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TUMOR MARKERS AND SIGNATURES

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Assessment of the EarlyCDT-Lung test as an early biomarker of lung cancer in ever-smokers: A retrospective nested case-control study in two prospective cohorts

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Abstract

The EarlyCDT-Lung test is a blood-based autoantibody assay intended to identify high-risk individuals for low-dose computed tomography lung cancer screening. However, there is a paucity of evidence on the performance of the EarlyCDT-Lung test in ever-smokers. We conducted a nested case-control study within two prospective cohorts to evaluate the risk-discriminatory performance of the EarlyCDT-Lung test using prediagnostic blood samples from 154 future lung cancer cases and 154 matched controls. Cases were selected from those who had ever smoked and had a prediagnostic blood sample <3 years prior to diagnosis. Conditional logistic regression was used to estimate the association between EarlyCDT-Lung test results and lung cancer risk. Sensitivity and specificity of the EarlyCDT-Lung test were calculated in all subjects and subgroups based on age, smoking history, lung cancer stage, sample collection time before diagnosis and year of sample collection. The overall lung cancer odds ratios were 0.89 (95% CI: 0.34-2.30) for a moderate risk EarlyCDT-Lung test result and 1.09 (95% CI: 0.48-2.47) for a high-risk test result compared to no significant test result. The overall sensitivity was 8.4% (95% CI: 4.6-14) and overall specificity was 92% (95% CI: 87-96) when considering a high-risk result as positive.

Abbreviations: CI, confidence interval; ELISA, enzyme-linked immunosorbent assay; EPIC, European Prospective Investigation into Cancer and Nutrition; ICER, incremental cost-effectiveness ratio; LDCT, low-dose computed tomography; NELSON, Nederlands Leuvens Longkanker Screenings Onderzoek; NLST, National Lung Screening Trial; NSHDS, Northern Swedish Health and Disease Study; OR, odds ratio.

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Stratified analysis indicated higher sensitivity (17%, 95% CI: 7.2-32.1) in subjects with blood drawn up to 1 year prior to diagnosis. In conclusion, our study does not support a role of the EarlyCDT-Lung test in identifying the high-risk subjects in ever-smokers for lung cancer screening in the EPIC and NSHDS cohorts.

KEYWORDS
biomarkers, EarlyCDT-Lung test, lung cancer, prediagnostic samples

What's new?

Low-dose computed tomography (LDCT) is a promising tool for the early detection of lung cancer. To improve its effectiveness, prescreening to identify individuals with high lung cancer risk may be necessary. Here, the authors examined the prescreening utility of the commercially available blood-based autoantibody EarlyCDT®-Lung test. Analysis of prediagnostic blood samples from cases and controls reveals low sensitivity for the EarlyCDT®-Lung test, with limited evidence supporting an association between a positive test result and lung cancer risk. The results indicate that the EarlyCDT®-Lung test is unlikely to be useful for identifying ever smokers with an elevated risk of lung cancer.

1 | INTRODUCTION

Lung cancer causes approximately 2 million deaths annually and is the most common cause of cancer death worldwide.[1] The prognosis for newly diagnosed lung cancer cases is overall poor and strongly influenced by disease stage at the primary diagnosis, with dismal survival rates for cases diagnosed at late stage.

Early detection is the most important strategy for improving lung cancer survival rates. With the intention of reducing lung cancer mortality through screening of high-risk populations, several randomized trials have now demonstrated the efficacy of screening with low-dose computed tomography (LDCT). The largest lung cancer screening study to date was the National Lung Screening Trial (NLST), which included 53 454 high-risk individuals and showed a 20% reduction in lung cancer mortality in subjects who were screened by LDCT compared to chest radiography.[2] The recently published Nederlands-Leuvens Longkanker Screenings Onderzoek (NELSON) study included 15 789 participants who were randomized to LDCT screening or no screening.[3] The NELSON study reported a 25% reduction in lung cancer mortality in the screened group compared to the control group in the high-risk population at 10 years of follow-up.[3] However, the screening studies have also highlighted several negative aspects associated with screening, including those related to invasive work-up and treatment of benign nodules and morbidity associated with potential overdiagnosis.[2,4] It is important to avoid screening people at low risk for lung cancer, as they may experience more harm than benefit due to potential work-up of benign nodules and cumulative radiation exposure during screening.

All lung cancer screening programs have focused on high-risk individuals who are identified primarily based on their tobacco exposure history. In many European countries, the recent results of the NELSON study have been awaited before a decision on implementation of national lung cancer screening programs is made. While lung cancer remains the most common cause of cancer death, smoking has decreased steadily over several decades which presents a challenge for identifying the target population.

Risk biomarkers may improve LDCT screening efficacy at least two key junctions; (a) in defining screening eligibility by providing more accurate information on lung cancer risk, thus improving the identification of high-risk individuals who are more likely to benefit from screening, and (b) in the diagnostic work-up of screenees who have presented with indeterminant or high-risk nodules on LDCT. The EarlyCDT-Lung test which measures a panel of circulating autoantibodies represents one of few commercial products available and is advertised as a tool to improve eligibility criteria for LDCT screening.[5-7] Whilst initial studies have shown some promising results consistent with high specificity and acceptable sensitivity, no studies have evaluated the risk-discriminatory performance of the EarlyCDT-Lung test in ever smoking individuals from the broad spectrum of lung cancer risk experienced in the general population. We aimed to evaluate the EarlyCDT-Lung test as a prescreening assessment tool in the general population of ever-smokers using prediagnostic blood samples.

2 | MATERIALS AND METHODS

2.1 | Study design

We conducted a nested case-control study within two prospective cohorts, European Prospective Investigation into Cancer and Nutrition (EPIC) and Northern Sweden Health and Disease Study (NSHDS). EPIC is a large cohort study, which enrolled over 521 000 participants between 1992 and 1999 from 23 centers in 10 European countries.[8] Detailed descriptions of study design, recruitment method, blood collection protocols and follow-up procedures have been published.[8,9] Information on lifestyle characteristics, including detailed smoking history was collected at recruitment.

Blood samples were stored in a central biorepository in liquid nitrogen (−196°C) at the International Agency for Research on Cancer—World Health Organization. NSHDS is an ongoing population-based cohort built up by three substudies, the Västerbotten Intervention Project, the Northern Sweden MONICA project and the Mammography screening project. Participants were asked to fill out an extensive questionnaire covering lifestyle, diet and health, and were invited to donate a blood sample at each visit. Blood sample was stored at −80°C at the Northern Sweden Biobank (Biobanken Norr) in Umeå, Sweden.

We first identified the incident lung cancer cases (ICD-O-2, C34) diagnosed between 1992 and 2013 from EPIC and between 1989 and 2017 from NSHDS among ever-smoking participants. Cases who had blood samples drawn <3 years prior to diagnosis were selected to evaluate the performance of the EarlyCDT-Lung test. For each case, one matched control who was alive and free of cancer (except nonmelanoma skin cancer) at the time of diagnosis of the index case was randomly selected. The matching criteria were cohort, study center, sex, date of blood collection (±1 month, relaxed to ±3 months for sets without available controls), date of birth (±1 year, relaxed to ±3 years) and smoking status in four categories: former smokers with <10 or ≥10 years since quitting, and current smokers with <15 or ≥15 cigarettes smoked per day. The final study sample included 154 cases and 154 matched controls.

2.2 | EarlyCDT-Lung immunoassay

The Oncimmune EarlyCDT-Lung enzyme-linked immunosorbent assay (ELISA) Lung test were purchased from the manufacturer (Oncimmune LTD, UK) who provided a protocol on how to perform the assay, but had no role in the design, analysis, interpretation, or writing of the study. Briefly, 20 μL of plasma was diluted and loaded onto wells already precoated with each of seven antigens (CAGE, GBU4-5, HuD, MAGE A4, NY-ESO-1, p53 and SOX2) and a control protein (VOL) at two dilutions (50 nM and 160 nM) followed by an incubation for 90 minutes at room temperature. The plates were washed and incubated further with secondary antibody for 60 minutes, followed by a series of washing steps and addition of substrate. The plate was then measured using the SpectraMAX i3x (Molecular Devices, San Jose, California) spectrophotometer at the wavelength of 650 nm. Each test was performed with the provided control samples and the raw optical densities were processed using the EarlyCDT-Lung test kit result calculation software (Oncimmune). The software reported each autoantibody as either "high," "moderate" or "no-significant" levels based on marker-specific cut-off values. Based on the manufacturer's recommendations, research participants with one or more autoantibodies above the high cut-off value were defined as high risk, whereas research participants with at least one autoantibody above the moderate cut-off (but none above the high cut-off) were defined as moderate risk. Research participants with all autoantibodies below the moderate threshold were categorized as no significant test result.

2.3 | Statistical analyses

We initially used conditional logistic regression to estimate odds ratios (OR) of developing lung cancer following a moderate or high EarlyCDT-Lung test. The validity of the EarlyCDT-Lung test was subsequently evaluated by estimating its sensitivity and specificity in discriminating lung cancer cases from controls. The sensitivity was estimated as the fraction of incident lung cancer cases defined as high or moderate risk based on the EarlyCDT-Lung test. The specificity was estimated as the fraction of control subjects who were not defined as high or moderate risk based on the EarlyCDT-Lung test. We calculated exact binomial (Clopper-Pearson) 95% confidence intervals (CIs) for the sensitivity and specificity estimates. The Fisher exact test was used to test the equivalence of sensitivities between two subgroups (eg, former smoker and current smoker). All analyses were performed using R software for statistical computing version 4.0.4.

3 | RESULTS

There were 154 cases and 154 matched controls in our study. In total, 90 cases (58.4%) were enrolled from the EPIC study and 64 cases (41.6%) were from the NSHDS study. Table 1 and Table S1 show the characteristics of included study participants. The average age at lung cancer diagnosis was 59.2 years (range: 39-78 years), 60% were men, 25% were diagnosed with stage I or stage II disease, 33% were diagnosed with adenocarcinoma and 17% were diagnosed with squamous cell carcinoma. The median time between blood draw and lung cancer diagnosis was 1.88 years, and ranged between 0 and 3 years by study design.

The OR of lung cancer in the overall study sample was estimated at 0.89 (95% CI: 0.34-2.30) following a moderate risk EarlyCDT-test result, and 1.09 (95% CI: 0.48-2.47) following a high-risk EarlyCDT-Lung test result, compared to no significant autoantibody levels (Table 2). The sensitivity of the EarlyCDT-Lung test in predicting lung cancer cases was 8.4% (95% CI: 4.6-14) when considering a high-risk result as positive, and the corresponding specificity was 92% (95% CI: 87-96) (Table 3). When also considering moderate risk as positive, the sensitivity was 14% (95% CI: 8.6-20) and the specificity was 86% (95% CI: 80-91.4). Stratified analysis (Table 3) indicated a sensitivity for a high-risk test result of 17% in blood drawn up to 1 year prior to diagnosis (95% CI: 7.2-32.1), compared to a sensitivity of 5.3% in blood drawn 1 to 3 years prior to diagnosis (95% CI: 2.0-11.2, *P* for difference: .04). We also observed some suggestive evidence that the sensitivity of the EarlyCDT-Lung test was higher in EPIC than in NSHDS, in former smokers, and in older study participants (Table 3). The characteristics of participants in two cohorts and the performance of EarlyCDT-Lung test in each cohort were shown in Tables S1-S3. We further sought to evaluate if the sample storage time influenced the EarlyCDT-Lung test sensitivity by stratifying by year of sample collection (Table 3), as well as visually (Figure S1), but did not observe any evidence that storage time influenced the results.

TABLE 1 Characteristics of lung cancer cases and controls in the European Prospective Investigation into Cancer and nutrition (EPIC) and Northern Sweden Health and Disease Study (NSHDS) cohorts

Characteristics	Cases	Controls
Cohort, n (%)		
EPIC	90 (58.4)	90 (58.4)
NSHDS	64 (41.6)	64 (41.6)
Age at diagnosis (years), mean (SD)	59.2 (7.42)	—
BMI (kg/m^2), mean (SD)	25.5 (3.85)	26.4 (4.12)
Sex, n (%)		
Male	92 (59.7)	92 (59.7)
Female	62 (40.3)	62 (40.3)
Education, n (%)		
Less than high-school	96 (62.3)	90 (58.4)
High-school graduate	40 (26.0)	38 (24.7)
Bachelor's degree or higher	18 (11.7)	26 (16.9)
Calendar year of sample collection, n (%)		
1988-1995	58 (37.7)	59 (38.3)
1996-2016	96 (62.3)	95 (61.7)
Sample collection time before diagnosis, n (%)		
≤1 year	41 (26.6)	
1-2 years	47 (30.5)	
2-3 years	66 (42.9)	
Age at sample collection, n (%)		
≤60 years	102 (66.2)	102 (66.2)
>60 years	52 (33.8)	52 (33.8)
Stage, n (%)		
I/II	19 (25.3)	
III	26 (34.7)	
IV	30 (40.0)	
Unknown	79	
Histology, n (%)[a]		
Squamous cell carcinoma	26 (16.9)	
Small cell carcinoma	24 (15.6)	
Adenocarcinoma	50 (32.5)	
Large cell carcinoma	2 (1.3)	
Others	52 (33.8)	
Smoking status, n (%)		
Former	54 (35.1)	56 (36.4)
Current	100 (64.9)	98 (63.6)
Smoking history, n (%)		
<20 pack-years	45 (29.2)	71 (46.1)
≥20 pack-years	109 (70.8)	83 (53.9)

[a]The histopathological classification of lung cancer is according to WHO classification.

4 | DISCUSSION

Our study represents the first evaluation of the validity of the EarlyCDT-Lung test in discriminating future lung cancer cases in pre-diagnostic samples from general population cohorts. Based on pre-diagnostic samples drawn up to 3 years prior to diagnosis with matched controls from two population cohort studies, we did not observe any clear evidence that the EarlyCDT-Lung test can predict incident lung cancer among ever smokers in a time period of relevance when treatment can improve survival.

Lung cancer screening with LDCT of high-risk individuals represents one of the most promising means to decrease lung cancer mortality, and is now being introduced in several countries. An important challenge in LDCT screening is how to identify individuals who are at sufficiently high risk of lung cancer to benefit from screening. Current eligibility criteria, mainly based on age and smoking habits, miss a large fraction of incident lung cancers. Complementary tools, such as circulating risk biomarkers, may be useful to inform eligibility criteria by improving risk discrimination, as well as to inform nodule management following a positive LDCT-screening test. The EarlyCDT-Lung test is one of few commercially available biomarker kits and is advertised as a tool to identify people at high lung cancer risk for triage into LDCT screening. The tool has been evaluated in several settings with promising results,[7,10,11] but two recent studies indicated an insufficient sensitivity of the EarlyCDT-Lung test to be used as a prescreening tool for selecting high-risk individuals for LDCT screening; in the German Lung Cancer Screening Intervention trial (LUSI)[12] a sensitivity of 13% was estimated in patients detected by LDCT and a recently published Danish study estimated a sensitivity of 33% in patients referred to hospital for suspected lung cancer.[13]

To date, no study has evaluated the EarlyCDT-Lung test in a study population reflective of the general population of ever-smokers. To address this question, we applied the EarlyCDT-Lung test in two European nested case-control studies with blood samples drawn up to 3 years prior to diagnosis. We estimated the sensitivity of the EarlyCDT-Lung test to be 8% if a high risk test result was considered as positive and 14% if a moderate-risk test result was considered as positive To test the performance of the EarlyCDT-Lung test in a subgroup of participants that would be eligible for LDCT screening based on current recommendations, we performed a sensitivity analysis in subjects aged more than 50 and at least a 20 pack-year smoking history. The sensitivity of the EarlyCDT-Lung test in our study was 8.3% (95% CI: 3.7-15.8) or 13.5% (95% CI: 7.4-22) when considering high level or high/moderate level of test results as positive, a result in line with the findings from the German LUSI (13%).[12] The Danish study showed that the overall sensitivity was 33% in subjects with a suspicion of lung cancer by their general practitioners but lower sensitivities were found in early-stage cancers (21%) or cancers diagnosed before age 60 (11%).[13] It indicated the limitation of using the EarlyCDT-Lung test as a pre-screening tool for triage. Interestingly, we also observed that the sensitivity seems to increase when time of

TABLE 2 Odds ratios (ORs) and 95% confidence intervals (CIs) for lung cancer in relation to EarlyCDT-Lung test results in different subgroups

	High vs no significant		Moderate vs no significant	
	OR (95% CI)	*P*-value	OR (95% CI)	*P*-value
Overall	1.09 (0.48-2.47)	.84	0.89 (0.34-2.30)	.81
Cohort				
EPIC	1.67 (0.61-4.59)	.32	0.83 (0.25-2.73)	.76
NSHDS	0.40 (0.08-2.06)	.27	1.00 (0.20-4.95)	1.00
Sex				
Male	0.75 (0.26-2.16)	.59	0.71 (0.23-2.25)	.57
Female	2.00 (0.50-8.00)	.33	1.50 (0.25-8.98)	.66
Smoking status				
Former smokers	1.50 (0.42-5.32)	.53	2.00 (0.37-10.92)	.42
Current smokers	0.83 (0.25-2.73)	.76	0.57 (0.17-1.95)	.37
Smoking history				
<20 pack-years	0.40 (0.08-2.06)	.27	1.00 (0.14-7.10)	1.00
≥20 pack-years	2.50 (0.49-12.89)	.27	0.67 (0.19-2.36)	.53
Sample collection time before diagnosis				
≤1 year	2.33 (0.60-9.02)	.22	0.67 (0.11-3.99)	.66
>1 year	0.63 (0.20-1.91)	.41	1.00 (0.32-3.10)	1.00
Age at sample collection				
≤60 years	0.56 (0.19-1.66)	.29	1.17 (0.39-3.47)	.78
>60 years	6.00 (0.72-49.84)	.10	0.50 (0.05-5.51)	.57
Age at diagnosis				
≤60 years	0.67 (0.19-2.36)	.53	0.80 (0.21-2.98)	.74
>60 years	1.60 (0.52-4.89)	.41	1.00 (0.25-4.00)	1.00
Calendar year of sample collection				
1988-1995	0.83 (0.25-2.73)	.76	2.50 (0.49-12.89)	.27
1996-2016	1.20 (0.37-3.93)	.76	0.43 (0.11-1.66)	.22

Abbreviations: EPIC, European Prospective Investigation into Cancer and Nutrition; NSHDS, Northern Sweden Health and Disease Study.

blood draw approaches the time of diagnosis, like in the Early Diagnosis of Lung Cancer Scotland (ECLS) study.[11] However, the findings of the current study do not support the good performance of EarlyCDT-Lung test as shown in the ECLS trial with a sensitivity of 45.5% (95% CI: 28.1-63.6) at 1 year after randomization. It should be noticed that the ECLS trial is designed to evaluate the EarlyCDT-Lung test plus LDCT to standard clinical care. Although the results demonstrated a significant reduction of advanced lung cancer cases, the question of using the EarlyCDT-Lung test for triage still remains. Furthermore, our study showed that the sum of sensitivity and specificity was equal to 1, indicating that the test is noninformative for lung cancer case status. Even though we observed some evidence for a higher sensitivity in samples drawn within 1 year of diagnosis, these discrimination estimates would not seem to justify the use of the EarlyCDT-Lung test as prescreening eligibility criteria. Indeed, we observed limited evidence that a positive EarlyCDT-Lung test is associated with lung cancer risk in the overall study sample.

The cost-effectiveness of EarlyCDT-Lung was evaluated by Sutton et al who estimated that an acceptable incremental cost-effectiveness ratio (ICER) was GBP 2417.[14] However, the validity of the ICER estimate is directly dependent on the assumptions of the sensitivity and specificity. Sutton et al assumed that EarlyCDT-Lung test has sensitivity of 41% and specificity of 93% based on the findings from Healey et al.[15] Considering the notable differences in sensitivity and our study which is more likely to reflect the sensitivity of the EarlyCDT-Lung test in the general population—the intended target population, an updated cost-effectiveness analysis would seem warranted.

Our study has potential limitations. First, A potential caveat in evaluating early detection biomarkers in prospective cohorts such as EPIC and NSHDS is that the biospecimens have been stored for many years prior to analysis, and may as such, be compromised. It has been shown that autoantibodies are highly stable proteins in circulation and are not subject to proteolysis.[16,17] Sossi described the potential of using frozen serum samples to measure the p53 autoantibody.[16] However, the long-term stability of autoantibodies has not been examined. We evaluated the potential impact of storage time for EarlyCDT-Lung test by performing stratified analysis by calendar year

TABLE 3 Performance of the EarlyCDT-Lung test in different subgroups

	High level as positive			High/Moderate level as positive		
	Sensitivity; n (95% CI)	*P*-value[a]	Specificity; n (95% CI)	Sensitivity; n (95% CI)	*P*-value[a]	Specificity; n (95% CI)
Overall	8.4%; 13/154 (4.6-14.0)	—	92.2%; 142/154 (86.8-95.9)	13.6%; 21/154 (8.6-20.1)	—	86.4%; 133/154 (79.9-91.4)
Cohort						
EPIC	11.1%; 10/90 (5.5-19.5)	.24	93.3%; 84/90 (86.1-97.5)	16.7%; 15/90 (9.6-26.0)	.24	86.7%; 78/90 (77.9-92.9)
NSHDS	4.7%; 3/64 (1.0-13.1)		90.6%; 58/64 (80.7-96.5)	9.4%; 6/64 (3.5-19.3)		85.9%; 55/64 (75.-93.4)
Sex						
Male	6.5%; 6/92 (2.4-13.7)	.38	91.3%; 84/92 (83.6-96.2)	12.0%; 11/92 (6.1-20.4)	.48	83.7%; 77/92 (74.5-90.6)
Female	11.3%; 7/62 (4.7-21.9)		93.5%; 58/62 (84.3-98.2)	16.1%; 10/62 (8.0-27.7)		90.3%; 56/62 (80.1-96.4)
Smoking status						
Former smokers	11.1%; 6/54 (4.2-22.6)	.38	92.9%; 52/56 (82.7-98.0)	18.5%; 10/54 (9.3-31.4)	.22	89.3%; 50/56 (78.1-96)
Current smokers	7.0%; 7/100 (2.9-13.9)		91.8%; 90/98 (84.5-96.4)	11.0%; 11/100 (5.6-18.8)		84.7%; 83/98 (76.0-91.2)
Smoking history						
<20 pack-years	6.7%; 3/45 (1.4-18.3)	.76	88.7%; 63/71 (79.0-95.0)	13.3%; 6/45 (5.1-26.8)	1.00	85.9%; 61/71 (75.6-93.0)
≥20 pack-years	9.2%; 10/109 (4.5-16.2)		95.2%; 79/83 (88.1-98.7)	13.8%; 15/109 (7.9-21.7)		86.7%; 72/83 (77.5-93.2)
Stage						
I/II	10.5%; 2/19 (1.3-33.1)	.64		21.1%; 4/19 (6.1-45.6)	.26	
III/IV	7.1%; 4/56 (2.0-17.3)			10.7%; 6/56 (4.0-21.9)		
Sample collection time before diagnosis						
≤1 year	17.1%; 7/41 (7.2-32.1)	.04	92.7%; 38/41 (80.1-98.5)	22.0%; 9/41 (10.6-37.6)	.11	85.4%; 35/41 (70.8-94.4)
>1 year	5.3%; 6/113 (2.0-11.2)		92.0%; 104/113 (85.4-96.3)	10.6%; 12/113 (5.6-17.8)		86.7%; 98/113 (79.1-92.4)
Age at sample collection						
≤60 years	5.9%; 6/102 (2.2-12.4)	.13	91.8%; 101/110 (85.0-96.2)	12.7%; 13/102 (7.0-20.8)	.63	85.5%; 94/110 (77.5-91.5)
>60 years	13.5%; 7/52 (5.6-25.8)		93.2%; 41/44 (81.3-98.6)	15.4%; 8/52 (6.9-28.1)		88.6%; 39/44 (75.4-96.2)
Age at diagnosis						
≤60 years	6.0%; 4/67 (1.7-14.6)	.39	91.0%; 61/67 (81.5-96.6)	11.9%; 8/67 (5.3-22.2)	.64	83.6%; 56/67 (72.5-91.5)
>60 years	10.3%; 9/87 (4.8-18.7)		93.1%; 81/87 (85.6-97.4)	14.9%; 13/87 (8.2-24.2)		88.5%; 77/87 (79.9-94.3)
Calendar year of sample collection						
1988-1995	10.3%; 6/58 (3.9-21.2)	.56	88.1%; 52/59 (77.1-95.1)	19.0%; 11/58 (9.9-31.4)	.15	84.7%; 50/59 (73.0-92.8)
1996-2016	7.3%; 7/96 (3.0-14.4)		94.7%; 90/95 (88.1-98.3)	10.4%; 10/96 (5.1-18.3)		87.4%; 83/95 (79.0-93.3)

[a]*P*-value comparing the difference of sensitivity between the levels of subgroups.

of sample collection and did not observe any indication that samples stored longer had poorer sensitivity (Table 3; Figure S1). We also note that samples from NSHDS have successfully been used in the past to identify and develop biomarkers that are now used in clinical practice, such as anti-CCP in rheumatoid arthritis.[18,19] Second, the results of subgroup analyses need to be interpreted with caution due to small sample sizes. Third, our study cannot inform on the potential benefit of using the EarlyCDT-Lung test in the management of solitary pulmonary nodules on LDCT scans. Finally, it is possible that the EarlyCDT-Lung test has a higher sensitivity very close to clinical diagnosis, and whilst we observed some evidence in support of this hypothesis, our limited sample size did not allow for a conclusive analysis stratified for cases that were diagnosed very soon after their blood draw (eg, <6 months).

One unanticipated finding was that higher sensitivity (16.7% vs 9.4%) was observed in the EPIC cohort than in the NSHDS cohort (Tables S2 and S3), especially in subjects with blood sample collection within 1 year before diagnosis (30.8% vs 6.7%). Compared to the EPIC cohort, NSHDS enrolled more subjects with recently collected samples, female sex, lower education and light smoking history. However, we do not observe any significant difference in the sensitivity or specificity between two cohorts. One notable factor is that the EPIC and NSHDS samples were stored in different temperatures. However, further studies are needed to investigate the stability of autoantibodies on the storage time and temperature.

In conclusion, our study does not support a role of the EarlyCDT-Lung test in predicting incident lung cancer in the general population of ever-smokers up to 3 years prior to diagnosis. This observation leads us to conclude that the EarlyCDT-Lung test is unlikely to be a useful tool in identifying individuals with elevated risk of lung cancer to be enrolled in lung cancer screening. The study does not inform on the potential of the EarlyCDT-Lung test in the management of pulmonary nodules detected on LDCT. We encourage future studies that aim to develop biomarkers for early detection of lung cancer but stress the importance of using a study design that reflect the intended target population in the development and validation phases.

AUTHOR CONTRIBUTIONS

Wendy Yi-Ying Wu: Formal analysis; data curation; visualization; writing—original draft preparation; writing—review and editing; **Zahra Haider:** Data curation; investigation; writing—review and editing; **Xiaoshuang Feng:** Validation; data curation; investigation; writing—review and editing; **Alicia K. Heath:** Resources; writing—review and editing; **Anne Tjønneland:** Resources; writing—review and editing; **Antonio Agudo:** Resources; writing—review and editing; **Giovanna Masala:** Resources; writing—review and editing; **Hilary A. Robbins:** Resources; writing—review and editing; **María-José Huerta:** resources; writing—review and editing; **Marcela Guevara:** Resources; writing—review and editing; **Matthias B. Schulze:** Resources; writing—review and editing; **Miguel Rodriguez-Barranco:** Resources; writing—review and editing; **Paolo Vineis:** Resources; writing—review and editing; **Rosario Tumino:** Resources; writing—review and editing; **Rudolf Kaaks:** Resources; writing—review and editing; **Renée T. Fortner:** resources; writing—review and editing; **Sabina Sieri:** Resources; writing—review and editing; **Salvatore Panico:** resources; writing—review and editing; **Therese Haugdahl Nøst:** Resources; writing—review and editing; **Torkjel M. Sandanger:** Resources; writing—review and editing; **Tonje Braaten:** Resources; writing—review and editing; **Mattias Johansson:** Conceptualization; writing—original draft preparation; writing—review and editing; supervision; funding acquisition; **Beatrice Melin:** Conceptualization; writing—original draft preparation; writing—review and editing; supervision; funding acquisition; **Mikael Johansson:** Conceptualization; resources; writing—original draft preparation; writing—review and editing; supervision; funding acquisition. The work reported in the article has been performed by the authors, unless clearly specified in the text.

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CONFLICT OF INTEREST

The authors have no conflicts to report.

DATA AVAILABILITY STATEMENT

The data that support the findings of our study are available from the corresponding author upon request.

ETHICS STATEMENT

Our study was conducted according to the principles expressed in the Declaration of Helsinki. Approval for the study (Dnr 2019-05964) was obtained from the ethical review boards of the International Agency for Research on Cancer and from all participating EPIC centres. All EPIC and NSHDS participants provided written informed consent at baseline for use of their blood samples and data in future research.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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