie, bronchial reactivity), and pack-y smoked (B) are shown.*

were associated with increased risk of COPD, and those of Curjuric et al,[7] who found a significant relationship between serum bilirubin and FEV_1 to FVC ratio in adult ever smokers. We extend these previous observations by demonstrating that serum bilirubin relates to lung function and progression of COPD in a large cohort of well-characterized patients with mild to moderate COPD.

There is a strong biologic rationale as to why bilirubin may have a modulatory role on lung function in COPD. The biochemical production of bilirubin begins when heme is degraded to biliverdin by heme oxygenase.[12] Biliverdin is subsequently reduced to bilirubin by biliverdin reductase.[12] The inducible isoform of heme oxygenase, heme oxygenase-1 (HO-1), has been shown to be up-regulated by oxidative stress,[13] and its expression is also increased by hypoxia.[14] Within the lung, HO-1 is expressed in type 2 pneumocytes and alveolar macrophages.[14] Additionally, genomic studies have found that the relative gene expression of heme oxygenase and biliverdin reductase is high in lung tissue.[15] Shinohara et al[16] investigated the role of heme oxygenase in a mouse model of pulmonary emphysema induced by intratracheal instillation of pancreatic elastase. Increasing HO-1 expression prior to pancreatic elastase instillation attenuated the severity of emphysema.[16] The HO-1 gene also contains a variable number of GT nucleic acid repeats in its flanking region. Individuals with fewer GT repeats have higher serum bilirubin concentrations[17] and a lower risk of COPD.[18] Although serum and tissue concentrations of bilirubin are relatively small compared with other antioxidants, in vitro studies suggest that tissue expression of bilirubin can be rapidly increased by up to 10,000-fold by biliverdin reductase, which reverts oxidized bilirubin to its reduced form.[19]

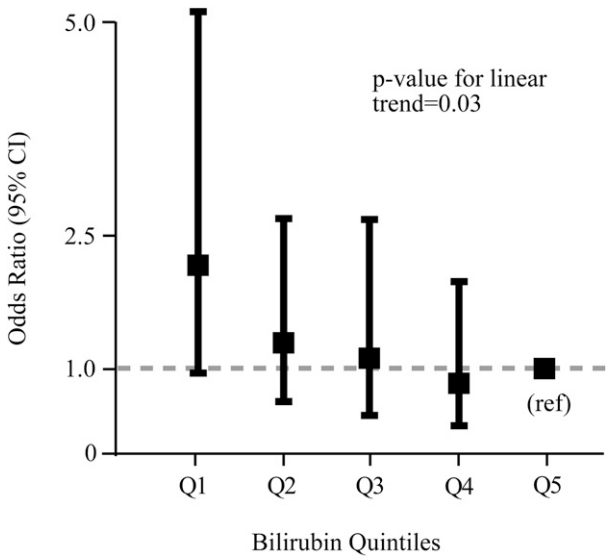

Figure 3 – *Adjusted ORs for coronary heart disease mortality by bilirubin quintile. ORs are adjusted for age, sex, BMI, race, and pack-y smoking. Quintile 5 was used as the logistic regression reference quintile. 95% CIs are displayed for each quintile.*

Oxidative stress in COPD is generated by both endogenously produced oxidants and exogenous sources such as cigarette smoke. Oxidants induce damage to proteins, DNA, and lipids.[4] Animal studies have revealed that bilirubin has a greater affinity in preventing oxidation of lipids than proteins,[20] and inhibition of bilirubin synthesis results in significant increases in lipid peroxidation products.[20] Within human lungs, lipid peroxidation causes damage to multiple cell membrane components and impairs cell structure and permeability.[21] Patients with COPD have higher levels of lipid peroxidation products in sputum,[22] and serum levels of these products are higher in patients with severe airflow limitation compared with those with

TABLE 3] Unadjusted and Adjusted ORs of Mortality End Points Divided by Quintiles of Serum Bilirubin Measured at Year 5

Mortality End Points	Quintile 1	Quintile 2	Quintile 3	Quintile 4	Quintile 5	*P* for Trend
Overall mortality						
Unadjusted	0.99 (0.70, 1.40)	1.11 (0.84, 1.47)	1.03 (0.73, 1.45)	1.03 (0.75, 1.41)	Reference	.89
Adjusted	1.15 (0.80, 1.65)	1.14 (0.85, 1.52)	0.99 (0.70, 1.41)	1.02 (0.74, 1.40)	Reference	.32
Death from CHD						
Unadjusted	1.44 (0.63, 3.28)	1.08 (0.51, 2.26)	1.06 (0.44, 2.58)	0.78 (0.32, 1.90)	Reference	.26
Adjusted	2.20 (0.95, 5.14)	1.30 (0.62, 2.74)	1.12 (0.46, 2.73)	0.83 (0.34, 2.01)	Reference	.03
Death from CVD						
Unadjusted	1.19 (0.63, 2.23)	1.07 (0.63, 1.84)	0.80 (0.40, 1.61)	0.86 (0.46, 1.62)	Reference	.42
Adjusted	1.60 (0.84, 3.06)	1.20 (0.69, 2.07)	0.81 (0.40, 1.63)	0.89 (0.47, 1.67)	Reference	.09
Cancer death						
Unadjusted	1.01 (0.62, 1.64)	1.21 (0.82, 1.79)	1.17 (0.73, 1.87)	1.14 (0.73, 1.76)	Reference	.88
Adjusted	1.03 (0.62, 1.71)	1.15 (0.77, 1.72)	1.06 (0.66, 1.71)	1.08 (0.69, 1.68)	Reference	.81
Lung cancer death						
Unadjusted	0.78 (0.41, 1.49)	1.09 (0.67, 1.79)	1.04 (0.57, 1.89)	1.12 (0.65, 1.92)	Reference	.46
Adjusted	0.79 (0.41, 1.55)	1.03 (0.62, 1.71)	0.94 (0.51, 1.71)	1.08 (0.62, 1.87)	Reference	.48
Respiratory death						
Unadjusted	1.31 (0.42, 4.09)	1.48 (0.57, 3.84)	0.86 (0.24, 3.08)	1.93 (0.72, 5.16)	Reference	.82
Adjusted	1.59 (0.50, 5.11)	1.57 (0.60, 4.11)	0.85 (0.24, 3.06)	1.97 (0.73, 5.29)	Reference	.58

Data presented as OR (95% CI) using bilirubin quintile 5 as the reference. ORs adjusted for age, sex, BMI, race, and pack-y smoking. CHD = coronary heart disease; CVD = cardiovascular disease.

moderate limitation.[23] Thus, bilirubin may protect the COPD lung by inhibiting lipid peroxidation.

Our data are also consistent with Horsfall et al,[24] who showed that serum bilirubin is an inverse risk factor for cardiovascular events in those treated with statins. Higher bilirubin concentration was associated with lower CHD mortality following adjustment for pack-years smoked; the relationship weakened after adjustment for current smoking status. Adjusting for pack-years smoked accounts for the wider variation in smoking exposure and may partially explain this difference. COPD is also associated with oxidative stress despite smoking cessation,[25] and this persistent inflammation may overwhelm the capacity of biliverdin reductase to recycle oxidized bilirubin, resulting in lower serum bilirubin in those with higher levels of oxidative stress regardless of smoking status. Smoking has also been associated with lower serum bilirubin[26] and may further deplete bilirubin, resulting in borderline significance when this relationship was adjusted for smoking status.

There were limitations to the present study. The LHS enrolled patients with COPD with mild to moderate airflow limitation based on spirometry, and thus the findings of this study may not be generalizable to patients with more severe disease. Second, only one blood sample was collected, and although bilirubin measurements are reported to be stable after adolescence,[27] a single measurement did not allow for evaluation of intraindividual variability. Additionally, it is not known if bilirubin changes with variation in lung function or if it is modifiable with therapeutic intervention. Third, we measured total serum bilirubin; direct and indirect fractions were not determined. Fourth, the determination of COPD severity was made using spirometry, and further clinical (eg, exacerbation frequency) and radiologic measures of severity were not used.

Conclusions

In summary, serum bilirubin is significantly associated with FEV_1 and FEV_1 decline in patients with mild to moderate COPD, irrespective of smoking history. Serum bilirubin is widely available, relatively inexpensive, and well standardized. Accordingly, it has the potential to be repurposed as a biomarker of disease progression and cardiac comorbidity in patients with COPD.

Acknowledgments

Author contributions: S. A. had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis. S. A., H. Y. P., D. T. H., S. F. P. M., and D. D. S. conceived of the project and study design; D. T., R. A. W., and J. E. C. obtained the blood samples and phenotyping data from the study participants; S. A., H. Y. P., and D. D. S. performed the statistical analysis and interpretation; S. A. and H. Y. P. wrote the first draft of the manuscript; D. D. S. obtained funding for the LHS Biomarker Study and supervised the study; D. T. H. and D. D. S. provided administrative, technical, or material support; and all authors contributed to critical revision of the manuscript for important intellectual content and approved of the final version.

Financial/nonfinancial disclosures: The authors have reported to *CHEST* the following conflicts of interest: Dr Holmes has sat on advisory boards for Pfizer Inc and EMD Serono, Inc to discuss diagnostic aspects of acromegaly. He has also been remunerated by IDS PLC for providing education on the diagnosis of secondary hypertension. Dr Tashkin has received fees for serving as an advisory board member for AstraZeneca, Novartis Corporation, and Sunovion Pharmaceuticals Inc; for serving as a consultant for Theravance Biopharma; and for serving as a speaker on behalf of AstraZeneca, Boehringer-Ingelheim, and Sunovion Pharmaceuticals Inc. His university has received research grants from Sunovion Pharmaceuticals Inc and Pearl Therapeutics Inc. Drs Apperley, Park, Man, Wise, Connett, and Sin have reported that no potential conflicts of interest exist with any companies/organizations whose products or services may be discussed in this article.

Role of sponsors: The funding organizations had no role in the design and conduct of the study; collection, management, analysis, and interpretation of the data; or preparation, review, or approval of the manuscript.

Other contributions: We thank the Lung Health Study (LHS) Investigators and the participants of the LHS.

Additional information: The e-Figures can be found in the Supplemental Materials section of the online article.

References

1. Buist AS, McBurnie MA, Vollmer WM, et al; BOLD Collaborative Research Group. International variation in the prevalence of COPD (the BOLD Study): a population-based prevalence study. *Lancet*. 2007;370(9589):741-750.
2. Lozano R, Naghavi M, Foreman K, et al. Global and regional mortality from 235 causes of death for 20 age groups in 1990 and 2010: a systematic analysis for the Global Burden of Disease Study 2010. *Lancet*. 2012;380(9859):2095-2128.
3. Yoon HI, Sin DD. Biomarkers of therapeutic response in patients with chronic obstructive pulmonary disease: a critical review of the literature. *Drugs*. 2011;71(14):1821-1837.
4. van Eeden SF, Sin DD. Oxidative stress in chronic obstructive pulmonary disease: a lung and systemic process. *Can Respir J*. 2013;20(1):27-29.
5. Stocker R, Glazer AN, Ames BN. Antioxidant activity of albumin-bound bilirubin. *Proc Natl Acad Sci U S A*. 1987;84(16):5918-5922.
6. Horsfall LJ, Rait G, Walters K, et al. Serum bilirubin and risk of respiratory disease and death. *JAMA*. 2011;305(7):691-697.
7. Curjuric I, Imboden M, Adam M, et al. Serum bilirubin is associated with lung function in a Swiss general population sample. *Eur Respir J*. 2014;43(5):1278-1288.
8. Anthonisen NR, Connett JE, Kiley JP, et al. Effects of smoking intervention and the use of an inhaled anticholinergic bronchodilator on the rate of decline of FEV1. The Lung Health Study. *JAMA*. 1994;272(19):1497-1505.
9. Enright PL, Johnson LR, Connett JE, Voelker H, Buist AS. Spirometry in the lung health study. 1. Methods and quality control. *Am Rev Respir Dis*. 1991;143(6):1215-1223.
10. Anthonisen NR, Connett JE, Murray RP. Smoking and lung function of Lung Health Study participants after 11 years. *Am J Respir Crit Care Med*. 2002;166(5):675-679.
11. Wise RA, Kanner RE, Lindgren P, et al; Lung Health Study Research Group. The effect of smoking intervention and an inhaled bronchodilator on airways reactivity in COPD: the Lung Health Study. *Chest*. 2003;124(2):449-458.
12. Tomaro ML, Batlle AM. Bilirubin: its role in cytoprotection against oxidative stress. *Int J Biochem Cell Biol*. 2002;34(3):216-220.
13. Carter EP, Garat C, Imamura M. Continual emerging roles of HO-1: protection against airway inflammation. *Am J Physiol Lung Cell Mol Physiol*. 2004;287(1):L24-L25.
14. Fredenburgh LE, Perrella MA, Mitsialis SA. The role of heme oxygenase-1 in pulmonary disease. *Am J Respir Cell Mol Biol*. 2007;36(2):158-165.
15. Burren OS, Adlem EC, Achuthan P, Christensen M, Coulson RM, Todd JA. T1DBase: update 2011, organization and presentation of large-scale data sets for type 1 diabetes research. *Nucleic Acids Res*. 2011;39(Database issue):D997-D1001.
16. Shinohara T, Kaneko T, Nagashima Y, Ueda A, Tagawa A, Ishigatsubo Y. Adenovirus-mediated transfer and overexpression of heme oxygenase 1 cDNA in lungs attenuates elastase-induced pulmonary emphysema in mice. *Hum Gene Ther*. 2005;16(3):318-327.
17. D'Silva S, Borse V, Colah RB, Ghosh K, Mukherjee MB. Association of (GT)n repeats promoter polymorphism of heme oxygenase-1 gene with serum bilirubin levels in healthy Indian adults. *Genet Test Mol Biomarkers*. 2011;15(4):215-218.
18. Yamada N, Yamaya M, Okinaga S, et al. Microsatellite polymorphism in the heme oxygenase-1 gene promoter is associated with susceptibility to emphysema. *Am J Hum Genet*. 2000;66(1):187-195.
19. Baranano DE, Rao M, Ferris CD, Snyder SH. Biliverdin reductase: a major physiologic cytoprotectant. *Proc Natl Acad Sci U S A*. 2002;99(25):16093-16098.
20. Sedlak TW, Saleh M, Higginson DS, Paul BD, Juluri KR, Snyder SH. Bilirubin and glutathione have complementary antioxidant and cytoprotective roles. *Proc Natl Acad Sci U S A*. 2009;106(13):5171-5176.
21. Repine JE, Bast A, Lankhorst I; Oxidative Stress Study Group. Oxidative stress in chronic obstructive pulmonary disease. *Am J Respir Crit Care Med*. 1997;156(2 pt 1):341-357.
22. Nowak D, Kasielski M, Antczak A, Pietras T, Bialasiewicz P. Increased content of thiobarbituric acid-reactive substances and hydrogen peroxide in the expired breath condensate of patients with stable chronic obstructive pulmonary disease: no significant effect of cigarette smoking. *Respir Med*. 1999;93(6):389-396.
23. Kluchová Z, Petrásová D, Joppa P, Dorková Z, Tkácová R. The association between oxidative stress and obstructive lung impairment in patients with COPD. *Physiol Res*. 2007;56(1):51-56.
24. Horsfall LJ, Nazareth I, Petersen I. Cardiovascular events as a function of serum bilirubin levels in a large, statin-treated cohort. *Circulation*. 2012;126(22):2556-2564.
25. Rutgers SR, Postma DS, ten Hacken NH, et al. Ongoing airway inflammation in patients with COPD who do not currently smoke. *Thorax*. 2000;55(1):12-18.
26. Jo J, Kimm H, Yun JE, Lee KJ, Jee SH. Cigarette smoking and serum bilirubin subtypes in healthy Korean men: the Korea Medical Institute study. *J Prev Med Pub Health*. 2012;45(2):105-112.
27. Wilding P, Rollason JG, Robinson D. Patterns of change for various biochemical constituents detected in well population screening. *Clin Chim Acta*. 1972;41:375-387.