Circulating tumour cells as a potential biomarker for lung cancer screening: a prospective cohort study

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Summary

Background Lung cancer screening with low-dose chest CT (LDCT) reduces the mortality of eligible individuals. Blood signatures might act as a standalone screening tool, refine the selection of patients at risk, or help to classify undetermined nodules detected on LDCT. We previously showed that circulating tumour cells (CTCs) could be detected, using the isolation by size of epithelial tumour cell technique (ISET), long before the cancer was diagnosed radiologically. We aimed to test whether CTCs could be used as a biomarker for lung cancer screening.

Methods We did a prospective, multicentre, cohort study in 21 French university centres. Participants had to be eligible for lung cancer screening as per National Lung Screening Trial criteria and have chronic obstructive pulmonary disease with a fixed airflow limitation defined as post-bronchodilator FEV1/FVC ratio of less than 0·7. Any cancer, other than basocellular skin carcinomas, detected within the previous 5 years was the main exclusion criterion. Participants had three screening rounds at 1-year intervals (T0 [baseline], T1, and T2), which involved LDCT, clinical examination, and a blood test for CTCs detection. Participants and investigators were masked to the results of CTC detection, and cytopathologists were masked to clinical and radiological findings. Our primary objective was to test the diagnostic performance of CTC detection using the ISET technique in lung cancer screening, compared with cancers diagnosed by final pathology, or follow up if pathology was unavailable as the gold standard. This study is registered with ClinicalTrials.gov identifier, number NCT02500693.

Findings Between Oct 30, 2015, and Feb 2, 2017, we enrolled 614 participants, predominantly men (437 [71%]), aged 65·1 years (SD 6·5), and heavy smokers (52·7 pack-years [SD 21·5]). 81 (13%) participants dropped out between baseline and T1, and 56 (11%) did between T1 and T2. Nodules were detected on 178 (29%) of 614 baseline LDCTs. 19 participants (3%) were diagnosed with a prevalent lung cancer at T0 and 19 were diagnosed with incident lung cancer (15 (3%) of 533 at T1 and four (1%) of 477 at T2). Extrapulmonary cancers were diagnosed in 27 (4%) of participants. Overall 28 (2%) of 1187 blood samples were not analysable. At baseline, the sensitivity of CTC detection for lung cancer detection was 26·3% (95% CI 11·8–48·8). ISET was unable to predict lung cancer or extrapulmonary cancer development.

Interpretation CTC detection using ISET is not suitable for lung cancer screening.

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Introduction

Lung cancer is the leading cause of cancer deaths worldwide. However, detection at an early stage can reduce mortality. In this regard, the national lung screening trial (NLST) reported a 20% reduction in lung cancer mortality after annual screening when using low-dose chest CT (LDCT) compared with annual chest radiography.[1] The Dutch-Belgian NELSON trial confirmed this result by showing that LDCT lung cancer screening reduced mortality.[2] The US Preventive Services Task Force (UPSTF) issued recommendations for lung cancer screening, and health insurance companies in the USA now reimburse LDCT for individuals meeting the NLST criteria. Despite this approach, the uptake of lung cancer screening remains poor[3,4] due, in particular, to the high number of false positives.[1,3] Implementation of lung cancer screening can be improved by refining the selection criteria of individuals undergoing screening and by developing novel blood signatures.[5] These blood biomarkers could become part of the lung cancer screening strategy; they could be used as a standalone screening tool,[6] as a marker to target the optimal population to be screened,[7–9] and as a complementary marker to guide the action to be taken towards screening-detected pulmonary nodules.[7–10]

We previously showed that circulating tumour cells (CTCs) can be isolated from peripheral venous blood from patients with early stage lung cancer undergoing

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See Online for appendix

Research in context

Evidence before this study

Low-dose chest CT lung cancer screening has been shown to reduce lung cancer mortality. However, implementation of this screening is hampered by the high number of false positives. To improve the performance of screening tools, tumour-derived blood biomarkers have been tested in patients at risk, particularly as part of screening programmes. Some biomarkers, such as circulating free DNA, microRNA, protein panels, or circulating tumour cells (CTCs) have shown promising results. In a previous observational study, we detected CTCs using the isolation by size of epithelial tumour cells technique (ISET), long before lung cancer was diagnosed radiologically. We carried out a literature search in MEDLINE through PubMed and Embase from their inception date to June 1, 2015, with the keywords "lung cancer", "early detection", "screening", "predictive", "biomarker", "circulating tumour cell", and "liquid biopsy". At the time of initiation of this study there were no published data regarding the use of CTCs as a biomarker for lung cancer screening.

Added value of this study

The AIR study is a prospective, multicentre, cohort study done in 21 university centres in France and is the largest cohort trial to test the performance of ISET as a lung cancer screening tool. However, this technique was not sufficiently reliable to recommend use for lung cancer screening, detection of interval cancers, characterisation of pulmonary nodules or prediction of the occurrence of lung cancer. The rate of lung cancer detection in our population was high, compared with other cohorts, reaching as high as 3·1% prevalent lung cancers and 2·8% 1-year incident lung cancers.

Implications of all the available evidence

The detection of lung cancer using blood biomarkers is still in progress. Many teams have tested various biomarkers with mixed results. It is likely that biological signatures alone will not be sufficient for screening. The solution might lie in integrating a triple clinical, biological, and radiological signature into lung screening programmes. Patients with chronic obstructive pulmonary disease have a particularly high risk of developing lung cancer and should be given special attention in screening programmes.

surgical resection when using the isolation by size of epithelial tumour cell technique (ISET).[11] Moreover, in a series of 168 patients with COPD and therefore at high risk of lung cancer, extracted from the Nice University Hospital human biobank (Nice, France, biobank BB-003-0002), five (3%) developed lung cancer 1–4 years after circulating non-haematological cells with malignant features (CNHC-malignant) had been detected in their blood.[12] On the basis of these preliminary results we hypothesised that blood biomarkers such as CTCs could play a role in lung cancer screening.

The objective of this study was to assess whether CTC detection could be used as a standalone screening tool. Therefore, we launched a national prospective study (the AIR study) to evaluate the performance of CTC search for early detection of lung cancer in a population of patients at high risk with COPD.

Methods

Study design and participants

The AIR study is a prospective, multicentre, cohort study done in 21 university centres in France.

To participate, volunteers had to satisfy the NLST-UPSTF criteria (aged 55–74 years, a 30 or more pack-year smoking history, and a current smoking status or having quit in the last 15 years)[1] plus have COPD defined as persistent respiratory symptoms and fixed aiflow limitation with a post-bronchodilator FEV_1/forced vital capacity ratio of less than 0·7.[13] Patients with COPD were classified according to the airflow limitation (GOLD grades 1 to 4) and to exacerbation history and symptoms (GOLD groups A, B, C, and D).[14] Any cancer, other than basocellular skin carcinoma, detected within the previous 5 years was the main exclusion criterion, the full exclusion criterion has been previously reported.[13] Participants were recruited via flyers distributed in medical practices and newspaper adverts and pre-screened in 21 university centres in France. Participants were enrolled by a designated investigator from each centre, after signing written informed consent for continued data collection until completion of their last study visit.

National ethics committee approval was obtained from the Comité de Protection des Personnes Sud Méditerranée V (registration 15.072) on July 8, 2015, and from the Agence Nationale de Sécurité du Médicament et des produits de santé (French Ministry of Health) on July 10, 2015. Liability Insurance was from Hospital Mutual Insurance Company (Lyon; France SHAM 145.017). The protocol of the AIR study has been previously published.[13]

Procedures

Participants were invited to undergo three screenings (T0 [baseline], T1, and T2) at 1-year intervals. Each screening round consisted of a clinical examination, a LDCT, and a blood test to detect CTCs (ISET Rarecells; Rarecells Diagnostics, Paris, France). Participants and investigators were masked to the ISET results; the four cytopathologists who examined the ISET filters were masked to the clinical and radiological data. All chest-CTs were read, anonymised, and stored in the Digital Imaging and Communications in Medicine format in a centralised databank. No further

screening was proposed when a definitive diagnosis of lung cancer was established (at final pathology or clinical follow-up and after consensus was obtained following a multidisciplinary team meeting in oncology, if pathology was unavailable, as the gold standard).

LDCT that revealed any non-calcified nodule measuring at least 5 mm in any diameter or 50 mm³ was classified as positive—ie, suspected lung cancer.[15,16] Additionally, clinically significant incidental findings such as mediastinal or hilar adenopathy, consolidation, parenchymal mass, atelectasis, pleural effusion, and distant metastases were also noted.

No specific recommendations were made as to the management of suspicious lesions, nonetheless all centres followed the strategies recommended by the Intergroupe Francophone de Cancérologie Thoracique and the Groupe d'Oncologie de Langue Française.[17]

CTCs were isolated by filtration of 10 mL of blood using ISET and then classified into three categories according to cytomorphological criteria:[11,18] CNHC-malignant, CNHC with uncertain malignant features (CNHC-uncertain), and CNHC with benign features (CNHC-benign). Briefly, CNHC-malignant are cells that have at least four of the following features: nuclei larger than three calibrated pore sizes of the filter (ie, >24 µm), anisonucleosis, irregular nuclei, high nucleo-cytoplasmic ratio, and the presence of three-dimensional sheets. CNHC-uncertain are cells that show one of the cytological features listed for CNHC-malignant cells. CNHC-benign are cells without these cytological features, but not corresponding morphologically to blood cells. Cells without visible cytoplasm or corresponding to apoptotic cells, or both, (presence of nuclear shrinkage and fragmented nuclei) were not counted as CNHC.[11,18] In this Article we use for simplicity the term CTCs for any circulating cell with a well defined nucleus and a cytoplasm that did not correspond morphologically to a blood cell.

EDTA tubes (BD Vacutainer, Le Pont-de-Claix, France), were used for blood sampling in hospitals in Nice, Tenon, Toulouse, and Nancy that were equipped with an ISET device. Blood was then filtered no more than 4 h after venipuncture. Streck Cell-Free DNA BCT tubes (Streck Inc, Omaha, NE, USA) were used for blood sampling in other hospital centres and immediately shipped to the human biobank at Nice University Hospital (Nice, France; biobank BB-0033-00025) for delayed blood filtration within 24 h. The post-hoc analysis comparing immediately filtered and delayed filtered blood samples has been previously described.[19] All filtrates were examined at the Laboratory of Clinical and Experimental Pathology (Nice University Hospital, Nice, France) by four senior thoracic cytopathologists who were masked to clinical and radiological data (including VH, MI, and PH). Discordant cases were reviewed using a multi-head microscope. Damaged, missing, or incorrectly identified tubes were considered as not analysable. Unmasking of the cytopathological results for the clinicians and of the clinical and radiological results for cytopathologists was done after completion of T2.

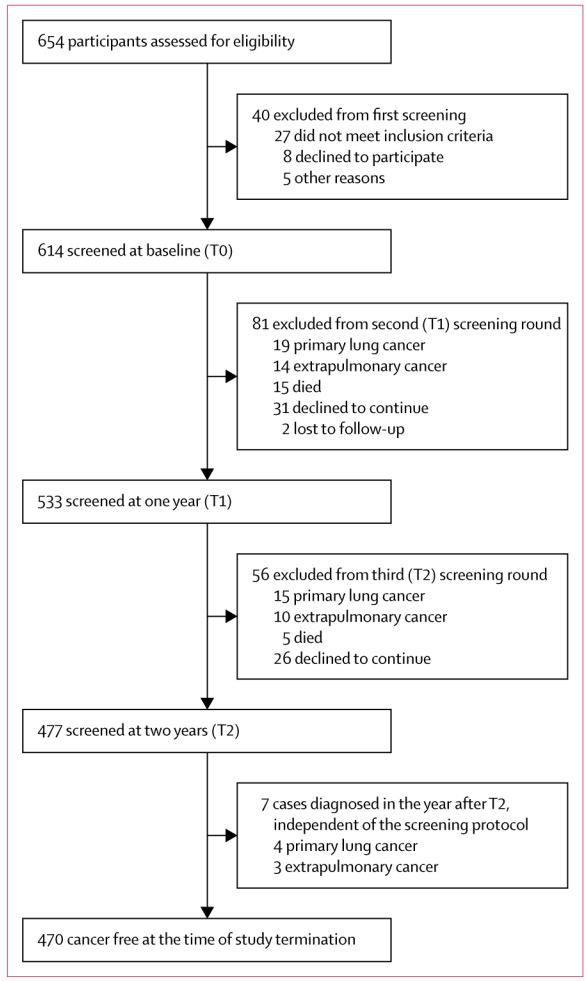

Figure: **Trial profile**

Outcomes

The primary endpoint of the study was the diagnostic performance of CTC detection as a biomarker for diagnosis of lung cancer at T0 in the context of lung cancer screening–ie, could CTC detection act as a screening tool? For this purpose, the detection of CNHC-malignant and CNHC-uncertain was considered as positive for cancer diagnosis.

Secondary endpoints were the performance of CTC detection to classify LDCT screening-detected pulmonary nodules (benign *vs* malignant); to diagnose extrapulmonary cancers; and to predict development of lung cancer and thus the need for specific follow-up in participants with a negative LDCT screening and the presence of CTCs.

Statistical analysis

The prevalence (baseline screening round) of lung cancer in this population at high-risk was estimated to

	Baseline (T0; n=614)
Age, years	65·1 (6·5)
Age group	
55–59 years	140 (23%)
60–69 years	328 (53%)
70–79 years	129 (21%)
≥ 80 years	17 (3%)
Sex	
Male	437 (71%)
Female	177 (29%)
Smoking status	
Current	309 (50%)
Former	305 (50%)
Pack years	52·7 (21·5)
BMI, kg/m^2	26·2 (5·2)
Cardiovascular comorbidity	326 (53%)
Coronary heart disease	146 (24%)
Hypertension	201 (33%)
Peripheral arterial disease	67 (11%)
Emphysema on initial LDCT	509 (83%)
FEV_1, % predicted	65·1 (22·1)
GOLD status*	
GOLD grade 1	169 (28%)
GOLD grade 2	267 (43%)
GOLD grade 3	146 (24%)
GOLD grade 4	32 (5%)
GOLD group A	106 (17%)
GOLD group B	370 (60%)
GOLD group C	14 (2%)
GOLD group D	124 (20%)

Data are mean (SD) or n (%). BMI=body-mass index. LDCT= low-dose computed tomography. *Patient classification is determined according to the airflow limitation severity on the basis of post bronchodilator FEV_1 (grades 1–4), the symptom burden (using a modified Medical Research Council Questionnaire), and risk of exacerbation (groups A–D).

Table 1: **Baseline characteristics**

	Baseline (T0; n=614)	T1 (n=533)	T2 (n=477)	Total
Lung cancers	19	15	4	38
Pathology				
NSCLC	17	11	4	32
Adenocarcinoma	7	8	4	19
SCC	5	1	0	6
Other or unknown	5	2	0	7
SCLC	2	4	0	6
Stage (NSCLC)				
Stage I	5	6	3	14
Stage IA	4	6	3	13
Stage IB	1	0	0	1
Stage II	1	1	0	2
Stage IIA	1	0	0	1
Stage IIB	0	1	0	1
Stage III	7	2	1	10
Stage IIIA	5	1	0	6
Stage IIIB	1	1	0	2
Stage IIIC	1	0	1	2
Stage IV	4	2	0	6
Stage (SCLC)				
Localised	1	0	0	1
Regional	0	2	0	2
Distant	1	2	0	3
Extrapulmonary cancers	14	10	3	27
Bladder	3	3	0	6
Gastro-oesophageal	2	1	0	3
Colon	2	0	0	2
Pancreas	0	1	1	2
Prostate	1	1	0	3
Head and neck	2	2	0	4
Other	4	1	2	7

NSCLC=non-small-cell lung carcinoma. SCC=squamous cell carcinoma. SCLC=small-cell lung cancer.

Table 2: **Characteristics of the primary lung tumours and extrapulmonary cancers**

be as high as 4%.[20] To assess the place of CTC detection in lung cancer screening, minimisation of false-negative results was prioritised. Our principal performance parameter was therefore sensitivity, on which we performed the sample size calculation. The sensitivity of LDCT for lung cancer screening is about 80%.[21–23] To meet expectations, a sensitivity of 80% was anticipated in direct comparison of CTC detection versus LDCT. The sample size target was 600 patients giving 24 confirmed lung cancer cases, allowing a 95% CI of 60·4–91·3% for CTC sensitivity. Continuous variables are presented as means with SDs, and categorical variables as n (%). Baseline characteristics between patients with or without lung cancer were compared using the Student *t* test or Wilcoxon-Mann Whitney for quantitative variables depending on the normality of distribution of the parameters or the χ^2 test for qualitative variables. The diagnostic performance of LDCT and CTC detection for lung cancer screening was determined as the sensitivity, specificity, positive predictive value, and negative predictive value using final pathology or clinical follow-up if pathology was unavailable as the gold standard. A true-positive result (LDCT or ISET blood test) was a positive result in a participant who was diagnosed with lung cancer through diagnostic work-up. A false-positive result was a positive result in the absence of lung cancer. A true negative result was a negative result in the absence of lung cancer, and a false-negative result was a negative result followed by diagnosis of interval cancer. The various tests were considered significant at a threshold of 5%. Analyses were done using the SPSS, version 25.0. The study is registered at ClinicalTrial.gov, NCT02500693.

Role of the funding source

The funders of the study had no role in the study design, data collection, data analysis, data interpretation, writing of the report, or the decision to submit the paper for publication. The corresponding authors had full access to all of the data and the final responsibility to submit for publication.

Results

654 volunteers were assessed for eligibility in 21 university centres in France, of whom 614 (94%) were included between Oct 30, 2015, and Feb 2, 2017 (figure, table 1). Participants were predominantly men (437 [71%]), aged 65·1 years (SD 6·5), and heavy smokers (52·7 pack-years [SD 21·5]). About half of the participants were active smokers. Cardiovascular comorbidities, and especially hypertension and coronary heart disease were present in more than half the individuals. Participants were found to be at a high risk of developing lung cancer with a mean COPD-Lung Cancer Screening Score[24] of 7·1 (SD 2·1) at baseline.

The rate of adherence to the screening protocol at the second visit (T1) was high: 533 (94%) of 566 participants who were eligible for T1 underwent screening (figure). However, given the high cancer prevalence and overall mortality in the study population, the dropout rate was 81 (13%) of 614 participants between T0 (baseline) and T1. The median follow-up time of the participants was 737 days (SD 188).

178 (29%) of 614 participants had a positive LDCT, among those, 154 had solid nodule(s) only, three had mixed nodule(s) only, eight had ground glass nodules only, and 13 had a combination of nodules of different types. The sensitivity of LDCT for malignant nodule detection was 82·6% (95% CI 61·2–95·0), with a specificity of 73·1% (69·3–76·6).

38 primary lung cancers were detected with LDCT. 19 (3%) of 614 participants were diagnosed with a prevalent lung cancer (tumour present at T0). 19 were diagnosed with an incident lung cancer (15 (3%) of 533 at T1 and four (1%) of 477 at T2; table 2). The diagnosis of lung cancer was confirmed by pathology in 35 patients and by follow-up plus multidisciplinary team meeting consensus in three patients. Patients with prevalent or incident lung cancer had similar baseline characteristics as the rest of the cohort for smoking duration, number of pack-years, COPD Assessment Test and Modified Medical Research Council scores, and FEV_1. The only statistically significant difference was the baseline body-mass index, an average of 24·30 kg/m² (SD 4·7) in participants with lung cancer versus 26·27 kg/m² (5·2) in participants without lung cancer (p=0·018).

During the study, extrapulmonary cancers were diagnosed in 27 (4%) of 614 participants (table 2); tumours were not diagnosed as a result of LDCT screening.

All blood samples were filtered with ISET and reading of the results was masked as per the study protocol.[13]

	Primary lung cancer present	Primary lung cancer absent	Total
CNHC-malignant or CNHC-uncertain	5	22	27
No CNHC detected	14	570	584
Total	19	592	611

Three patients were not included in this analysis because their blood samples were not analysable. CNHC-malignant=circulating non-haematological cells with malignant features. CNHC-uncertain=circulating non-haematological cells with uncertain malignant features.

Table 3: **Diagnostic performance of circulating tumour cell detection as a biomarker to diagnose lung cancer at baseline (T0) in the context of lung cancer screening**

	T0			T1			T2		
	LC+	EPC+	LC– EPC–	LC+	EPC+	LC– EPC–	LC+	EPC+	LC– EPC–
CNHC-malignant	3	0	4	0	3	1	0	0	1
CNHC-uncertain	2	0	18	0	0	11	0	0	0
No CNHC detected	14	14	556	15	7	474	2	0	32
Not analysable	0	0	3	0	0	22	2	0	1

LC+=presence of primary lung cancer. LC–=absence of primary lung cancer. EPC+=presence of extra pulmonary cancer. EPC–=absence of extra pulmonary cancer. CNHC-malignant=circulating non-haematological cells with malignant features. CNHC-uncertain=circulating non-haematological cells with uncertain malignant features.

Table 4: **Presence of circulating tumour cells and matched oncological status over the study period**

Overall, 28 (2%) of 1187 blood samples were not analysable for technical reasons. At baseline (T0), seven participants had CNHC-malignant, 20 participants had CNHC-uncertain, zero patients had CNHC-benign, the remainder had no CNHC, and three blood sample results were not interpretable because of numerous artifacts (table 3, appendix p 3). Discordant cases occurred in five patients because two of the four cytopathologists classified the detected cells as CNHC-uncertain while the two others considered these cells to be CNHC-malignant. After simultaneous analysis using a multihead microscope, a consensus was made to classify these five cases as CNHC-uncertain, because some important cytological criteria (eg, the presence of a large irregular nuclei) were not present to be able to characterise these cells as CNHC-malignant.

At baseline (T0), the sensitivity of CTC detection as a biomarker for lung cancer detection was 26·3% (95% CI 11·8–48·8) when considering both CNHC-malignant and CNHC-uncertain as positive results (table 3). The sensitivity did not depend on whether the blood sample was filtered immediately on site (Nice, Tenon, Toulouse, or Nancy) or within 24 h of being shipped to the Nice University Hospital biobank from the other centres.[23] The blood test did not detect four interval cancers that had been missed by LDCT at T0. At baseline (table 3), when considering both the CNHC-malignant and CNHC-uncertain as positive, the CTC detection specificity for lung cancer detection was 96·2% (95% CI 94·4–97·5), the negative predictive value was 97·6%

	Extrapulmonary cancer present	Extrapulmonary cancer absent	Total
CNHC-malignant or CNHC-uncertain	0	27	27
No CNHC detected	14	570	584
Total	14	597	611

Three patients were not included in this analysis because their blood samples were not analysable. CNHC-malignant=circulating non-haematological cells with malignant features. CNHC-uncertain=circulating non-haematological cells with uncertain malignant features.

Table 5: **Presence of circulating tumour cells according to the presence or absence of extrapulmonary cancers at baseline**

	Lung cancer present (T1)	Lung cancer absent (T1)	Extrapulmonary cancer present (T1)	Extrapulmonary cancer absent (T1)
CNHC-malignant (T0)	0	2	0	2
CNHC-uncertain (T0)	2	15	0	17
No CNHC detected (T0)	13	498	10	501
Not analysable	0	3	0	3

CNHC-malignant=circulating non-haematological cells with malignant features. CNHC-uncertain=circulating non-haematological cells with uncertain malignant features.

Table 6: **Presence of circulating tumour cells at baseline (T0) as a predictor of lung or extrapulmonary cancer development within 2 years in patients without a lung tumour at T0**

	Malignant nodules	Benign nodules
CNHC-malignant (T0)	1	0
CNHC-uncertain (T0)	1	8
No CNHC detected (T0)	13	154
Not analysable	0	1

CNHC-malignant=circulating non-haematological cells with malignant features. CNHC-uncertain=circulating non-haematological cells with uncertain malignant features.

Table 7: **Relationship between the circulating tumour cells status at baseline (T0) and the nature of the lung nodules**

(96·9–98·2), and the positive predictive value was 18·42% (8·7–34·7).

During rounds of follow-up (T1 and T2) five participants had CNHC-malignant, 11 had CNHC-uncertain, and 25 were not analysable because of numerous artifacts (table 4). None of the 17 patients with incident lung cancer and an available matched blood sample had a positive CTC detection test (table 4).

None of the extrapulmonary cancers had a positive biomarker (CTC detected) at baseline (table 5). Three of the ten extrapulmonary cancers diagnosed at T1 were CNHC-malignant (table 4).

The ability of ISET to predict the subsequent development of pulmonary or extrapulmonary cancers was low, only two of 13 lung cancers and none of the 13 (10 at T1 and 3 at T2) extrapulmonary cancers detected within 2 years had a positive CTC detection test at baseline (table 6).

The presence of CTC in screening-detected pulmonary nodules was highly specific for lung cancer (table 7).

Discussion

The ISET Rarecells test used in this study had too low a sensitivity to be used as a reliable lung cancer screening tool for patients at high-risk. We deliberately chose to study CTC as a potential biomarker for lung cancer screening in a population of patients with COPD because our previous work in this patient population suggested that this biomarker might be of interest for screening,[12] and this population is at a high risk of developing lung cancer and so deserves special attention when screening for this cancer.[20,25]

The age and smoking status of the participants was comparable to that reported in the NLST, NELSON, DLST, ITALUNG, and LSS studies.[1,2,26–28] The male-to-female ratio of 71% to 29% in this study was comparable to that reported in the European NELSON trial.[15] The majority of participants had a mild or moderate airflow limitation (GOLD grade 1 and 2). Despite this, most participants had substantial symptom burden because they belonged to group B (60%) or group D (20%) of the 2017 GOLD classification.

The baseline prevalence of lung cancer was 1·0% in the NLST study, 0·9% in the NELSON study, 0·8% in the DLCST study, 1·5% in the ITALUNG study, and 1·8% in the LSS study.[1,2,26–28] The baseline prevalence of lung cancer in the present study was about three times higher than in these studies, which were done in participants whose risk was primarily defined by their smoking history, without the presence or absence of COPD being a criterion for inclusion. Our results are in line with the numerous epidemiological studies that showed that COPD is an independent risk factor (2–4 times higher than in participants without COPD) for lung cancer development.[20]Although not part of the original objective, to our knowledge this study is the first to report the incidence of lung cancer in a screened COPD population and shows that the 1-year incidence (2·8%) is particularly high, in the order of four times that observed in habitual smokers without a formal diagnosis of COPD.[1,2,27,28]

Baseline body-mass index was significantly lower in participants who had lung cancer at baseline or who developed lung cancer during the study. Weight loss could probably be attributed in this context to malignancy, especially because most of the diagnosed lung cancers were advanced (stage III and IV).

Because the presence of extrapulmonary cancers might negatively interfere with the search for CTCs, we paid special attention to the recording of these malignancies. These extrapulmonary malignancies were within the upper range usually reported in lung cancer screening programmes.[29] Thus, the centralised double readings of LDCT, the high rate of adherence to the screening protocol coupled with the high level of prevalence and incidence rates of lung and extrapulmonary cancers, allowed us to confidently conclude that none or very few cancers escaped our attention. To avoid bias due to the preanalytical phase of cytological assessment, we

previously compared the potential effect of the time between venous puncture and blood filtration using EDTA and Streck buffers.[19]

This study, although disappointing, does not rule out the validity of our previous results from a 2014 study.[12] Indeed, in this previous study, we selected patients from the Nice University Hospital human biobank who are at high risk of developing lung cancer—ie, those with COPD. From this population, we retrospectively extracted the 168 patients for whom we had clinical and radiological follow-up. Of this highly selected population five (3%) of 168 patients had CNHC-malignant on blood sampling and developed lung cancer 1–4 years later and three (2%) of 168 had CNHC-uncertain. None of the patients with CNHC-uncertain developed lung cancer. Similarly, 160 patients with COPD, as well as healthy smokers and non-smokers in whom CNHC-malignant were not detected did not develop lung cancer. We concluded from our 2014 study that the biological expression of some lung tumours could result in the presence of CTCs before detection by conventional radiological methods.[12] Although we were probably a little too optimistic to believe that CTC detection might serve as a standalone screening tool. However, because of the potential biases in the 2014 study, we did not infer that CTC detection should be integrated into the lung cancer screening arsenal. To reach this conclusion, a methodologically sound multicentre and large prospective study (the present AIR study) was done. This study showed a very low sensitivity for CTCs detection in identifying lung cancer at baseline, which argues against its value as a screening tool. However, we used only one method for CTCs detection in this study, the ISET technology. The overall negative results of this study do not imply that these results would be the same using one of the other methods for CTCs detection.[30,31] Generally, the number of CTCs in the blood in early stage NSCLC is low, which means that this approach is probably not the most suitable for early lung cancer detection.[31,32]

A tumour-derived blood biomarker could also be used to identify a population at risk, particularly as part of a screening programme.[7–9] In the present study, as with our 2014 study, some patients (2 [13%] of 15) had sentinel CTCs—that is, were cancer-free and had positive CTCs detection at baseline and subsequently developed lung cancer.

The probability of developing cancer in a patient with a screening-detected nodule is usually a function of its volume, its doubling time as assessed by repeat screening at 3 months, the combination of the two, and the prevalent or incident nature of the nodule.[16] A tumour-derived biomarker might also be useful in the characterisation of a screening-detected nodule and thus avoid repeat LDCTs.[7] When considering both CNHC-malignant and CNHC-uncertain detection as positive results, CTC detection was worse than other biomarkers such as micro-RNA signature in characterising screening-detected lung nodules.[7] When we consider the entire cohort, ISET was highly specific for lung cancer reaching as high as 96·2%. Nonetheless, when we considered patients with lung cancer, ISET missed 13 out of 15 malignant nodules. It can therefore and by no means be presently used as an adjunct to LDCT in individuals with screening-detected pulmonary nodules of undetermined nature.

Despite previous promising results, the one-dimensional approach to lung cancer screening is certainly not the answer. Although radiological screening has clearly been shown to reduce lung cancer mortality, its implementation based on a radiological signature alone—that is, the presence of a nodule larger than a predefined threshold on LDCT—is hampered by the high number of false positives.

The development of more complex radiological signatures is certainly necessary.[33,34] The development of a blood test that detects cancer faces even greater difficulties as illustrated by this study. Beside the use of CTC detection in an early stage, which has already been shown by some previous studies,[10,11,35] many other blood biomarkers (such as cfDNA, miRNA, or protein signatures)[36–41] are currently and very actively under consideration as more promising screening tools for lung cancer detection. However, it is likely that even more complex biological signatures[6–8] will not be sufficient alone for screening. The solution might be based on a holistic approach of screening that integrates clinical, biological, and radiological signatures into lung screening programmes.[5,25,42,43]

Contributors

C-HM, JBo, JBe, PH, BP, and VH designed the study and protocol submission. All authors participated to the acquisition of data. JBo, C-HM, JBe, MI, VH, and PH analysed and interpreted the data. JBo, JBe, MF, BP, JP, CP, FB, VF, NG, MI, and DB revised the manuscript.

Declaration of interests

We declare no competing interests.

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