Serum-based biomarkers associated with lung cancer risk and cause-specific mortality in the German randomized Lung Cancer Screening Intervention (LUSI) trial

Francisco O. Cortés-Ibáñez[1,2], Theron Johnson[1], Mario Mascalchi[1,3,4], Verena Katzke[1], Stefan Delorme[5], Rudolf Kaaks[1,2]^

[1]Division of Cancer Epidemiology (C020), German Cancer Research Center (DKFZ), Heidelberg, Germany; [2]Translational Lung Research Center Heidelberg (TLRC-H), the German Center for Lung Research (DZL), Heidelberg, Germany; [3]Division of Epidemiology and Clinical Governance, Institute for Study, Prevention and Network in Oncology (ISPRO), Florence, Italy; [4]Department of Clinical and Experimental Biomedical Sciences "Mario Serio", University of Florence, Florence, Italy; [5]Division of Radiology, German Cancer Research Center (DKFZ), Heidelberg, Germany

Contributions: (I) Conception and design: R Kaaks, S Delorme, FO Cortés-Ibáñez; (II) Administrative support: R Kaaks, S Delorme, T Johnson, V Katzke; (III) Provision of study materials or patients: R Kaaks, S Delorme; (IV) Collection and assembly of data: R Kaaks, S Delorme, T Johnson; (V) Data analysis and interpretation: R Kaaks, FO Cortés-Ibáñez; (VI) Manuscript writing: All authors; (VII) Final approval of manuscript: All authors.

Correspondence to: Rudolf Kaaks, PhD. Division of Cancer Epidemiology (C020), German Cancer Research Center (DKFZ), Im Neuenheimer Feld 280, 69120 Heidelberg, Germany; Translational Lung Research Center Heidelberg (TLRC-H), the German Center for Lung Research (DZL), Heidelberg, Germany. Email: r.kaaks@dkfz.de.

Background: Lung cancer (LC) screening can be optimized using individuals' estimated risks of having a detectable lung tumor, as well as of mortality risk by competing causes, to guide decisions on screening eligibility, ideal screening intervals and stopping ages. Besides age, sex and smoking history, blood-based biomarkers may be used to improve the assessment of LC risk and risk of mortality by competing causes.

Methods: In the German randomized Lung Screening Intervention Trial (LUSI), we measured growth/differentiation factor-15 (GDF-15), interleukin-6 (IL-6), C-reactive protein (CRP) and N-terminal pro-brain natriuretic protein (NT-proBNP), in blood serum samples collected at start of the trial. Participants in the computed tomography (CT)-screening arm also had a pulmonary function test. Regression models were used to examine these markers as predictors for impaired lung function, LC risk and mortality due to LC or other causes, independently of age, sex and smoking history.

Results: Our models showed increases in LC risk among participants with elevated serum levels of GDF-15 [odds ratio (OR)$_{Q4-Q1}$ =2.47, 95% confidence interval (CI): 1.49–4.26], IL-6 [OR$_{Q4-Q1}$ =2.36 (1.43–4.00)] and CRP [OR$_{Q4-Q1}$ =1.81 (1.08–2.75)]. Likewise, proportional hazards models showed increased risks for LC-related mortality, hazard ratio (HR)$_{Q4-Q1}$ of 4.63 (95% CI: 2.13–10.07) for GDF-15, 3.56 (1.72–7.37) for IL-6 and 2.34 (1.24–4.39) for CRP. All four markers were associated with increased risk of mortality by causes other than LC, with strongest associations for GDF-15 [HR$_{Q4-Q1}$ =3.04 (2.09–4.43)] and IL-6 [HR$_{Q4-Q1}$ =2.98 (2.08–4.28)]. Significant associations were also observed between IL-6, CRP, GDF-15 and impaired pulmonary function [chronic obstructive pulmonary disease (COPD), preserved ratio impaired spirometry (PRISm)]. Multi-marker models identified GDF-15 and IL-6 as joint risk predictors for risk of LC diagnosis, without further discrimination by CRP or NT-proBNP. A model based on age, sex, smoking-related variables, GFD-15 and IL-6 provided moderately strong discrimination for prediction of LC diagnoses within 9 years after blood sampling [area under the curve (AUC) =74.3% (57.3–90.2%)], compared to 67.0% (49.3–84.8%) for a model without biomarkers. For mortality by competing causes, a model including biomarkers resulted in an AUC of 76.2% (66.6–85.3%)], compared to 70.0% (60.9–77.9%) a model including age, sex and smoking variables.

^ ORCID: 0000-0003-3751-3929.

Conclusions: Serum GDF-15 and IL-6 may be useful indicators for estimating risks for LC and competing mortality among long-term smokers participating in LC screening, to optimize LC screening strategies.

Keywords: Serum biomarkers; lung cancer screening (LC screening); lung function impairment (spirometry); mortality; risk modeling

Introduction

Randomized trials have conclusively shown that screening by low-dose computed tomography (CT) can reduce lung cancer (LC) mortality among long-term smokers. However, the optimal approaches for targeting screening to those individuals that may have greatest benefit (life years gained) compared to the financial costs of screening and the risks of possible clinical harms (false-positive screening tests and over-diagnosis) are still being debated (1,2). Current guidelines in North America and Europe recommend annual LC screening for all individuals who meet eligibility criteria based on age limits and minimal lifetime smoking exposure (3,4). Ongoing research, however, focuses on the development of strategies that use more refined model predictions of an individuals' absolute risks of, on the one hand, having or developing a detectable LC (5-8) and, on the other hand, the risk of imminent (e.g., 5- or 10-year) mortality by competing causes (9-11), to guide decisions on individually optimized screening scenarios. In these scenarios, screening eligibility and individually optimized screening intensity (frequency) should depend on minimum thresholds for an individual's short-term (e.g., 5-year) LC risk, combined with a maximum threshold for the short- to medium-term (e.g., 5- or 10-year) risk of death by causes other than LC.

Basic information to estimate a person's LC and mortality risks include age (as a continuous risk factor), sex and smoking history (5,12). In addition, for those who are already participating in LC screening, risk estimates for LC and all-cause mortality can be updated and further improved by including relevant risk indicators derived from CT images, concurrent pulmonary function tests or blood-based biomarkers (13). In the US National Lung Screening Trial and in the International Lung Cancer Cohort Consortium (LC3), proteomics-based explorations of extensive series of candidate serum proteins identified interleukin 6 (IL-6), C-reactive protein (CRP), and growth/differentiation factor-15 (GDF-15) as biomarkers that can significantly predict the risk of an imminent LC diagnosis, independently of age, sex and detailed smoking histories (14,15). In parallel studies, we and others found that these same, inflammation-related markers are also associated with biological aging processes (16-20), pulmonary function impairments [chronic obstructive pulmonary disease (COPD), preserved ratio impaired spirometry (PRISm), pulmonary fibrosis (21-24)], risk of cancers other than LC, and risk of cardiovascular events (myocardial infarction, stroke) and all-cause mortality (25-29). Another important marker associated with aging-related diseases and all-cause mortality, but not with LC risk, is N-terminal pro-brain natriuretic protein (NT-proBNP) (25-29).

Highlight box

Key findings
- Growth/differentiation factor-15 (GDF-15) and interleukin-6 (IL-6) are useful predictors of lung cancer risk and competing mortality in individuals eligible for lung cancer screening.
- Models including both biomarkers improved individual risk prediction, compared to models based only on age, smoking history and pulmonary function.

What is known and what is new?
- Lung cancer screening can be optimized using individuals' estimated risks of having a detectable lung tumor and mortality risk by competing causes, to guide decisions on screening eligibility, ideal screening intervals and stopping ages.
- This study indicates that GDF-15 and IL-6 can be used to improve these risk estimates.

What is the implication, and what should change now?
- In lung cancer screening settings, the assessments of individuals' risks of lung cancer and mortality by competing causes may be improved using measurements of serum GDF-15 and IL-6.

2462

Cortés-Ibáñez et al. Serum biomarkers in LC screening participants

We here report findings from the German Lung Cancer Screening Intervention (LUSI) trial, to confirm the capacity of GDF-15, IL-6, CRP or NT-proBNP to predict LC risk and risk of all-cause and competing-cause mortality when added to known risk factors such as age, sex and detailed smoking history in an actual population-based screening setting. We discuss findings in view of the potential utility of measuring these biomarkers for more personalized targeting of CT screening. We present this article in accordance with the STROBE reporting checklist (available at https://tlcr.amegroups.com/article/view/10.21037/tlcr-23-548/rc).

Methods

Study design and participants

The German LUSI study (30,31) is a registered randomized trial (ISRCTN30604390). The recruitment phase took place in the metropolitan area of Heidelberg between October 23rd 2007 and April 11th 2011. For this, 292,000 men and women aged 50–69 years were extracted from population registers and were asked by mailed questionnaires about their past and current smoking habits. To be eligible for the study, participants had to fulfill the following criteria: ≥25 years of smoking of ≥15 cigarettes per day, ≥30 years smoking of ≥10 cigarettes per day or ≤10 years since smoking cessation before invitation to screening. The presence of LC, or also of any other major disease associated with elevated short-term risk of death, was an exclusion criterion. These eligibility criteria are similar to those of the Dutch-Belgian NELSON trial (32). A total of 89,722 participants filled in and returned the pre-baseline smoking questionnaire, and among these respondents 4,708 were eligible by the established criteria, were willing to participate in the study and were invited to the German Cancer Research Center (DKFZ) in Heidelberg to participate in the trial. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). The LUSI study was approved by the local ethical review board of Heidelberg University (073/2001) and by the radiation protection authority (BfS, 22462/2, 2006-045). All study participants provided informed consent.

A total of 4,052 participants finally accepted and were randomized into a screening intervention arm (n=2,029) involving five annual CT screenings, and a control arm (n=2,023) without screening. The CT screening arm comprised a low-dose CT (LDCT) examination at the time of randomization plus four annual follow-up LDCT examinations. Participants were actively screened between October 23rd 2007 and May 25th 2016. Prospective ascertainment of LC incidence and overall (cause-specific) mortality is being performed continuously until to date. For all LC cases, detailed information from medical records (pathology reports, medical letters from responsible physicians on diagnosis and treatment and radiology reports, with their exact dates) was obtained by contacting the treating clinics, and coded to International Classification of Diseases for Oncology, 3rd (ICD-O-3) for tumor histology and stage. The vast majority of participants are of Caucasian ethnic ancestry. Broader descriptions of the study design and results for mortality reduction have been published previously (30,31).

Laboratory methods

At the baseline recruitment (time of randomization) all LUSI participants (both study arms) were asked to provide blood samples, which were processed into aliquots of serum, plasma, buffy coat, and erythrocytes and stored in –80 ℃ freezers. Aliquots were thawed for the first time for the current analyses. Electrochemiluminescence immunoassays were carried out using the Quickplex SQ 120 instrument (Meso Scale Discoveries, Rockville, Maryland, USA) to measure circulating plasma concentrations of GDF-15, IL-6, CRP and NT-proBNP. Standard protocols followed for each kit as provided by the manufacturer. Within-batch and between-batch coefficients of variation (CV) were: 3.15% and 7.08% for IL-6; 3.6% and 16.2% for NT-proBNP; 2.7% and 12.8% for CRP; and 3.0% and 11.1% for GDF-15. The percentages of missing values were below 5% for all biomarker measurements. Samples from cases and control participants were randomly distributed across analytical batches, and the batch mean-centering method was used for batch standardization (33). The case/control status of all samples was blinded for all laboratory measurements.

Spirometry

The participants in the CT screening arm were also offered a spirometry test, on the occasion of their baseline CT scan, and a total of 2,007 participants took part in this. Pre-bronchodilator spirometry was performed using a MasterScreen IOS (VIASYS Healthcare, Hoechberg, Germany) spirometer. The forced expiratory volume to forced vital capacity (FEV1/FVC) ratios were calculated from the largest FEV1 and FVC values recorded in any one

of two repeated assessments. Individual's predicted FEV1 and FVC values for a given age, sex, body height, and race (FEV1% predicted, FVC% predicted) were calculated using previously established equations (34). Participants with FEV1/FVC <0.70 were classified as having COPD and the severity of their airflow impairment was further graded into stages 1 (FEV1 ≥80% predicted), 2 (50%≤ FEV1 <80% predicted), or 3–4 (FEV1 <50% predicted) following the Global Initiative for Obstructive Lung Disease (GOLD) criteria (35). Participants with FEV1/FVC ≥0.70 but with FEV1% <80% were classified as having PRISm (36,37).

Analytical cohort; LC diagnoses and mortality outcomes

For the present analyses, we excluded participants who had reported a past diagnosis of any type of cancer (n=105), those with none of the biomarkers measured (n=406) and those with unknown vital status (n=11). This left a total of 3,530 LUSI participants who fulfilled the inclusion criteria for the present analyses; of these 1,873 were in the CT arm and 1,657 in the control arm. Till July 2021, a total of 155 cases of LC were diagnosed among the included LUSI participants whose biomarkers had been measured; of these, 88 were diagnosed in the CT screening arm whereas 67 LC cases were diagnosed in the control arm. Furthermore, amongst the LUSI participants with biomarker measurements, a total of 422 cases of death were documented (all causes combined) of which 221 were in the CT arm and 201 in the control arm. Of these, a total of 80 were directly caused by LC, of which 36 occurred in the screening arm and 44 in the control arm. A schematic overview (flow diagram) of data selected for the present analyses is in Figure S1.

Statistical analyses

For all biomarkers, missing values were imputed by multiple imputation by chained equations using the MICE package in R (Vienna, Austria). Means [standard deviation (SD)], medians [25th/75th percentiles (interquartile range (IQR)] or calculated proportions were used to describe sociodemographic and health-related characteristics of the selected study participants. Spearman rank correlations were used to examine associations among the four serum biomarkers and between biomarkers, body mass index (BMI) and smoking-related covariates. Logistic regression models were used to evaluate the associations between quartile levels of IL-6, GDF-15, NT-ProBNP

and CRP to the risk of having spirometry-based lung function impairments (COPD, PRISm) or risk of having or developing LC (prevalent and incident). Likewise, multivariable Cox proportional hazard models were used to quantify the association between all-cause mortality and LC mortality, with levels of the serum markers, using the age of participants as the time scale. In all Cox models, the time scale (age) was left-censored by the date of baseline recruitment and randomization into the screening or the control arm, whereas the time point of right-censoring was determined by the date of death, or April 4th, 2021, whichever came first. All logistic regression and Cox models were adjusted for age, sex, BMI and smoking history (lifetime smoking duration, time since smoking cessation, average cigarettes per day). The capacity to improve the prediction of future diagnoses of LC or cases of death, additionally to age, sex, BMI and smoking history, was examined using receiver operating curve (ROC) analyses, with the area under ROC curves (AUC) as an overall measure for discrimination capacity. The ROC analyses were performed for risk scores derived from logistic regression models (for LC diagnosis as outcome) and from Cox models (for mortality outcomes), fitted age, sex, BMI and smoking history, and additionally to the marker measurements as continuous variables (markers were added in order, according to their relevance in forward selection models). Log-likelihood ratio tests were used to test for the improvement in model fit with progressive additions of biomarkers to a base model for age, sex, BMI, and smoking history (lifetime smoking duration, average number of cigarettes smoked per day, time since smoking cessation for ex-smokers). Improvements in discrimination per marker added were evaluated by integrated discrimination improvement (IDI) indices, and by the progressive increases in the overall AUC for entire risk models.

All statistical analyses were performed using R, version R-4.3.0 (38), and SAS (version 9.4, SAS Institute, NC, USA).

Results

Of the 3,530 LUSI participants who fulfilled the inclusion criteria for the present analyses about two thirds (65.2%) were men. The median age at time of blood sampling was 56.9 years (IQR, 52.9–62 years) for men and 56.1 years (IQR, 52.7–60.9 years) for women. Among the men 59% were still current smokers, compared to 65.7% among the women, and all other participants were ex-smokers who had quitted smoking less than 10 years ago. About 60% of

2464

Cortés-Ibáñez et al. Serum biomarkers in LC screening participants

the participants reported a lifetime smoking duration of 30–40 years. Among the men, at the time of trial enrolment 10.5% were diabetic, 35.5% suffered from arterial hypertension, 10.3% reported having had a previous heart attack, and 4.6% a previous stroke. Among the women, the prevalence of diabetes (5.8%), arterial hypertension (28.2%), or previous heart attack (2.4%) or stroke (2.4%) was lower (*Table 1*). In the CT arm, among the 1,873 individuals retained for the present analyses spirometry measurements identified a total of 295 participants (15.8%) with PRISm, and 258 (13.8%) with moderate-to-severe

(GOLD stage 2–4) COPD.

Correlations between the biomarkers, and of biomarkers with pulmonary functional impairment (COPD and PRISm)

In the whole group of LUSI participants, adjusted for age and sex, there were only weak correlations between the plasma concentrations of IL-6, GDF-15, NT-proBNP and CRP (pairwise Spearman correlations all below 0.30), and very weak correlations between CRP and BMI (r=0.15) and between GDF-15 and lifetime smoking duration (r=0.12)

Table 1 Characteristics of the study population in both arms of the German randomized LUSI trial*

Characteristics	Male (N=2,304)	Female (N=1,226)	Overall (N=3,530)
Baseline age, years			
Mean (SD)	58.0 (5.59)	57.3 (5.14)	57.8 (5.44)
Median [IQR]	56.9 [52.9, 62]	56.1 [52.7, 60.9]	56.6 [52.8, 61.7]
BMI (kg/m^2)			
Mean (SD)	27.3 (3.83)	25.8 (4.68)	26.7 (4.20)
Median [IQR]	26.8 [17.5, 48.4]	25.0 [17.0, 52.1]	26.3 [17.0, 52.1]
Smoking status, n (%)			
Current smoker	1,359 (59.0)	806 (65.7)	2,165 (61.3)
Former smoker	945 (41.0)	420 (34.3)	1,365 (38.7)
Smoking duration (years), n (%)			
26–30	385 (16.7)	252 (20.6)	637 (18.0)
31–35	702 (30.5)	408 (33.3)	1,110 (31.4)
36–40	684 (29.7)	367 (29.9)	1,051 (29.8)
+40	533 (23.1)	199 (16.2)	732 (20.7)
Time since smoking cessation, n (%)			
Have not stopped	1,351 (58.6)	800 (65.3)	2,151 (60.9)
Less than 1 year	133 (5.8)	62 (5.1)	195 (5.5)
From 1 to 5 years	428 (18.6)	215 (17.5)	643 (18.2)
More than 5 years	391 (17.0)	148 (12.1)	539 (15.3)
Not answered	1 (0.04)	1 (0.1)	2 (0.1)
Average cigs. per day, n (%)			
11–15	300 (13.0)	278 (22.7)	578 (16.4)
16–20	695 (30.2)	430 (35.1)	1,125 (31.9)
21–25	557 (24.2)	283 (23.1)	840 (23.8)
26–30	264 (11.5)	108 (8.8)	372 (10.5)
+30	488 (21.2)	127 (10.4)	615 (17.4)

Table 1 (*continued*)

Table 1 (*continued*)

Characteristics	Male (N=2,304)	Female (N=1,226)	Overall (N=3,530)
Heart attack (prior to screening), n (%)			
No	2,067 (89.7)	1,197 (97.6)	3,264 (92.5)
Yes	237 (10.3)	29 (2.4)	266 (7.5)
Stroke (prior to screening), n (%)			
No	2,197 (95.4)	1,196 (97.6)	3,393 (96.1)
Yes	107 (4.6)	30 (2.4)	137 (3.9)
Diabetes (baseline), n (%)			
No	2,026 (87.9)	1,128 (92.0)	3,154 (89.3)
Yes	242 (10.5)	71 (5.8)	313 (8.9)
I don't know	31 (1.3)	17 (1.4)	48 (1.4)
Not answered	5 (0.2)	10 (0.8)	15 (0.4)
Hypertension (baseline), n (%)			
No	1,405 (61.0)	849 (69.2)	2,254 (63.9)
Yes	818 (35.5)	346 (28.2)	1,164 (33.0)
I don't know	80 (3.5)	25 (2.0)	105 (3.0)
Not answered	1 (0.04)	6 (0.5)	7 (0.2)
Concentration NT-proBNP (pg/mL)			
Median [Min, Max]	161 [14.0, 13,800]	218 [9.88, 17,100]	180 [9.88, 17,100]
Concentration GDF-15 (pg/mL)			
Median [Min, Max]	912 [22.9, 22,300]	840 [64.5, 5,580]	883 [22.9, 22,300]
Concentration CRP (ng/mL)			
Median [Min, Max]	3,010 [20.3, 53,600]	2,620 [120, 52,600]	2,820 [20.3, 53,600]
Concentration IL-6 (pg/mL)			
Median [Min, Max]	1.07 [0.180, 265]	0.987 [0.155, 371]	1.03 [0.155, 371]
Lung cancer, n (%)			
No lung cancer detected	2,198 (95.4)	1,177 (96.0)	3,375 (95.6)
lung cancer detected	106 (4.6)	49 (4.0)	155 (4.4)
Vital status, n (%)			
Alive	1,960 (85.1)	1,148 (93.6)	3,108 (88.0)
Dead	344 (14.9)	78 (6.4)	422 (12.0)
Lung cancer death, n (%)			
Alive	1,960 (85.2)	1,148 (93.6)	3,108 (88.0)
Death other cause	284 (12.2)	58 (4.7)	342 (9.7)
Lung cancer death	60 (2.6)	20 (1.6)	80 (2.3)

*, participants with available blood sample to measure biomarkers concentrations, further excluding those with a history of other cancer diagnosis and unknown vital status. LUSI, Lung Cancer Screening Intervention; SD, standard deviation; IQR, interquartile range; BMI, body mass index; cigs, cigarettes; CRP, C-reactive protein; NT-proBNP, N-terminal pro-brain natriuretic protein; Min, minimum; Max, maximum; GDF-15, growth/differentiation factor-15; IL-6, interleukin 6.

(Figure S2).

In the CT arm, logistic regression models adjusting for sex, age, and smoking history showed significant associations of IL-6 and CRP with moderate-to-severe COPD, with odds ratios (ORs) of 2.49 [95% confidence interval (CI): 1.63–3.86, P<0.001] and 1.97 (1.33–2.96, P<0.001), respectively, comparing highest to lowest quartiles of biomarker measurements. In relation to PRISm, models showed positive associations for IL-6 and GDF-15, with ORs of 2.02 (1.38–3.00) and 2.32 (1.58–3.45) respectively (Table S1).

Associations of biomarkers with LC risk

In logistic regression models, adjusting for age, sex, BMI and smoking history, higher blood levels of GDF-15, IL-6 and CRP were all significantly associated with increased risk of having prevalent, or later being diagnosed with incident LC. In the two study arms combined, and comparing highest with lowest marker quartiles, the highest OR estimate was for GDF-15 [OR =2.47 (95% CI: 1.49–4.26)] and slightly lower ORs were estimated for IL-6 [2.36 (1.43–4.00)] and CRP [1.81 (1.08–2.75)]. Considering histologic tumor subtypes, the associations of GDF-15, IL-6 and CRP with LC risk appeared to be stronger for non-adenocarcinomas (i.e., mostly squamous and small cell tumors) than for adenocarcinomas (*Table 2*), although differences in strength of association were not statistically significant in formal tests for heterogeneity (P values not shown in table). For NT-proBNP, no significant associations with LC risk was found. A separate analysis for early-stage LC (stages I and II) showed a positive association to the highest quartiles of IL-6 (Table S2).

As previously reported in greater detail (39), spirometry measurements in the CT arm showed increased risk of LC also for participants with moderate-to-severe COPD (GOLD stages 2–4) or PRISm, with ORs of 2.36 (95% CI: 1.35–4.1) and 3.30 (1.9–5.7), respectively, for subjects included in the present analyses (results not shown in table). However, additional adjustments for COPD or PRISM (further to age, sex, BMI and smoking history) resulted in only minimal attenuation of the associations of LC risk with the serum biomarker levels (Table S3).

Associations of biomarkers with mortality due to LC and other causes

Related to LC death, Cox proportional hazards models

adjusted by sex, age, and smoking history showed strong associations especially for GDF-15 [for highest compared to lowest quartile level hazard ratio (HR) =4.63 (95% CI: 2.13–10.07)] and IL-6 [HR =3.56 (1.72–7.37)], and a more moderate association for CRP [HR =2.34 (1.24–4.39)] (*Table 3*).

In relation to overall (all-cause) mortality, proportional hazards models showed associations with all four biomarkers, with HRs of 3.41 (95% CI: 2.43–4.79) for GDF-15, 3.17 (2.29–4.38) for IL-6, 1.80 (1.37–2.36) for CRP and 1.74 (1.30–2.32) for NT-proBNP, comparing between highest and lowest quartile levels. Similar estimates were obtained for mortality by causes other than LC (*Table 3*).

In the CT arm, we further examined whether markers predicted mortality independently of COPD or PRISm. The proportional hazard models showed associations similar to those found in the complete cohort after adjustment for spirometry categories, with still a strong association for GDF-15 [HR =3.08 (95% CI: 1.94–4.96)], and IL-6 [2.60 (95% CI: 1.60–4.34)]; however, the associations to CRP and NT-proBNP were no longer significant [1.51 (95% CI: 0.99–2.33); 1.31 (95% CI: 0.86–2.01), respectively] (Table S4).

Multimarker modelling evaluating predictive performance

Multi-marker modeling, using forward selection, identified GDF-15 and IL-6 as joint risk predictors for risk of LC diagnosis, whereas in multi-marker models CRP (and also NT-proBNP) did not further add significantly to overall model fit and risk discrimination. Examined over the entire study follow-up (till April 2021), a joint model based on age, sex, smoking-related variables, GFD-15 and IL-6 provided moderate discrimination for risk of having a LC diagnosis [AUC =64.9% (49.5–77.8%)]; the discrimination, however, was stronger [AUC =72.0% (59.8–83.4%)] for LC diagnosed within the first 5 years after baseline blood donation, as compared to an AUC of 67.0% (49.3–84.8%) for a model based on age, sex, BMI and smoking history only (*Table 4*).

Exploring marker combinations, all four biomarkers were significantly and jointly associated with risk of overall mortality, as well as mortality by causes other than LC (*Table 4*), although GDF-15 was the one marker providing strongest discrimination, followed by IL-6, NT-proBNP and CRP (*Table 4*). Considering deaths over the entire follow-up period (i.e., till April 2021, median follow-up

Table 2 Risk of lung cancer by quartiles of biomarker measurements in the screening arm (n=88), control arm (n=67) and in both arms combined (n=155)

Biomarkers	CT screening arm[1]				Control arm[1]				CT screening arm + control arm[1]			
	$N_{cases}/N_{non-cases}$	OR	95% CI	P_{trend}	$N_{cases}/N_{non-cases}$	OR	95% CI	P_{trend}	$N_{cases}/N_{non-cases}$	OR	95% CI	P_{trend}
All lung cancer cases (n=155)												
Quartile 1 GDF-15 ref	n=11/560				n=10/302				n=21/862			
Quartile 2 GDF-15	n=19/455	1.88	0.89–4.16		n=11/398	0.70	0.29–1.73		n=30/853	1.29	0.73–2.31	
Quartile 3 GDF-15	n=25/409	2.42	1.18–5.24		n=13/435	0.67	0.28–1.65		n=38/844	1.50	0.87–2.65	
Quartile 4 GDF-15	n=33/361	3.36	1.67–7.23	0.001	n=33/455	1.52	0.71–3.46	0.091	n=66/816	2.47	1.49–4.26	<0.001
Quartile 1 IL-6 ref	n=12/457				n=12/402				n=24/860			
Quartile 2 IL-6	n=17/451	1.37	0.64–3.00		n=18/396	1.41	0.66–3.09		n=35/847	1.45	0.85–2.50	
Quartile 3 IL-6	n=28/440	2.35	1.18–4.93		n=13/401	1.05	0.46–2.44		n=41/841	1.76	1.05–3.03	
Quartile 4 IL-6	n=31/437	2.45	1.23–5.15	0.004	n=24/390	2.01	0.96–4.39	0.110	n=55/827	2.36	1.43–4.00	<0.001
Quartile 1 CRP ref	n=19/502				n=14/385				n=33/887			
Quartile 2 CRP	n=15/467	0.75	0.34–1.60		n=16/372	1.27	0.60–2.77		n=31/839	1.00	0.60–1.65	
Quartile 3 CRP	n=29/433	1.83	0.97–3.56		n=12/396	0.90	0.39–2.05		n=41/829	1.36	0.84–2.20	
Quartile 4 CRP	n=25/383	1.74	0.91–3.41	0.017	n=25/437	1.77	0.87–3.74	0.183	n=50/820	1.81	1.08–2.75	0.010
Quartile 1 NT-proBNP ref	n=24/488				n=10/377				n=34/865			
Quartile 2 NT-proBNP	n=25/480	0.73	0.40–1.35		n=19/353	1.83	0.85–4.18		n=44/833	1.12	0.70–1.79	
Quartile 3 NT-proBNP	n=13/420	0.42	0.20–0.82		n=16/428	1.16	0.52–2.70		n=29/848	0.65	0.38–1.08	
Quartile 4 NT-proBNP	n=26/397	0.67	0.36–1.23	0.111	n=22/432	1.34	0.61–3.12	0.344	n=48/829	0.90	0.56–1.46	0.301
Adenocarcinomas (n=82)												
Quartile 1 GDF-15 ref	n=8/563				n=6/306				n=14/869			
Quartile 2 GDF-15	n=15/459	2.05	0.87–5.18		n=4/405	0.44	0.11–1.58		n=19/864	1.20	0.59–2.47	
Quartile 3 GDF-15	n=15/419	1.95	0.81–5.00		n=5/443	0.47	0.13–1.66		n=20/862	1.17	0.58–2.43	
Quartile 4 GDF-15	n=17/377	2.33	0.98–6.00	0.096	n=12/476	1.03	0.35–3.26	0.613	n=29/853	1.60	0.82–3.24	0.180
Quartile 1 IL-6 ref	n=9/460				n=8/407				n=17/867			
Quartile 2 IL-6	n=14/454	1.58	0.68–3.88		n=10/404	1.19	0.45–3.23		n=24/858	1.47	0.78–2.85	
Quartile 3 IL-6	n=13/455	1.57	0.65–3.93		n=4/410	0.52	0.13–1.73		n=17/865	1.11	0.55–2.26	
Quartile 4 IL-6	n=19/449	2.14	0.94–5.22	0.093	n=5/409	0.65	0.18–2.12	0.276	n=24/858	1.56	0.81–3.07	0.327
Quartile 1 CRP ref	n=11/510				n=10/389				n=21/899			
Quartile 2 CRP	n=11/471	1.06	0.45–2.53		n=7/381	0.80	0.28–2.14		n=18/852	0.91	0.47–1.73	
Quartile 3 CRP	n=21/441	2.29	1.09–5.06		n=4/404	0.43	0.12–1.34		n=25/845	1.33	0.73–2.44	
Quartile 4 CRP	n=12/396	1.34	0.56–3.21	0.176	n=6/456	0.59	0.19–1.71	0.218	n=18/852	0.97	0.50–1.88	0.745
Quartile 1 NT-proBNP ref	n=15/497				n=3/384				n=18/881			
Quartile 2 NT-proBNP	n=17/488	0.84	0.41–1.77		n=8/364	2.58	0.73–11.91		n=25/852	1.17	0.63–2.21	
Quartile 3 NT-proBNP	n=9/424	0.51	0.21–1.17		n=7/437	1.84	0.50–8.70		n=16/861	0.69	0.34–1.37	
Quartile 4 NT-proBNP	n=14/409	0.63	0.28–1.40	0.158	n=9/445	2.04	0.57–9.63	0.526	n=23/854	0.81	0.42–1.59	0.278

Table 2 (*continued*)

2468

Cortés-Ibáñez et al. Serum biomarkers in LC screening participants

Table 2 (*continued*)

Biomarkers	CT screening arm[1]				Control arm[1]				CT screening arm + control arm[1]			
	$N_{cases}/N_{non\text{-}cases}$	OR	95% CI	P_{trend}	$N_{cases}/N_{non\text{-}cases}$	OR	95% CI	P_{trend}	$N_{cases}/N_{non\text{-}cases}$	OR	95% CI	P_{trend}
Non-adenocarcinomas (n=73)												
Quartile 1 GDF-15 ref	n=3/568				n=4/308				n=7/876			
Quartile 2 GDF-15	n=4/470	1.33	0.29–6.86		n=7/402	1.10	0.32–4.29		n=11/872	1.32	0.51–3.64	
Quartile 3 GDF-15	n=10/424	3.12	0.91–14.29		n=8/440	0.99	0.30–3.85		n=18/864	1.88	0.77–4.96	
Quartile 4 GDF-15	n=16/378	4.97	1.53–22.35	0.003	n=21/467	2.18	0.76–7.92	0.078	n=37/845	3.52	1.52–8.94	<0.001
Quartile 1 IL-6 ref	n=3/466				n=4/411				n=7/877			
Quartile 2 IL-6	n=3/465	0.82	0.15–4.48		n=8/406	1.85	0.57–7.05		n=11/871	1.38	0.54–3.81	
Quartile 3 IL-6	n=15/453	3.66	1.15–16.31		n=9/405	2.12	0.67–8.05		n=24/858	2.93	1.29–7.51	
Quartile 4 IL-6	n=12/456	2.74	0.82–12.50	0.033	n=19/395	4.55	1.60–16.35	0.003	n=31/851	3.62	1.62–9.23	<0.001
Quartile 1 CRP ref	n=8/513				n=4/395				n=12/908			
Quartile 2 CRP	n=4/478	0.49	0.13–1.59		n=9/379	2.45	0.78–9.17		n=13/857	1.11	0.50–2.50	
Quartile 3 CRP	n=8/454	1.06	0.38–2.94		n=8/400	2.07	0.64–7.90		n=16/854	1.36	0.64–2.98	
Quartile 4 CRP	n=13/395	1.78	0.73–4.65	0.103	n=19/443	4.56	1.64–16.20	0.007	n=32/838	2.69	1.39–5.56	0.002
Quartile 1 NT-proBNP ref	n=9/503				n=7/380				n=16/883			
Quartile 2 NT-proBNP	n=8/497	0.71	0.26–1.92		n=11/361	1.55	0.60–4.29		n=19/858	1.08	0.55–2.14	
Quartile 3 NT-proBNP	n=4/429	0.37	0.10–1.18		n=9/435	0.92	0.33–2.63		n=13/864	0.65	0.30–1.38	
Quartile 4 NT-proBNP	n=12/411	0.92	0.35–2.46	0.761	n=13/441	1.10	0.42–3.11	0.839	n=25/852	1.05	0.54–2.11	0.847

[1], logistic regression models adjusted by age, sex, body mass index and smoking history. CT, computed tomography; OR, odds ratio; CI, confidence interval; GDF-15, growth/differentiation factor-15; IL-6, interleukin 6; CRP, C-reactive protein; NT-proBNP, N-terminal pro-brain natriuretic protein.

Table 3 Hazard ratios for all-cause (n=422) and lung cancer-specific mortality (N=80) by quartiles of biomarker measurements

Biomarkers	$N_{cases}/N_{non\text{-}cases}$	CT screening arm + control arm[1]		
		HR	95% CI	P_{trend}
All-cause mortality				
Quartile 1 GDF-15 ref	n=45/838			
Quartile 2 GDF-15	n=62/821	1.20	0.81–1.76	
Quartile 3 GDF-15	n=113/769	1.99	1.40–2.84	
Quartile 4 GDF-15	n=202/680	3.41	2.43–4.79	<0.001
Quartile 1 IL-6 ref	n=51/833			
Quartile 2 IL-6	n=82/800	1.44	1.01–2.05	
Quartile 3 IL-6	n=107/775	1.80	1.28–2.54	
Quartile 4 IL-6	n=182/700	3.17	2.29–4.38	<0.001
Quartile 1 CRP ref	n=87/833			
Quartile 2 CRP	n=89/781	1.08	0.80–1.45	
Quartile 3 CRP	n=100/770	1.18	0.88–1.57	

Table 3 (*continued*)

Table 3 (*continued*)

Biomarkers	N_{cases}/N_{non-cases}	CT screening arm + control arm[1]		
		HR	95% CI	P_{trend}
Quartile 4 CRP	n=146/724	1.80	1.37–2.36	<0.001
Quartile 1 NT-proBNP ref	n=74/825			
Quartile 2 NT-proBNP	n=86/791	1.07	0.78–1.46	
Quartile 3 NT-proBNP	n=87/790	0.97	0.71–1.33	
Quartile 4 NT-proBNP	n=175/702	1.74	1.30–2.32	<0.001
Mortality (other than lung cancer mortality)				
Quartile 1 GDF-15 ref	n=37/846			
Quartile 2 GDF-15	n=51/832	1.18	0.77–1.80	
Quartile 3 GDF-15	n=98/784	1.99	1.35–2.94	
Quartile 4 GDF-15	n=156/726	3.04	2.09–4.43	<0.001
Quartile 1 IL-6 ref	n=41/845			
Quartile 2 IL-6	n=66/814	1.40	0.95–2.08	
Quartile 3 IL-6	n=88/795	1.74	1.19–2.54	
Quartile 4 IL-6	n=147/734	2.98	2.08–4.28	<0.001
Quartile 1 CRP ref	n=72/848			
Quartile 2 CRP	n=69/801	1.00	0.72–1.40	
Quartile 3 CRP	n=86/784	1.20	0.88–1.65	
Quartile 4 CRP	n=115/755	1.66	1.23–2.25	<0.001
Quartile 1 NT-proBNP ref	n=61/838			
Quartile 2 NT-proBNP	n=67/810	1.02	0.72–1.44	
Quartile 3 NT-proBNP	n=72/805	0.99	0.70–1.39	
Quartile 4 NT-proBNP	n=142/735	1.73	1.26–2.38	<0.001
Lung cancer mortality				
Quartile 1 GDF-15 ref	n=8/875			
Quartile 2 GDF-15	n=11/872	1.23	0.49–3.07	
Quartile 3 GDF-15	n=15/867	1.53	0.64–3.64	
Quartile 4 GDF-15	n=46/836	4.63	2.13–10.07	<0.001
Quartile 1 IL-6 ref	n=10/874			
Quartile 2 IL-6	n=16/866	1.52	0.69– 3.37	
Quartile 3 IL-6	n=19/863	1.89	0.87–4.13	
Quartile 4 IL-6	n=35/847	3.56	1.72–7.37	<0.001
Quartile 1 CRP ref	n=15/905			
Quartile 2 CRP	n=20/850	1.42	0.73–2.79	
Quartile 3 CRP	n=14/856	1.01	0.48–2.10	
Quartile 4 CRP	n=31/839	2.34	1.24–4.39	0.017
Quartile 1 NT-proBNP ref	n=13/886			
Quartile 2 NT-proBNP	n=19/858	1.25	0.62–2.56	
Quartile 3 NT-proBNP	n=15/862	0.91	0.43–1.94	
Quartile 4 NT-proBNP	n=33/844	1.75	0.89–3.46	0.137

[1], Cox regression models adjusted by age, sex, body mass index and smoking history. CT, computed tomography; HR, hazard ratio; CI, confidence interval; GDF-15, growth/differentiation factor-15; IL-6, interleukin 6; CRP, C-reactive protein; NT-proBNP, N-terminal pro-brain natriuretic protein.

Table 4 Overall discrimination for multi-marker models to predict risk of all-cause mortality, lung cancer mortality or lung cancer

Variables	Both study arms combined		
	Log-likelihood ratio test (χ^2; df; P)	IDI (95% CI)***	AUC (95% CI) ***
Lung cancer*			
Entire follow-up time (n_{cases} =155/$n_{non\text{-}cases}$ =3,375)			
a) Model 1 age, sex, BMI and smoking history			58.2 (44.4–71.4)
b) Model 1 + GDF-15 vs. Model 1a	16.1; 1; <0.001	IDI: 0.009 (0.00, 0.01)[¶]	63.5 (50.4–76.8)
c) Model 1 + GDF-15 + IL-6 vs. Model 1b	8.8; 1; 0.002	IDI: 0.006 (0.00, 0.02)[¶]	64.9 (49.5–77.8)
≤2 years follow-up (n_{cases} =50/$n_{non\text{-}cases}$ =3,480)			
a) Model 2 age, sex, BMI and smoking history			65.6 (47.6–82.1)
b) Model 2 + GDF-15 vs. Model 2a	1.22; 1; 0.26	IDI: 0.000 (−0.001, 0.002)	72.4 (47.8–90.8)
c) Model 2 + GDF-15 + IL-6 vs. Model 2b	0.82; 1; 0.36	IDI: 0.000 (0.000, 0.002)	73.5 (45.9–87.7)
≤5 years follow-up (n_{cases} =85/$n_{non\text{-}cases}$ =3,445)			
a) Model 3 age, sex, BMI and smoking history			68.2 (55.5–80.9)
b) Model 3 + GDF-15 vs. Model 3a	6.4; 1; 0.01	IDI: 0.002 (0.00, 0.01)[¶]	70.1 (55.6–84.8)
c) Model 3 + GDF-15 + IL-6 vs. Model 3b	3.6; 1; 0.05	IDI: 0.001 (−0.001, 0.004)	72.0 (59.8–83.4)
≤9 years follow-up (n_{cases}=121/$n_{non\text{-}cases}$ =3,409)			
a) Model 4 age, sex, BMI and smoking history			67.0 (49.3–84.8)
b) Model 4 + GDF-15 vs. Model 4a	7.0; 1; 0.008	IDI: 0.004 (0.00, 0.01)[¶]	73.0 (55.5–88.9)
c) Model 4 + GDF-15 + IL-6 vs. Model 4b	4.31; 1; 0.03	IDI: 0.002 (−0.001, 0.012)	74.3 (57.3–90.2)
Lung cancer mortality**			
Entire follow-up time (n_{cases} =80/$n_{non\text{-}cases}$ =3,450)			
a) Model 1 age, sex, BMI and smoking history			64.9 (47.1–81.9)
b) Model 1 + GDF-15 vs. Model 1	26.38; 1; <0.001	IDI: 0.010 (0.001, 0.04)[¶]	71.2 (52.3–84.7)
c) Model 1 + GDF-15 + IL-6, vs. Model 1a	5.9; 1; 0.01	IDI: 0.005 (−0.004, 0.01)	72.1 (53.5–85.6)
≤5 years follow-up (n_{cases}=46/$n_{non\text{-}cases}$ =3,484)			
a) Model 2 age, sex, BMI and smoking history			66.1 (50.0–83.3)
b) Model 2 + GDF-15 vs. Model 2a	12.7; 1; <0.001	IDI: 0.002 (−0.002, 0.007)	70.1 (56.5–83.5)
c) Model 2 + GDF-15 + IL-6 vs. Model 2b	1.94; 1; 0.16	IDI: 0.001 (−0.003, 0.008)	71.1 (51.9–82.5)
≤9 years follow-up (n=50/3,480)			
a) Model 3 age, sex, BMI and smoking history			65.8 (43.9–83.3)
b) Model 3 + GDF-15 vs. Model 3a	17.7; 1; <0.001	IDI: 0.010 (0.004, 0.03)[¶]	71.9 (52.8–87.1)
c) Model 3 + GDF-15 + IL-6, vs. Model 3b	2.3; 1; 0.12	IDI: 0.000 (−0.005, 0.01)	72.3 (49.1–90.6)
Mortality, all causes except lung cancer**			
Entire follow-up time (n_{cases} =342/$n_{non\text{-}cases}$ =3,188)			
a) Model 1: Age, sex, BMI and smoking history			68.1 (60.5–75.6)
b) Model 1 + GDF-15 vs. Model 1a	88.2; 1; <0.001	IDI: 0.030 (0.01, 0.05)[¶]	72.3 (65.0–79.4)
c) Model 1 + GDF-15 + IL-6 vs. Model 1b	18.3; 1; <0.001	IDI: 0.008 (0.002, 0.016)[¶]	73.2 (65.9–80.3)
d) Model 1 + GDF-15 + IL-6 + NT-ProBNP vs. Model 1c	18.6; 1; <0.001	IDI: 0.012 (0.003, 0.027)[¶]	73.7 (66.7–80.7)
e) Model 1 + GDF-15 + IL-6 + NT-ProBNP + CRP vs. Model 1d	2.45; 1; 0.11	IDI: 0.002 (0.00, 0.008)	73.9 (66.2–81.7)

Table 4 (*continued*)

　　　　　　　　　Transl Lung Cancer Res 2023;12(12):2460-2475 | https://dx.doi.org/10.21037/tlcr-23-548

Table 4 (*continued*)

Variables	Both study arms combined		
	Log-likelihood ratio test (χ^2; df; P)	IDI (95% CI)***	AUC (95% CI) ***
≤5 years follow-up (n_{cases} =80/$n_{non\text{-}cases}$ =3,450)			
a) Model 2 age, sex, BMI and smoking history			69.5 (59.4–77.3)
b) Model 2 + GDF-15 *vs.* Model 2a	12.11; 1; 0.008	IDI: 0.003 (0.000, 0.007)¶	71.6 (61.0–78.8)
c) Model 2 + GDF-15 + IL-6 *vs.* Model 2b	6.29; 1; 0.01	IDI: 0.005 (0.001, 0.010)¶	73.2 (64.1–82.2)
d) Model 2 + GDF-15 + IL-6 + NT-ProBNP *vs.* Model 2c	5.71, 1, 0.01	IDI: 0.011 (0.002, 0.019)¶	73.3 (63.1–80.1)
e) Model 2 + GDF-15 + IL-6 + NT-ProBNP + CRP *vs.* Model 2d	0.17, 1, 0.67	IDI: 0.000 (–0.002, 0.001)	73.1 (62.9–81.3)
≤9 years follow-up (n_{cases} =171/$n_{non\text{-}cases}$ =3,359)			
a) Model 3 age, sex, BMI and smoking history			70.0 (60.9–77.9)
b) Model 3 + GDF-15 *vs.* Model 3a	72.6; 1; <0.001	IDI: 0.030 (0.01, 0.06)¶	75.4 (66.1–84.7)
c) Model 3 + GDF-15 + IL-6 *vs.* Model 3b	9.6; 1; 0.001	IDI: 0.006 (0.001, 0.012)¶	76.2 (66.6–85.3)
d) Model 3 + GDF-15 + IL-6 + NT-ProBNP *vs.* Model 3c	12.1; 1; <0.001	IDI: 0.010 (0.002, 0.024)¶	76.3 (67.5–84.1)
e) Model 3 + GDF-15 + IL-6 + NT-ProBNP + CRP *vs.* Model 3d	0.21; 1; 0.64	IDI: 0.001 (–0.001, 0.006)	76.4 (66.8–85.1)

*, logistic regression models; **, Cox regression models; ***, models were internally cross-validated on 1,000 bootstrapped samples to correct for overfitting. ¶, P<0.05. IDI, integrated discrimination index; CI, confidence interval; AUC, area under the receiver operator curve; BMI, body mass index; GDF-15, growth/differentiation factor-15; IL-6, interleukin 6.

time of 11.8 years), a model for mortality other than LC mortality, including age, sex, smoking history, GDF-15 and IL-6 resulted in an AUC of 73.2% (65.9–80.3%), compared to an AUC of 68.1 (60.5–75.6%) for a model based on age, sex, BMI and smoking history alone. A model including all four markers provided only slightly higher discrimination [AUC of 73.9% (66.2–81.7%)]. The prediction of mortality by causes other than LC was somewhat stronger for deaths occurring within the first 9 years of prospective follow-up with an AUC of 76.4% (66.8–85.1%) (*Table 4*). Finally, for LC-related mortality, only GDF-15 and IL-6 were jointly and significantly associated with risk, and for the risk model including both biomarkers the AUC was 72.1% (53.5–85.6%) and did not appear to differ for deaths occurring before the follow-up time of 9 years 72.3% (49.1–90.6%).

Discussion

The optimization of LC screening programs requires personalized strategies that account for an individual's estimated risk of having a detectable lung tumor (LC risk), as well as the risk of short-term mortality by competing causes, as indicators to guide decisions on screening eligibility, frequency and stopping ages (12). Using a minimal threshold for LC risk as a general eligibility criterion ensures that

financial costs, as well as the number of individuals that needs to be screened (NNS) and that exposed to the possible of harms of screening (radiation exposures; risk of false-positive findings), remains within acceptable limits relative to the number of LC cases detected within a screening program. For individuals already participating in a screening program (i.e., who meet a minimal-risk criterion for having LC) estimates of LC risk can be used further to determine individually more optimized time intervals between successive screenings (40-42). Finally, having a sufficiently low risk estimate for short- to medium-term (5- to 10-year) mortality (i.e., having a sufficiently high remaining life expectancy) can be used as a criterion for individualized recommendations as to whether LC screening should be further pursued or dis-continued.

Basic information to estimate an individual's risks of having LC and of short-term mortality include a person's age, sex, BMI and smoking history. Additional risk information may be derived from CT images and pulmonary function tests (for persons who have already attended first screening visit), or from blood-based biomarker measurements that can be programmed as part of a more general, accompanying health check-up.

In this population-based screening trial, we found that especially GDF-15 and IL-6 may be potentially useful

2472

Cortés-Ibáñez et al. Serum biomarkers in LC screening participants

predictors for an individual's short- to medium-term risk (e.g., over the next 5–10 years) of receiving an LC diagnosis, as well as of mortality by LC or by other causes. These two biomarkers significantly improved risk models based on age, smoking history or pulmonary function tests, which are established predictors for the risks of LC and all-cause mortality. In multi-marker models, the two other markers examined, CRP and NT-proBNP, showed no further significant associations with LC risk.

Our findings for GDF-15 and IL-6 confirm those from recent large-scale proteomics studies, which also found these to be among the strongest serum-based predictors for LC risk (15,25). Our data suggest that GDF-15 and IL-6 might both be associated more strongly with the risk of small cell or squamous cell LC, which are generally more aggressive tumor sub-types with poorer prognosis, as compared to adenocarcinomas, which may have better long-term curability rates. However, our study was too small to assess this possible heterogeneity in risk association with sufficient accuracy. Accessorily, we found that GDF-15 and IL-6 were also associated with presence of airflow limitations (COPD, PRISm). GDF-15 and IL-6 both are inflammation-related cytokines, and have also been described as involved in physiological responses such as fibrosis or apoptosis, which are related to various disease conditions, including but not limited to the development of cancer (43,44).

In analyses stratified by lag time between baseline recruitment (time of marker measurement) and cancer or mortality outcomes, we found that especially for LC risk the risk prediction by age, smoking history and serum measurements of GFD-15 and IL-6 was stronger when considering incidence within the first 5–9 years of follow-up, as compared to incidence after a longer time lag. This may be explained by variability and systematic changes in marker levels, but also smoking data, over time and indicates that in practice the information about risk predictors (smoking status, marker levels) should be repeated at regular, e.g., 5-year, intervals.

While it is generally accepted that LC screening needs to be reserved mostly to long-term smokers who are at increased risk of harboring or developing a lung tumor, a point not so often emphasized is that that screening participants should be still also in good enough health to expect a meaningful gain in life years in case of early-stage tumor detection and treatment. While guidelines for screening eligibility generally indicate a maximum age until which screening should be allowed, it is insufficiently

appreciated that, among individuals with long-term smoking history, there can be wide variation in health status and remaining life expectancy. Besides LC risk, age and smoking history (lifetime duration, average number of cigarettes per day, time since quitting for ex-smokers) are also major determinants for the risk of competing-cause mortality, even among long-term smokers, and are key predictors in models and tables for the estimation of absolute mortality risk and residual life expectancy. Our present findings show that the same is also true for GDF-15 and IL-6, and that these two markers may further improve the predictive discrimination of such models, so as to improve assessments of individuals' general eligibility for LC screening. Individualized estimates of short to medium-term risk of mortality, overall and by causes other than LC, may help identify screening participants whose residual life expectancy would be too low to allow a meaningful gain in life years in case of early LC detection, who are also at elevated risk for over-diagnosis. The determination of short-term mortality risk may be particularly relevant for older, still smoking screening participants, notably above the age of 70 years, and especially within this older age group, it may be used to determine whether continuation of CT screening would be expected to bring sufficient net clinical benefit.

Strengths of our study are that it is based on a representative population of LC screening participants, recruited from the general population, and that the serum markers could be evaluated jointly with detailed questionnaire data on smoking history. A limitation of our study, however, is that serum markers were measured only at a single point in time, in blood samples collected at the start of the LUSI trial. A further limitation is that the study, and incidence case numbers for LC and deaths, were far too small for the development of a reliable model for absolute risks for LC or mortality by age groups and sex. Thus, our analyses do not directly inform about possible risk thresholds to be used for the individualized optimization of intervals between successive screenings (based on LC risk), or for recommendations to discontinue screening in case of elevated short-term morality risk.

Further, and larger, studies may be needed to examine GDF-15 and IL-6 in combination with other biomarkers that were not evaluated in our present analyses (45,46), and to clarify whether there is significant heterogeneity in the relationships of these and other markers with LC risk by histologic sub-types. Further work will also be needed for the integration of biomarker measurements in model

algorithms based on age, sex, and smoking history that can be used to provide properly calibrated estimates for absolute risk for LC and competing-cause mortality for screening populations in different countries and screening contexts.

Conclusions

Our results confirm the potential utility of GDF-15 and IL-6 measurements as additional predictors for the estimation of LC risk, as well as competing-cause mortality, in view of optimizing individualized LC screening strategies. The biomarkers could be measured as part of a more general health check-up, to guide decisions on the general eligibility for entry into a LC screening program, on individually optimized screening intervals, or also on the optimal time point for screening participants to discontinue screening.

Acknowledgments

Footnote

References

1. Hunger T, Wanka-Pail E, Brix G, et al. Lung Cancer Screening with Low-Dose CT in Smokers: A Systematic Review and Meta-Analysis. Diagnostics (Basel) 2021;11:1040.

2. Kaaks R, Delorme S. Lung Cancer Screening by Low-Dose Computed Tomography - Part 1: Expected Benefits, Possible Harms, and Criteria for Eligibility and Population Targeting. Rofo 2021;193:527-36.

3. US Preventive Services Task Force; Krist AH, Davidson KW, et al. Screening for Lung Cancer: US Preventive Services Task Force Recommendation Statement. JAMA 2021;325:962-70.

4. Kauczor HU, Baird AM, Blum TG, et al. ESR/ERS statement paper on lung cancer screening. Eur Radiol 2020;30:3277-94.

5. Ten Haaf K, Jeon J, Tammemägi MC, et al. Risk prediction models for selection of lung cancer screening candidates: A retrospective validation study. PLoS Med 2017;14:e1002277.

6. Hüsing A, Kaaks R. Risk prediction models versus simplified selection criteria to determine eligibility for lung cancer screening: an analysis of German federal-wide survey and incidence data. Eur J Epidemiol 2020;35:899-912.

7. Tammemägi MC, Ruparel M, Tremblay A, et al. USPSTF2013 versus PLCOm2012 lung cancer screening eligibility criteria (International Lung Screening Trial): interim analysis of a prospective cohort study. Lancet Oncol 2022;23:138-48.

8. Toumazis I, Bastani M, Han SS, et al. Risk-Based lung cancer screening: A systematic review. Lung Cancer 2020;147:154-86.

9. Cheung LC, Berg CD, Castle PE, et al. Life-Gained-Based Versus Risk-Based Selection of Smokers for Lung Cancer Screening. Ann Intern Med 2019;171:623-32.

10. Ten Haaf K, Bastani M, Cao P, et al. A Comparative Modeling Analysis of Risk-Based Lung Cancer Screening Strategies. J Natl Cancer Inst 2020;112:466-79.

11. Meza R, Jeon J, Toumazis I, et al. Evaluation of the Benefits and Harms of Lung Cancer Screening With Low-Dose Computed Tomography: Modeling Study for the US Preventive Services Task Force. JAMA 2021;325:988-97.

12. Ten Haaf K, van der Aalst CM, de Koning HJ, et al. Personalising lung cancer screening: An overview of risk-stratification opportunities and challenges. Int J Cancer 2021;149:250-63.

13. Ostrin EJ, Sidransky D, Spira A, et al. Biomarkers for Lung Cancer Screening and Detection. Cancer Epidemiol Biomarkers Prev 2020;29:2411-5.

14. Shiels MS, Pfeiffer RM, Hildesheim A, et al. Circulating inflammation markers and prospective risk for lung cancer. J Natl Cancer Inst 2013;105:1871-80.

15. The blood proteome of imminent lung cancer diagnosis. Nat Commun 2023;14:3042.

16. Nadruz W Jr, Gonçalves A, Claggett B, et al. Influence of cigarette smoking on cardiac biomarkers: the Atherosclerosis Risk in Communities (ARIC) Study. Eur J Heart Fail 2016;18:629-37.

17. Klopack ET, Carroll JE, Cole SW, et al. Lifetime exposure to smoking, epigenetic aging, and morbidity and mortality in older adults. Clin Epigenetics 2022;14:72.

18. Mamoshina P, Kochetov K, Cortese F, et al. Blood Biochemistry Analysis to Detect Smoking Status and Quantify Accelerated Aging in Smokers. Sci Rep 2019;9:142.

19. Daskalopoulou C, Stubbs B, Kralj C, et al. Associations of smoking and alcohol consumption with healthy ageing: a systematic review and meta-analysis of longitudinal studies. BMJ Open 2018;8:e019540.

20. Petrie K, Abramson MJ, Cross AJ, et al. Predicting life expectancy of older people using respiratory symptoms and smoking status: Data from the Australian Longitudinal Study of Ageing. Respirology 2020;25:267-74.

21. Wan Y, Fu J. GDF15 as a key disease target and biomarker: linking chronic lung diseases and ageing. Mol Cell Biochem 2023. [Epub ahead of print]. doi: 10.1007/s11010-023-04743-x.

22. Sanders JL, Putman RK, Dupuis J, et al. The Association of Aging Biomarkers, Interstitial Lung Abnormalities, and Mortality. Am J Respir Crit Care Med 2021;203:1149-57.

23. Rydell A, Nowak C, Janson C, et al. Plasma proteomics and lung function in four community-based cohorts. Respir Med 2021;176:106282.

24. Regan EA, Hersh CP, Castaldi PJ, et al. Omics and the Search for Blood Biomarkers in Chronic Obstructive Pulmonary Disease. Insights from COPDGene. Am J Respir Cell Mol Biol 2019;61:143-9.

25. Srour B, Kaaks R, Johnson T, et al. Ageing-related markers and risks of cancer and cardiovascular disease: a prospective study in the EPIC-Heidelberg cohort. Eur J Epidemiol 2022;37:49-65.

26. Potok OA, Ix JH, Shlipak MG, et al. The Difference Between Cystatin C- and Creatinine-Based Estimated GFR and Associations With Frailty and Adverse Outcomes: A Cohort Analysis of the Systolic Blood Pressure Intervention Trial (SPRINT). Am J Kidney Dis 2020;76:765-74.

27. Hart A, Blackwell TL, Paudel ML, et al. Cystatin C and the Risk of Frailty and Mortality in Older Men. J Gerontol A Biol Sci Med Sci 2017;72:965-70.

28. Srour B, Hynes LC, Johnson T, et al. Serum markers of biological ageing provide long-term prediction of life expectancy-a longitudinal analysis in middle-aged and older German adults. Age Ageing 2022;51:afab271.

29. Justice JN, Ferrucci L, Newman AB, et al. A framework for selection of blood-based biomarkers for geroscience-guided clinical trials: report from the TAME Biomarkers Workgroup. Geroscience 2018;40:419-36.

30. Becker N, Motsch E, Gross ML, et al. Randomized study on early detection of lung cancer with MSCT in Germany: study design and results of the first screening round. J Cancer Res Clin Oncol 2012;138:1475-86.

31. Becker N, Motsch E, Trotter A, et al. Lung cancer mortality reduction by LDCT screening-Results from the randomized German LUSI trial. Int J Cancer 2020;146:1503-13.

32. de Koning HJ, van der Aalst CM, de Jong PA, et al. Reduced Lung-Cancer Mortality with Volume CT Screening in a Randomized Trial. N Engl J Med 2020;382:503-13.

33. Lazar C, Meganck S, Taminau J, et al. Batch effect removal methods for microarray gene expression data integration: a survey. Brief Bioinform 2013;14:469-90.

34. Quanjer PH, Stanojevic S, Cole TJ, et al. Multi-ethnic reference values for spirometry for the 3-95-yr age range: the global lung function 2012 equations. Eur Respir J 2012;40:1324-43.

35. GOLD Report. 2021 Global Strategy for Prevention, Diagnosis and Management of COPD 2021. Available online: https://staging.goldcopd.org/2021-gold-reports/

36. Higbee DH, Granell R, Davey Smith G, et al. Prevalence, risk factors, and clinical implications of preserved ratio impaired spirometry: a UK Biobank cohort analysis. Lancet Respir Med 2022;10:149-57.

37. Wan ES, Balte P, Schwartz JE, et al. Association Between Preserved Ratio Impaired Spirometry and Clinical Outcomes in US Adults. JAMA 2021;326:2287-98.

38. R Core Team. The R package for statistical computing: R: A language and environment for statistical computing. Version 3.4.4 Vienna, Austria; cited 2013. R Foundation for Statistical Computing. Available online: https://www.R-project.org/

39. Kaaks R, Christodoulou E, Motsch E, et al. Lung function impairment in the German Lung Cancer Screening Intervention Study (LUSI): prevalence, symptoms, and associations with lung cancer risk, tumor histology and all-cause mortality. Transl Lung Cancer Res 2022;11:1896-911.

40. Robbins HA, Berg CD, Cheung LC, et al. Identification of Candidates for Longer Lung Cancer Screening Intervals Following a Negative Low-Dose Computed Tomography Result. J Natl Cancer Inst 2019;111:996-9.

41. Schreuder A, Schaefer-Prokop CM, Scholten ET, et al. Lung cancer risk to personalise annual and biennial follow-up computed tomography screening. Thorax 2018;thoraxjnl-2017-211107.

42. González Maldonado S, Hynes LC, Motsch E, et al. Validation of multivariable lung cancer risk prediction models for the personalized assignment of optimal screening frequency: a retrospective analysis of data from the German Lung Cancer Screening Intervention Trial (LUSI). Transl Lung Cancer Res 2021;10:1305-17.

43. Breit SN, Brown DA, Tsai VW. The GDF15-GFRAL Pathway in Health and Metabolic Disease: Friend or Foe? Annu Rev Physiol 2021;83:127-51.

44. Banerjee I, Fuseler JW, Intwala AR, et al. IL-6 loss causes ventricular dysfunction, fibrosis, reduced capillary density, and dramatically alters the cell populations of the developing and adult heart. Am J Physiol Heart Circ Physiol 2009;296:H1694-704.

45. Integrative Analysis of Lung Cancer Etiology and Risk (INTEGRAL) Consortium for Early Detection of Lung Cancer, Guida F, Sun N, et al. Assessment of Lung Cancer Risk on the Basis of a Biomarker Panel of Circulating Proteins. JAMA Oncol 2018;4:e182078.

46. Fahrmann JF, Marsh T, Irajizad E, et al. Blood-Based Biomarker Panel for Personalized Lung Cancer Risk Assessment. J Clin Oncol 2022;40:876-83.

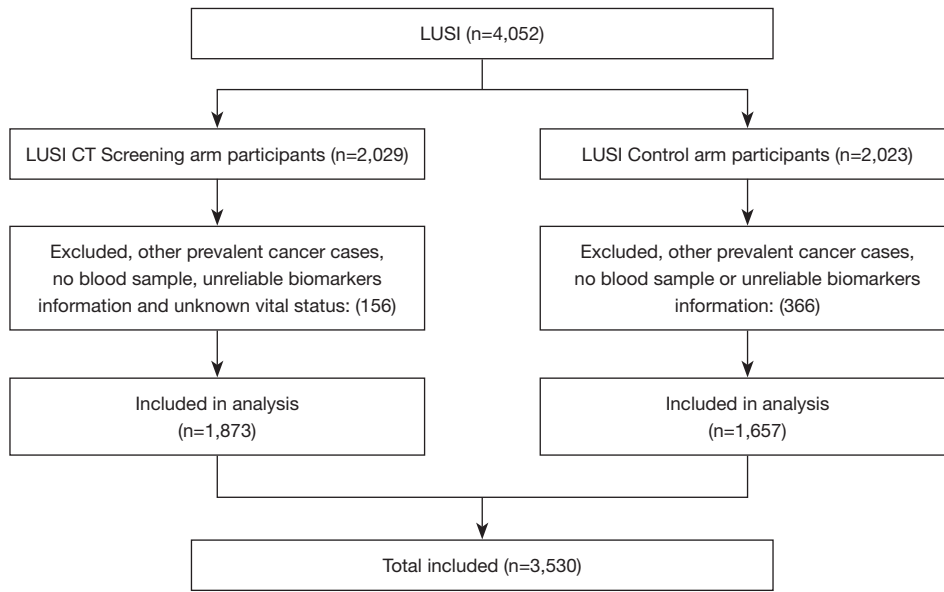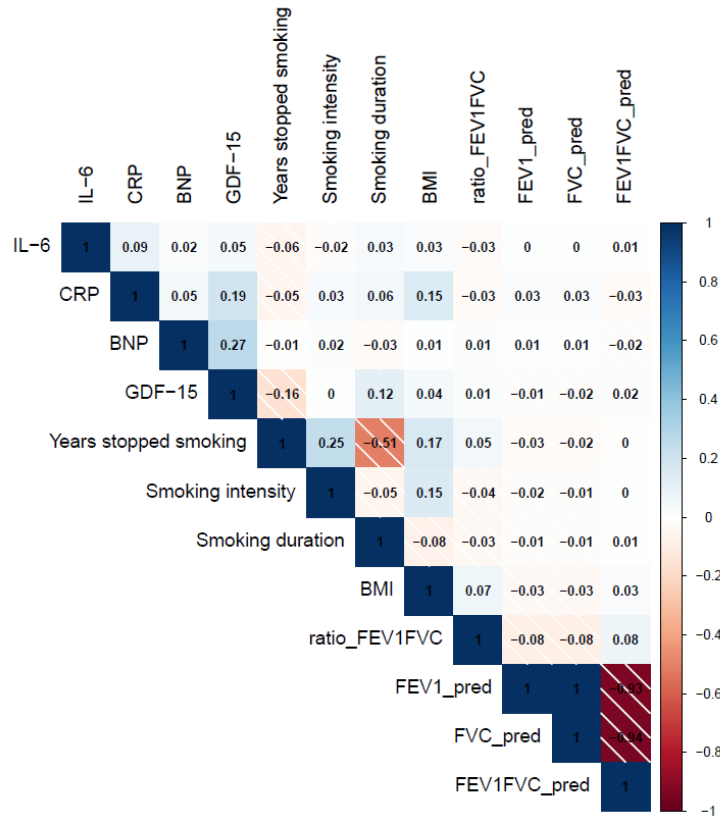**Figure S1** Flowchart, exclusion/inclusion criteria for the present study.

Figure S2 Spearman's partial correlations adjusted by age and sex among plasma levels of interleukin 6 (IL-6), growth/differentiation factor-15 (GDF-15), N-terminal pro-brain natriuretic protein (NT-proBNP) and C-reactive protein (CRP), sociodemographic covariates and smoking history. The calculations were made within the subcohort (n=1,678).

Table S1 Odds Ratios for moderate-severe COPD [stages 2-4] (n=258) and PRISm (n=295) across quartiles of biomarkers from the LUSI cohort [CT screening arm]

Biomarkers		Unadjusted			Adjusted[1]		
		OR	95%CI	P_{trend}	OR	95%CI	P_{trend}
COPD (stages 2-4)							
Quartile 1 GDF-15 ref	n=48						
Quartile 2 GDF-15	n=62	1.34	0.90–2.00		1.12	0.74–1.69	
Quartile 3 GDF-15	n=68	1.49	1.01–2.22		1.09	0.72–1.65	
Quartile 4 GDF-15	n=80	1.81	1.24–2.67	0.002	1.11	0.73–1.70	0.686
Quartile 1 IL-6 ref	n=37						
Quartile 2 IL-6	n=52	1.46	0.94–2.28		1.38	0.88–2.18	
Quartile 3 IL-6	n=81	2.44	1.63–3.73		2.24	1.47–3.47	
Quartile 4 IL-6	n=88	2.71	1.82–4.12	<0.001	2.49	1.63–3.86	<0.001
Quartile 1 CRP ref	n=50						
Quartile 2 CRP	n=60	1.32	0.88–1.98		1.31	0.87–1.99	
Quartile 3 CRP	n=61	1.35	0.91–2.03		1.29	0.85–1.96	
Quartile 4 CRP	n=87	2.06	1.41–3.03	<0.001	1.97	1.33–2.96	0.001
Quartile 1 BNP ref	n=53						
Quartile 2 BNP	n=60	1.20	0.81–1.80		1.05	0.70–1.58	
Quartile 3 BNP	n=64	1.30	0.88–1.93		1.06	0.71–1.59	
Quartile 4 BNP	n=81	1.72	1.18–2.55	0.005	1.19	0.80–1.80	0.419
PRISm							
Quartile 1 GDF-15 ref	n=49						
Quartile 2 GDF-15	n=60	1.26	0.84–1.88		1.23	0.82–1.85	
Quartile 3 GDF-15	n=79	1.76	1.19–2.56		1.69	1.14–2.51	
Quartile 4 GDF-15	n=107	2.50	1.77–3.69	<0.001	2.32	1.58–3.45	<0.001
Quartile 1 IL-6 ref	n=49						
Quartile 2 IL-6	n=63	1.33	0.90–1.99		1.25	0.84–1.87	
Quartile 3 IL-6	n=81	1.79	1.23–2.64		1.54	1.04–2.30	
Quartile 4 IL-6	n=102	2.40	1.67–3.49	<0.001	2.02	1.38–3.00	<0.001
Quartile 1 CRP ref	n=69						
Quartile 2 CRP	n=66	1.10	0.75–1.59		1.00	0.68–1.46	
Quartile 3 CRP	n=69	1.16	0.80–1.68		0.99	0.68–1.46	
Quartile 4 CRP	n=91	1.61	1.14–2.31	0.007	1.32	0.91–1.91	0.146
Quartile 1 NT-proBNP ref	n=74						
Quartile 2 NT-proBNP	n=63	0.89	0.62–1.28		0.89	0.60–1.27	
Quartile 3 NT-proBNP	n=69	0.95	0.66–1.37		0.98	0.68–1.41	
Quartile 4 NT-proBNP	n=89	1.32	0.94–1.86	0.085	1.33	0.92–1.91	0.102

[1], logistic regression models adjusted by age, sex body mass index and smoking history; GDF-15, growth/differentiation factor-15; IL-6, interleukin 6; CRP, C-reactive protein; NT-proBNP, N-terminal pro-brain natriuretic protein.

Table S2 Risk of lung cancer by quartiles of biomarker measurements in the screening arm (n=52), control arm (n=13) and in both arms combined (n=65) for those early stage lung cancer cases

Biomarkers	CT screening arm[1]				Control arm[1]				CT screening arm + control arm[1]			
	$N_{cases}/N_{non-cases}$	OR	95% CI	P_{trend}	$N_{cases}/N_{non-cases}$	OR	95% CI	P_{trend}	$N_{cases}/N_{non-cases}$	OR	95% CI	P_{trend}
Lung cancer cases												
Quartile 1 GDF-15 ref	n=7/564				n=4/308				n=11/872			
Quartile 2 GDF-15	n=14/460	2.04	0.83–5.49		n=2/407	0.31	0.04–1.65		n=16/867	1.20	0.55–2.70	
Quartile 3 GDF-15	n=14/420	2.00	0.80–5.42		n=2/446	0.24	0.03–1.34		n=16/866	1.04	0.47–2.36	
Quartile 4 GDF-15	n=17/377	2.36	0.96–6.42	0.108	n=5/483	0.52	0.12–2.44	0.531	n=22/860	1.25	0.58–2.81	0.67
Quartile 1 IL-6 ref	n=6/463				n=2/415				n=8/878			
Quartile 2 IL-6	n=10/458	1.59	0.58–4.75		n=5/407	2.29	0.48–16.35		n=15/865	1.78	0.76–4.48	
Quartile 3 IL-6	n=16/452	2.51	0.99–7.21		n=3/412	1.44	0.23–11.37		n=19/864	2.22	0.98–5.51	
Quartile 4 IL-6	n=20/448	3.09	1.25–8.79	0.010	n=3/410	1.40	0.21–11.25	0.971	n=23/858	2.64	1.18–6.53	0.022

[1], Cox regression models adjusted by age, sex, Body Mass Index and smoking history. GDF-15, growth/differentiation factor-15; IL-6, interleukin 6.

Table S3 Risk of lung cancer by quartiles of biomarker measurements in the screening arm (n=88) additionally adjusted by lung function impairment (COPD or PRISm)

Biomarkers	$N_{cases}/N_{non-cases}$	CT screening arm[1]		
		OR	95% CI	P_{trend}
Lung cancer cases (n=88)				
Quartile 1 GDF-15 ref	n=11/560			
Quartile 2 GDF-15	n=19/455	1.79	0.84–3.97	
Quartile 3 GDF-15	n=25/409	2.19	1.06–4.79	
Quartile 4 GDF-15	n=33/361	2.84	1.40–6.17	0.001
Quartile 1 IL-6 ref	n=12/457			
Quartile 2 IL-6	n=17/451	1.34	0.63–2.95	
Quartile 3 IL-6	n=28/440	2.03	1.01–4.33	
Quartile 4 IL-6	n=31/437	1.98	0.97–4.25	0.042
Quartile 1 CRP ref	n=19/502			
Quartile 2 CRP	n=15/467	0.80	0.39–1.61	
Quartile 3 CRP	n=29/433	1.72	0.93–3.23	
Quartile 4 CRP	n=25/383	1.42	0.75–2.75	0.022
Quartile 1 NT-proBNP ref	n=24/488			
Quartile 2 NT-proBNP	n=25/480	0.83	0.45–1.51	
Quartile 3 NT-proBNP	n=13/420	0.43	0.21–0.87	
Quartile 4 NT-proBNP	n=26/397	0.70	0.37–1.31	0.121

[1], logistic regression models additionally adjusted by lung function impairment (COPD or PRISm). GDF-15, growth/differentiation factor-15; IL-6, interleukin 6; CRP, C-reactive protein; NT-proBNP, N-terminal pro-brain natriuretic protein.

Table S4 Hazard Ratios for all-cause mortality in the screening arm (n=221) by quartiles of biomarker measurements

Biomarkers	$N_{cases}/N_{non\text{-}cases}$	CT screening arm[1]			CT screening arm[2]		
		HR	95% CI	P_{trend}	HR	95% CI	P_{trend}
All-cause mortality							
Quartile 1 GDF-15 ref	n=31/540						
Quartile 2 GDF-15	n=39/435	1.35	0.82–2.24		1.24	0.75–2.07	
Quartile 3 GDF-15	n=60/374	2.14	1.34–3.47		1.82	1.13–2.97	
Quartile 4 GDF-15	n=91/303	3.75	2.39–6.02	<0.001	3.08	1.94–4.96	<0.001
Quartile 1 IL-6 ref	n=26/443						
Quartile 2 IL-6	n=43/425	1.55	0.93–2.63		1.42	0.85–2.42	
Quartile 3 IL-6	n=62/406	2.13	1.31–3.57		1.71	1.03–2.88	
Quartile 4 IL-6	n=90/378	3.44	2.14–5.67	<0.001	2.60	1.60–4.34	<0.001
Quartile 1 CRP ref	n=49/472						
Quartile 2 CRP	n=44/438	0.92	0.59–1.43		0.89	0.56–1.35	
Quartile 3 CRP	n=61/401	1.47	0.97–2.24		1.37	0.90–2.17	
Quartile 4 CRP	n=67/341	1.81	1.19–2.76	0.001	1.51	0.99–2.33	0.015
Quartile 1 NT-proBNP ref	n=51/461						
Quartile 2 NT-proBNP	n=44/461	0.70	0.45–1.09		0.70	0.44–1.09	
Quartile 3 NT-proBNP	n=42/391	0.71	0.45–1.11		0.70	0.44–1.10	
Quartile 4 NT-proBNP	n=84/339	1.35	0.90–2.05	0.301	1.31	0.86–2.01	0.091

[1], Cox regression models adjusted by age, sex, body mass index and smoking history; [2], Cox regression models adjusted by age, sex, body mass index, smoking history, and spirometry (COPD and PRISm. Abbreviations: GDF-15, growth/differentiation factor-15; IL-6, interleukin 6; CRP, C-reactive protein; NT-proBNP, N-terminal pro-brain natriuretic protein.