**Assessment of lung cancer risk based on a biomarker panel of circulating proteins**

**Front page group name:** The INTEGRAL consortium for early detection of lung cancer

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**Key points:**

**Question:** Can a panel of circulating protein biomarkers improve upon a traditional risk prediction model for lung cancer?

**Findings:** Abiomarker-based risk-prediction model consisting of four protein markers that was developed in a high-risk US cohort outperformed a model based on smoking history alone when blindly validated using pre-diagnostic samples from two European cohorts.

**Meaning:** Biomarker-based risk profiling has the potential to improve eligibility criteria for screening.

**Importance:**  There is an urgent need to improve lung cancer risk assessment as current screening criteria miss a large proportion of cases.

**Objective:** To determine if a panel of selected circulating protein biomarkers can contribute to lung cancer risk assessment and outperform current US screening criteria.

**Design, Setting and Participants:**  Pre-diagnostic samples from ever-smoking cases diagnosed within one year of blood collection and smoking-matched controls from the Carotene and Retinol Efficacy Trial (CARET) cohort were used to develop a biomarker risk-score based on 4 proteins (CA125, CEA, CYFRA 21-1 and Pro-SFTPB). The biomarker score was subsequently validated blindly using absolute risk-estimates in ever-smoking cases diagnosed within one year of blood collection and matched controls from two large European population-based cohorts; the European Prospective Investigation into Cancer and nutrition (EPIC) study and the Northern Sweden Health and Disease Study (NSHDS).

**Main Outcome and Measures**: Model validity in discriminating between future lung cancer cases and controls. Discrimination estimates were weighted to reflect the background populations of EPIC and NSHDS validation studies (area under receiver-operating characteristics curve [AUC], sensitivity and specificity).

**Results:** In the validation study, an integrated risk-prediction model combining smoking exposure with the biomarker score yielded an AUC of 0.83 (95% CI: 0.76-0.90) compared to 0.73 (95% CI: 0.64-0.82) for a model based on smoking exposure alone (P=0.003 for difference in AUC). At an overall specificity of 0.83 based on the USPSTF screening criteria, the sensitivity of the integrated risk-prediction model (biomarker) model was 0.63 compared to 0.43 for the smoking model. Conversely, at an overall sensitivity of 0.42 (USPSTF), the integrated risk-prediction model yielded a specificity of 0.95 compared to 0.86 for the smoking model.

**Conclusions and Relevance:** This study provided a proof-of-principle in demonstrating that a panel of circulating protein biomarkers can improve lung cancer risk assessment and may be used to define eligibility for CT-screening.

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The National Lung Screening Trial (NLST) findings suggested that screening with low-dose computed tomography (LDCT) can reduce lung cancer mortality.1 As a result, the US Preventive Services Task Force (USPSTF) recommends LDCT screening for lung cancer among individuals aged 55-80 years who have smoked 30 pack-years with up to 15 years since quitting smoking.1,2 However, LDCT screening results in a large number of indeterminate nodules,1 and less than 50% of incident lung cancer cases are eligible for screening.3 Biomarkers may improve lung cancer risk assessment over and beyond that of traditional smoking-based risk models and improve current screening eligibility criteria.4,5

We demonstrated that the precursor form of surfactant protein B (Pro-SFTPB) is predictive of lung cancer risk.5,6 Other markers that have been shown to be useful for the work-up and diagnosis of lung cancer include cancer antigen 125 (CA125), cytokeratin-19 fragment (CYFRA 21-1), carcinoembryonic antigen (CEA), and Human Epididymis Protein 4 (HE4).7-12 However, there is limited data regarding their performance in discriminating between future lung cancer cases and controls.

The current study aimed to assess the potential of these 5 protein biomarkers to inform about lung cancer risk when tested blindly using pre-diagnostic samples.

**Methods**

A full account of the methods are provided in the Supplement.

Briefly, samples from ever-smoking lung cancer cases diagnosed within one year following blood collection (N=108) and smoking-matched controls (N=216) from the US CARET cohort were used to develop a biomarker score based on circulating measures of Pro-SFTPB, CA125, CEA, HE4 and CYFRA 21-1 using logistic regression.

The extent to which the biomarker score improved discrimination of incident lung cancer cases and controls was validated externally using ever-smoking lung cancer cases diagnosed within one year following blood collection (N=63) and matched controls (N=90) from the European EPIC and NSHDS cohorts (eFigure 1). Absolute 1-year risks of lung cancer were estimated for each study participant in the validation study by modelling the cumulative hazards of lung cancer using flexible parametric survival models.13 Two models were evaluated, *i)* a traditional smoking history-based risk model, and *ii)* an integrated risk-prediction model combining the smoking model and the biomarker score. Model discrimination was assessed by receiver operating characteristics (ROC) analysis using the predicted 1-year lung cancer risks as scoring rule. Discrimination estimates included area under ROC curve (AUC), sensitivity and specificity that were weighted to reflect the background populations.

Statistical significance was assumed at a two-sided P-value below 0.05. All study participants gave written informed consent to participate in the study and the research was approved by the local ethics committees.

**Results**

Details of the biomarker score and discrimination estimates in the CARET training study are available in eTable 1 and 2, eFigure 2 and 3.

In the validation study, the predicted 1-year risk for a 60-year old male with 30 pack-years of smoking history was estimated at 0.37% using the smoking model (Figure 1). In comparison, using the integrated risk-prediction model we estimated 1-year risks at 0.07% and 1.56% for the same man assuming a biomarker score in the 1st and 4th quartile, respectively. The 1-year lung cancer risk estimates for each study participant in the validation study according to the smoking and integrated risk-prediction models are depicted in Figure 2. In comparison to the smoking model, the median 1-year risk estimates from the integrated risk-prediction model increased for cases from 0.27% to 0.45%, and decreased for controls 0.12% to 0.04%.

In the validation study, the population-weighted AUC was 0.73 (95% CI: 0.64-0.82) for the smoking model and 0.83 (95% CI: 0.76-0.90) for the integrated risk-prediction model (P=0.003 for difference in AUC, Figure 3-A). The AUCs were consistently higher for the integrated model than for the smoking model across relevant strata (eTable 3). At an overall specificity of 0.83 based on the USPSTF screening criteria, the integrated risk-prediction model yielded a sensitivity of 0.63 (95% CI: 0.49-0.76) compared to 0.43 (95% CI: 0.23-0.65) for the smoking model. Similarly, at an overall sensitivity of 0.42 (USPSTF), the integrated risk-prediction model yielded a specificity of 0.95 (95% CI: 0.85-0.99) compared to 0.86 (95% CI: 0.72-0.94) for the smoking model. The improvement in AUC for the integrated risk prediction model (AUC: 0.80, 95% CI: 0.75-0.85) over the smoking model (AUC: 0.73, 95% CI: 0.68-0.79) was more modest when considering cases diagnosed up to 2 years after blood draw (eFigure 4).

A full account of all conducted analyses are provided in the Supplement.

**Discussion**

This is the first study in which a blood-based biomarker score was developed using one cohort and externally validated using pre-diagnostic samples from other independent cohorts. We observed a notable improvement in discrimination between future lung cancer cases and controls over a traditional smoking-based risk-prediction model by incorporating information from a biomarker score consisting of four circulating proteins.

In our validation study, 42% of the incident lung cancer cases would have qualified for CT screening according to USPSTF criteria. Using the biomarker score together with smoking information, we estimated that 63% of *all* cases could be identified, without increasing the number of false positives. The data further suggested that the biomarker score could alternatively be used to reduce screening of subjects not destined to develop lung cancer from 14% to 5%, without affecting the uptake of future lung cancer cases. These improvements in sensitivity and specificity were consistently observed across each evaluated stratum. Our findings also indicated that the improvement in discrimination afforded by the biomarker score is more modest beyond the initial year after blood draw, suggesting that an annual biomarker test may be necessary in a a screening program.

**Strengths and Limitations**

Naïve discrimination estimates, as typically provided in a matched nested case-control setting, are inherently biased. An important strength of our study was the use of absolute risks and population-based discrimination estimates, which are necessary to evaluate the impact of applying a biomarker tool in a practical screening scenario.

A limitation of our study was that three variables originally included in a validated risk prediction model (PLCOM2012) were not available in our validation studies. However, using the original PLCO data, excluding these variables from the PLCOM2012 model only nominally decreased its performance, suggesting that our risk prediction model represented a valid comparison for the biomarkers score (eMethods, eFigure 5).14

Whilst the current study provided a proof-of-principle of the potential of using biomarkers in lung cancer risk assessment to define screening eligibility, validating and calibrating the integrated risk-prediction model using larger sample size with pre-diagnostic samples is clearly needed before such a risk prediction tool can be used in practice. A larger sample size will also allow stratified analysis aiming to evaluate the performance of the biomarker panel in predicting lung cancer cases of different characteristics, in particular by stage at diagnosis and histological subtype. Further, our study was limited to a select panel of circulating proteins, and we note that other types of biomarkers may also be informative.4,5 We also note that the population that would most benefit from a biomarker test prior to undergoing LDCT screening remains to be defined. A thorough cost-effectiveness assessment based on a large study sample is warranted to determine the threshold in absolute risk for developing lung cancer over a specific time-period, above which the benefits of screening outweigh the harms.15

**Conclusion**

The current study provided a proof-of-principle in demonstrating that circulating biomarkers have the potential to inform lung cancer risk assessment and substantially improve upon current criteria for CT-screening.**Acknowledgment**

*Contributions*

SH, MJ, and PBr initiated, acquired the main funding, and designed this investigation. SH,NS, AT, DD, DK, and NP led the laboratory analysis. FG and DM conducted the statistical analysis of EPIC and NSHDS data under the supervision of MJ. LB and QY conducted the statistical analysis of the CARET data under supervision of ZF. MJ, PBr, NS, LB, FG, ZF and SH drafted the first version of the manuscript. All authors were involved with collection of data, data interpretation, critical revisions of the paper, and approval of the final version. SH had had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis. ER is the overall coordinator of the EPIC study, which he designed and implemented in collaboration with the main investigators in the collaborating centers.

*Conflicts of Interest and Financial Disclosures***:** The authors declare no conflict of interest.

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**Figure Legend**

**Figure 1**. 1-year predicted lung cancer probability for a male from NSHDS according to his smoking history.

A: predicted lung cancer probability from the smoking risk prediction model based on age (in years) and smoking history. The “rug plot” shows the observed distribution of age in the validation study (EPIC and NSHDS, ever smokers). B: predicted lung cancer probability from the integrated risk prediction model according to the biomarker score and the smoking history. The “rug plot” shows the observed distribution of the biomarker score in the validation study (EPIC and NSHDS, ever smokers). The vertical lines correspond to the quartiles threshold for biomarker score among controls (Q1, Q2, Q3, Q4).

**Figure 2**. 1-year predicted lung cancer probabilities from the smoking and integrated risk prediction models in the validation study (EPIC and NSHDS, ever smokers).

The validation samples consist of EPIC and NSHDS ever smoking subjects diagnosed within 1 year of blood collection. For the controls, the size of the points is proportional to the number of eligible subjects represented (corresponding to 1/sampling probability). The right panel represents a zoomed version of the full figure.**Figure 3**. Receiver Operating Characteristics (ROC) curve analysis in the validation study (EPIC and NSHDS, ever smokers).

A: ROC curve analysis in the validation study (EPIC and NSHDS ever smoker subjects diagnosed within 1 year of blood collection) for two risk prediction models, smoking variables only, and an integrated model with the smoking variables and the biomarker score combined. B: Sensitivity and specificity in relation to the 1-year predicted lung cancer probability from the integrated model.