

Review Article

Screening approaches for lung cancer by blood-based biomarkers: Challenges and opportunities

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Abstract. Lung cancer (LC) is one of the leading causes for cancer-related deaths in the world, accounting for 28% of all cancer deaths in Europe. Screening for lung cancer can enable earlier detection of LC and reduce lung cancer mortality as was demonstrated in several large image-based screening studies such as the NELSON and the NLST. Based on these studies, screening is recommended in the US and in the UK a targeted lung health check program was initiated. In Europe lung cancer screening (LCS) has not been implemented due to limited data on cost-effectiveness in the different health care systems and questions on for example the selection of high-risk individuals, adherence to screening, management of indeterminate nodules, and risk of overdiagnosis. Liquid biomarkers are considered to have a high potential to address these questions by supporting pre- and post- Low Dose CT (LDCT) risk-assessment thereby improving the overall efficacy of LCS. A wide variety of biomarkers, including cfDNA, miRNA, proteins and inflammatory markers have been studied in the context of LCS. Despite the available data, biomarkers are currently not implemented or evaluated in screening studies or screening programs. As a result, it remains an open question which biomarker will actually improve a LCS program and do this against acceptable costs. In this paper we discuss the current status of different promising biomarkers and the challenges and opportunities of blood-based biomarkers in the context of lung cancer screening.

Keywords: Lung cancer screening, biomarkers

1. Introduction

Lung cancer (LC) is one of the leading causes for cancer-related deaths in the world, accounting for 28% of all cancer deaths in Europe. About 70% of lung cancer patients are diagnosed at an advanced stage, resulting in a 15% five-year survival. In line with existing screening programs, screening for lung cancer can enable earlier detection of LC and a reduction in lung cancer mortality. The potential of lung cancer screening (LCS) was demonstrated in several imaging-based screening studies of which the largest are the NELSON trial [1] and the NLST [2]. These, and a number of other studies [3–5]

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have proven the value of a Low Dose Computed Tomography (LDCT)-based LCS. In the NELSON trial, mortality was reduced to 26% in men and to 39–61% in women [1]. One of the driving factors of this reduction was the observed stage shift in detected cancers from approximately 21% of stage I/II tumors in the control arm to 60% in the screening approach. In addition, the NELSON trial introduced a volumetric nodule management protocol which reduced the false positive LDCT results from 25.3% as reported in the NLST trial to 1.2% [1, 2]. The final positive predictive value (PPV) for lung cancer of a positive LDCT was 43.5% [1]. Further efforts are ongoing to improve the efficacy of LCS [6–8]. Based on the results from the NLST trial, LCS is recommended in the US (age 50–88 y and at least 30 pack-years), but the annual uptake remains limited at approximately 14% of the eligible individuals [9]. In the UK a targeted lung health check programme in 10 regions has recently been initiated using different selection criteria (people aged 55 to 75 that have ever smoked) compared to the US [10]. In Europe LCS has not been implemented because of the limited data on cost-effectiveness in the different health care systems and questions on the selection of high-risk individuals, adherence to screening, management of indeterminate nodules, and risk of overdiagnosis [11–19].

Liquid biomarkers are considered to have a high potential to address some of these questions by supporting pre- and post-LDCT risk-assessment and as a result could improve overall efficacy of an LCS program [13–15]. Ideally, robust biomarkers would allow evidence-based refinement of selection criteria and support clinical decisions in case of indeterminate or positive LDCT scans. Table 1 describes the potential impact of biomarkers in an LCS program. A wide variety of biomarkers has been studied in the context of LCS. But there is still no consensus on which biomarker will actually improve an LCS program with respect to for example the performance of risk models, selection criteria, PPV of indeterminate and positive LDCT scans and do this against acceptable costs [20]. Evaluating and comparing reported biomarker performance is not straightforward. Biomarkers in screening can be used as (1) a stand-alone (multi-cancer) test to detect cancer, (2) as a marker to select participants for downstream evaluation, or (3) to improve interpretation and performance of a LDCT result. Each of these approaches will require different biomarker test specifications [21, 22]. For example, a test to detect cancer will have to be set to a high specificity and PPV in order to limit the number of false positive results while selection of participants will require a high sensitivity and NPV, accepting false positive results but limiting or preventing false negatives. Additional variables that hamper a direct comparison of biomarkers are differences in the criteria for risk, prevalence of cancer in the studied population, the included stages of disease and technical factors such as analytical and pre-analytical variables. In this paper we discuss the current status and application of cell-free DNA (cfDNA) methylation and fragmentation, combining blood-based biomarkers, microRNA, proteins, auto-antibodies and inflammatory biomarkers in the context of LCS.

2. Biomarkers in lung cancer screening

2.1. Cell-free DNA

cfDNA are DNA fragments complexed with nucleosomes that circulate in the blood [23]. Although the majority of these fragments originate from white blood cells, cfDNA can originate from every cell in the body and is significantly increased in patients with cancer [24]. The fraction of cfDNA originating from the tumor is called circulating tumor DNA (ctDNA). Analysis of ctDNA has been shown to be a promising and an effective strategy to for example identify targetable mutations [24, 25], determine treatment response or disease progression [22, 26–28] and residual disease [29, 30]. Conceptually, ctDNA levels will depend on the shedding of DNA into the blood (correlated with the tumor volume [31] and the rate of cell death in the tumor), clearance of circulating DNA, the total

Table 1
Potential role and impact of biomarkers in the setting of a LCS program

	Detect	Select	Improve Indeterminate LDCT	Positive LDCT	Negative LDCT
Current approach	Low Dose CT	Risk models, based (minimally) on age, pack-years (for example PLCOm2012)	Volume doubling time via a second LDCT scan at a 3-month time interval	Cancer diagnosis based on tissue biopsy	Regular follow-up by LDCT
Potential merits of a biomarker in a LCS program	<ul style="list-style-type: none"> - Identification of LC as part of a multi-cancer detection test - Reduce burden for health care system and participant 	<ul style="list-style-type: none"> - Increase % of LC in eligible population thereby increase cost-effectiveness of LCS - Reduce burden of LCS (society) - More objective criteria for selecting participants compared to self-reported parameters - Possibility to broaden selection criteria while limiting total volume of the screening population 	<ul style="list-style-type: none"> - Increase % of LC in indeterminate scans by excluding non-cancer lesions (determine risk of cancer of the observed lesion) - Reduce burden of LCS (participant and society) 	<ul style="list-style-type: none"> - Increase % of LC in positive LDCT scans by excluding non-cancer lesions - Prevent unnecessary invasive procedures 	<ul style="list-style-type: none"> - Risk-based (personalized) frequency of follow-up by LDCT - Reduction in burden of LCS (societal and participant) - Increase adherence to LCS
When is a biomarker successful?	<ul style="list-style-type: none"> - Improve detection of LC (or multiple cancers) compared to LDCT or cancer screening programs against lower cost and burden. - Increase stage shift by earlier detection 	<ul style="list-style-type: none"> - Cost for a biomarker should balance the reduced burden of the total program - No negative effect on mortality or stage distribution 	<ul style="list-style-type: none"> - Increase on performance of VDT without significant increase in costs - Rule-out individuals without lung cancer - No negative effect on mortality or stage distribution 	<ul style="list-style-type: none"> - Any reduction in unnecessary invasive follow-up by increasing the PPV - No negative effect on mortality or stage distribution 	<ul style="list-style-type: none"> - No negative effect on mortality or stage distribution - Increase long-term adherence to the screening program

(Continued)

Table 1
(Continued)

	Detect	Select	Improve Indeterminate LDCT	Positive LDCT	Negative LDCT
Approaches	<ul style="list-style-type: none"> - Multi-cancer detection assays based on blood-based biomarkers - Biomarker set at high specificity (low false positive rate) 	<ul style="list-style-type: none"> - blood-based biomarkers, combinations of markers and/or clinical parameters - Companion test to select for LDCT: high sensitivity (very low false negative rate) 	<ul style="list-style-type: none"> - Step-wise approach biomarker refines the indeterminate scan - Combined analysis of biomarker and imaging as one biomarker 	<ul style="list-style-type: none"> - Step-wise approach biomarker refines the positive scan - Combined analysis of biomarker and imaging as one biomarker or algorithm 	<ul style="list-style-type: none"> - Biomarker as part of a risk model to determine screening frequency by LDCT - Interval biomarker assessment to select participants eligible for LDCT evaluation
Limitations	<ul style="list-style-type: none"> - Sensitivity for smaller tumors - Cost of the biomarker - Requires further confirmation and diagnostic work-up 	<ul style="list-style-type: none"> - Sensitivity for smaller tumors - Potential detection of other cancers than lung cancer (“true false positive”) - No comparative data on biomarkers: no clear “best” biomarker - Cost of the biomarker in relation to the LCS screening 	<ul style="list-style-type: none"> - High sensitivity (96,4%) and specificity (99,9%) of VDT - Limited data on the addition of different biomarkers 	<ul style="list-style-type: none"> - Potential false negative results 	<ul style="list-style-type: none"> - Sensitivity for smaller tumors - Potential detection of other cancers than lung cancer (“true false positive”) - No comparative data on the performance of biomarkers

Abbreviations: LDCT = Low-dose CT, PLCOm2012 = Prostate, Lung, Colorectal, and Ovarian Cancer Screening Trial model 2012, LC = Lung cancer, LCS = Lung Cancer Screening, VDT = Volume Doubling Time, PPV = Positive Predictive Value.

blood volume, and the collected volume of blood [32]. Technically, cfDNA is mostly analysed using (droplet) digital PCR, which can detect single or, in a multicolour set-up, multiple targets or NGS-based approaches to analyse multiple genes. Mutation detection in cfDNA can be challenging due to pre-analytical variables, mutations from clonal hematopoiesis, mutation calling accuracy at low variant allele frequencies (<1%), low number of input molecules, etcetera. As a result, the sensitivity for small tumors will be limited, posing a relevant challenge for cfDNA mutation detection in LCS. Sensitivity will mostly be affected by the number of detectable alterations. Increasing the number of detectable alterations will improve the overall chance of detection [33]. Several strategies have been developed in the area of LCS: analysis of methylation, fragmentation of cfDNA and combining data from of different biomarkers.

2.2. cfDNA methylation

DNA methylation is an important epigenetic regulator of gene transcription. Alterations in DNA methylation are common in carcinogenesis and occur early in the development of a tumor. Based on differential DNA methylation of cancer cells and healthy cells, analysis of DNA methylation in cfDNA could provide a marker for early disease [34]. Several approaches have been developed to exploit these differences ranging from whole genome to more targeted approaches.

In the context of detection of multiple cancers, the circulating cell-free Genome Atlas (CCGA) consortium has done the most extensive evaluation of DNA methylation as a biomarker for >50 tumor types [35]. Analyzing several key informative DNA methylation regions in a cohort of 4077 participants (2823 with cancer and 1254 non-cancer participants) this approach reached a sensitivity of 67.3% for stages I–III in a pre-specified set of 12 cancer types with a specificity of 99.5%. Sensitivity was dependent on stage increasing from 16.8%, 40.4%, 77.0 % to 90.1% for stage I, II, III and IV, respectively. The SUMMIT trial in the UK is now using this DNA methylation test (Grail's blood test) in a prospective observational cohort study enrolling 25,000 participants (men and women 50–77 y) to clinically validate the detection of multiple cancers at an early stage and to examine the feasibility of selecting high-risk individuals for LDCT based LC screening. In the US the PATHFINDER study is using the same DNA methylation test (Grail's blood test) in a prospective interventional design. Participants with a positive test undergo further diagnostic work-up following standard guidelines. This study is currently in its follow-up stage [36]. A planned interim analysis of the PATHFINDER study over 4011 participants indicated that the PPV of the test was 43.3% and a NPV of 99.7%, detecting 9 different cancers [37], reaching comparable PPV to LDCT screening for lung cancer. The potential of DNA methylation is further underlined by the PanSeer test based on the methylation of 477 genomic regions. PanSeer was retrospectively evaluated on biobanked samples from 123,115 subjects in the Taizhou Longitudinal Study and reached a cancer detection rate of 95% over 5 common types (stomach, esophageal, colorectal, lung and liver cancers) up to four years before diagnosis. Sensitivity before diagnosis of cancer was 91% with a set specificity of 95% [38]. Due to some limitations of the study further prospective studies will have to determine the clinical impact of this test.

An alternative approach to detect lung cancer is based on the analysis of single DNA methylation sites in cfDNA. DNA Methylation of *SHOX2*, *RASSF1A* and *PTGER4* genes reached a sensitivity of 82.5% and specificity of 90.5% for stage I/II LC in a training and validation cohort [39]. When using *CDO1*, *TAC1* and *HOXA7* combined with clinical predictors in 150 stage I/IIA LCs and 60 controls, sensitivity of 93% and specificity of 63% was reached [40]. In addition, this latter combination was able to detect tumor sizes <1.0 cm with a sensitivity of 64% and specificity of 82% [41]. Other combinations of DNA methylation sites have also been evaluated in small selected cohorts in the context of nodule risk management [42–44]. The Lung Epicheck, which uses DNA methylation of six sites, was trained (120 cases and 265 controls) and validated (209 cases and 152 controls) to a sensitivity for stage I LC

of 87.2% and specificity of 64.2%, or when preferring specificity over sensitivity 90.5% and 74.3%, respectively [45]. One of the limitations of these studies is the small number of cases and controls used in the evaluation (ranging from 101 to 351). As a result, data for these markers in a full LCS population is lacking.

DNA Methylation could also improve selection criteria for screening. Based on 9206 individuals collected in the Copenhagen City Heart Study including a 5-year follow-up for lung cancer, the addition of AHHR methylation (cut-off <55%) to the criteria used in the NLST led to a high specificity of 84%, a sensitivity of 62% and a 21.9% reduction in the burden of screenings (which was defined as the number of individuals included per detected LC in 5-year follow-up). These findings were reproduced in a second cohort of 5334 individuals [46]. These results indicate the potential of the detection of cfDNA methylation to improve selection criteria to rule out individuals with the lowest risk [46]. Considering DNA methylation of specific sites, data primarily comes from small selected cohorts. As a result, their performance in a screening population remains unclear. Larger population studies such as PATHFINDER and the SUMMIT trail will, in the coming years, provide more insight in the impact of cfDNA methylation in LCS.

2.3. cfDNA fragment length

Fragment size of cfDNA is approximately 166 bp. This represents an overall fragment length coinciding with the DNA length wrapped around a nucleosome and is consistent with cellular apoptosis as the major source for cfDNA. The majority of cfDNA originates from white blood cells. cfDNA originating from other cells or tissues have shorter fragment lengths [47]. Although the exact mechanism is not clear, this is probably due to tissue-specific processes for example tissue-specific differences in nucleosome wrapping [23] or differences in tissue- and blood endonucleases [48]. This observation led to a wide variety of assays exploiting these differences including the actual length, end motifs, preferred ends, nucleosome footprints, jagged ends or DNA topology [49]. Of those, breakpoint motif profiling has been described in the context of the detection of stage I Lung cancer [50].

In the context of LCS two studies have explored the application of cfDNA fragmentation by using shallow whole genome sequencing (SWGs) of cfDNA. Differences in length between cancer-derived cfDNA and cfDNA from non-cancer cells were used to develop an algorithm to differentiate “healthy cell” DNA fragments from those of tumor cells, expressed in a score (DELFI score) [47, 51]. This approach was prospectively validated in a cohort of 385 individuals at risk for lung cancer and 46 lung cancer patients. ROC analysis showed an AUC for the detection of lung cancer in a population without prior cancer, age 50–80 and a smoking history of 0.78, 0.86, 0.93 and 0.99 for stage I, II, III and IV lung cancer, respectively. Combining the fragmentation with clinical risk factors and CEA levels detected 94% of the CT identified lung cancers. The DELFI score correlated with size and invasiveness of the tumor and individuals with higher scores (>0.5) and a significantly shorter survival with lower scores (<0.5). Further improvement of the assay is to be expected from the DELFI lung cancer training study (DELFI-L101) [52].

The impact of a DELFI score in the selection of participants for LDCT was modelled for a population of 100.000 high-risk individuals. Using the NLST data as basis and assuming 60% uptake of screening due to the introduction of a blood test this would result in 8 times increase in the detection of lung cancer. Thereby increasing the PPV of LDCT from 1.9% (NLST cohort) to 3.9%. The question remains whether this impact is due to the assumed increase in screening uptake, the performance of the test itself or a combination. Currently, this combined approach is evaluated in the CASCADE-LUNG trial in the US, a multi-center trial that will include up to 15,000 participants at the time of their LDCT screening. In addition, the DELFI score will be evaluated in the participants of the 4ITLR LCS study in Europe

[8]. These large population-based studies are ongoing and will provide more definitive information on the performance of the DELFI score in the coming years.

2.4. Combinations of blood-based biomarkers

Combining information from different biomarkers or materials is an alternative method to increase sensitivity. The most extensive data is available for the CANCERSEEK test which combines eight selected proteins with mutational data from cfDNA on 16 genes. This test was set up to detect 8 different cancer types. Their initial evaluation of 1005 patients with eight common nonmetastatic cancers, reached a sensitivity of 69–98% for different cancers at a specificity of 99%. Sensitivity for lung cancer in this population was just below 60% [53]. The feasibility and safety of this test in a multi cancer screening setting was evaluated in the DETECT-A study [53]. In total 10,006 women (60–75 y) not previously known to have cancer were included, all positive blood tests were confirmed by PET-CT. Twenty-six cancers were detected by blood testing of which 9 lung cancers; 17 were other local or regional cancers and in total 5 stage I cancers were detected. Twenty-four additional cancers were found in the standard screening programs, of which 3 were lung cancers. 1% of the blood tests proved to be false positive (initially 4.9% which was reduced to 1.35% after a second confirmational blood test); 0.22% of participants underwent futile invasive diagnostics. At this moment the final performance (esp. the specificity) of the CANCERSEEK test in the DETECT-A study will depend on the long-term follow-up of the participants.

Also other combinations have been explored. The methylation of 5 DNA sites combined with 3 proteins (CEA, CA125 and CA19-9) has been evaluated in a cohort of 180 patients (36 lung cancer) and 257 matched controls. An overall sensitivity of 87% at 95% specificity was reported for 6 tumor types [54, 55]. Although this approach will have to be validated and evaluated in a screening population it underlines the potential of combining DNA sequence or methylation markers and protein-based information and its potential in LCS.

Another approach was explored by The ITALUNG biomarker study which looked at loss of heterozygosity, microsatellite instability and cfDNA plasma values (cut-off 5 ng/ml) on DNA extracted from blood and sputum from asymptomatic high-risk participants (age 55–69 and 20 pack-years). The ITALUNG Biomarker Panel (IBP) was positive if at least one of the two panel biomarkers was positive. In total 1406 participants were screened and 1356 blood and sputum samples were collected. Sensitivity at baseline screening was 90%, with a specificity of 71% (LDCT) and 61% (IBP as single test), which improved to 89% for the combined test leading to an increase in PPV of a baseline LDCT from 4.3% to 10.6% in a combined approach [56]. Currently, more multi-modal approaches are being explored, using information from cfDNA, proteins, cfRNA, methylation, etcetera. A number of tests are in development and their performance in a screening setting is still unknown. Large population studies are ongoing, for example the SHIELD lung trial which will recruit 10,000 participants in the US and Europe to determine the sensitivity and specificity of the multimodal Guardant LUNAR-2 assay.

Impact of combinations of biomarkers have also been reported in the context of risk-stratification of indeterminate lung nodules. The combination of the MAYO clinic risk score with hs-Cyfra 21-1 and a radiomics risk score could for example reduce the number of participants requiring invasive procedures from 62.9% to 50.6% in a cohort ($n = 456$) recalibrated to a prevalence of LC of 0.33 [57]. Similar approaches of radiomics and blood-based biomarkers [58, 59] or based on multiple proteins [60] are under development. These strategies have not directly been evaluated against image-based approaches such as volume doubling time (VDT) of pulmonary nodules.

At this moment, there is data indicating that combining blood-based biomarkers has the potential to improve the performance over the use of individual markers and more multimodal biomarkers are

likely to be developed and evaluated. The actual performance of the current combinations in LCS will become available in the coming years.

3. Other blood-based markers

3.1. MicroRNA

MicroRNA are circulating non-coding small RNA molecules which participate in regulation of gene expression processes. Changes in their quantity and quality are associated with initiation and progression of cancer. Circulating miRNAs reflecting tumor-host interactions have emerged as potential biomarkers for cancer diagnosis and prognosis irrespective of tumor stage and mutational burden [61]. Multiple studies have shown miRNA to be a promising biomarker in lung cancer detection, although between studies different miRNAs were used, and only a limited number of miRNA panels have been evaluated prospectively [62]. Both pre-analytical and analytical features often lack harmonization and should be cautiously evaluated when comparing miRNA performance [63, 64]. The role of miRNA-based liquid biopsies in the context of screening with LDCT was mainly assessed in two large Italian retrospective validation studies, MILD and COSMOS [65–67]. In the context of selecting high-risk participants the MILD study evaluated a 24 miRNA panel in 4119 heavy smokers. The miRNA signature classifier (MSC) was used to stratify patients in high, intermediate (MSC+), or low levels of risk (MSC–). Combined with an LDCT result four groups were identified (CT–/MSC–, CT–/MSC+, CT+/MSC– and CT+/MSC+). MSC+ participants had a 2-fold higher chance of having LC. The MSC status in the LC+ group was correlated with incidence, stage and mortality. The findings after 5.3 years of follow-up showed that combined microRNA biomarkers and LDCT could reduce unnecessary LDCT repeats without any potential damage in terms of stage I LC, resection rates, interval cancer [66]. The data from the ITALUNG trial was used for a model-based economic evaluation simulating 7 different screening strategies varying selection criteria including the use of MSC in the China 1947 to 1971 birth cohort. This study showed that a combination of LDCT and MSC screening beginning at age 70 to 74 with 20 pack-year smoking history was most cost-effective [68].

In the COSMOS lung cancer screening program, a 13 miRNA miR-Test (of which 5 miRNAs are also used in the MSC test in the BIOMILD trial) was validated in 1115 enrolled high-risk individuals, which were divided in a calibration set (12 non-LC and 12 LC) and a validation set (972 non LC, 36 LC, 38 non calcified lung nodules stable during 5 years of follow-up, 16 COPD, 24 pneumonia and 5 benign) [65]. The miR-Test reached an overall accuracy of 74.9%, with a sensitivity of 77.8% and a specificity of 74.8%. Although this test was further optimized and standardized, no information is published on its performance in a screening setting [69].

Recently other miRNAs have been reported in the context of the selection of participants [70] and risk-assessment of indeterminate nodules [71]. For the selection of participants three miRNAs (miR-142-3p, miR-148a-3p and miR-451a) were combined with pack-years into a miR risk score [70]. The impact of this score was compared to criteria used in large screening studies. When maintaining equal numbers of LC-free participants as non-eligible for screening, the miR score could increase the proportion of LC cases identified as eligible for screening from 10.5 to 16.1% compared to the trial criteria. Discrimination of early-stage NSCLC from benign solitary pulmonary nodules (SPNs) based on a three miRNA panel (miRs21, 31 and 210) in sputum reached 82.9% sensitivity and 87.8% specificity in 2 cohorts of 136 patients and 155 patients. Validation of this test in two external cohorts confirmed a PPV of 84% and a NPV of 81%. Prospective data in a LCS screening population is lacking [71]. Other studies have looked into different combinations of 3 to 10 miRNAs resulting in sensitivities between 34 and 89.9% and specificities between 60 and 90.9% to discriminate early stage NSCLC

from benign lesions [72]. All these studies indicate that there is a potential for miRNA to improve an LCS program.

3.2. Proteins

Several studies have looked into the application of blood protein markers in the detection of lung cancer. For optimal sensitivity panels of proteins have been evaluated, often in combination with clinical data. Proteins include routinely used tumor markers, such as CEA, CA15.3, SSC, CA19.9, NSE and ProGRP but also less common proteins [73–79]. The majority of these studies did not use a screening population, but for example in a population referred based on the suspicion of lung cancer [74], selected retrospective cohorts [73, 77, 78], or patients with pulmonary nodules [75, 77, 79, 80].

In the context of the selection of individuals for screening “the consortium for early detection of lung cancer” evaluated whether a panel of selected protein biomarkers could outperform a traditional prediction model used in US screening (INTEGRAL U19 project) [81]. Based on 108 patients diagnosed with lung cancer 1 year after blood collection and 216 matched controls a biomarker risk score was developed based on CEA, Cyfra21-1, CA125 and a precursor of surfactant B (Pro-SFTPB). In combination with smoking exposure this score increased sensitivity from 43% (smoking history alone) to 63%, at a specificity of 83% [81]. In addition, it was shown that this panel could improve the characterization of indeterminate nodules [82]. This 4-protein panel was further evaluated in combination with a lung cancer risk prediction model (PLCOm2012) to evaluate its impact on the risk assessment for lung cancer screening. Based on 1299 sera collected before lung cancer diagnosis and 8709 non-case sera, the sensitivity of the 4-protein panel was 83.5% with a specificity of 69.3% for the diagnosis of lung cancer within 1 year of the test in the absence of screening vs 77.6% and 65.4%, respectively, for PLCOm2012 risk model alone. The combined approach of 4-protein panel and PLCOm2012 would have identified 9.2% more lung cancer cases and reduce referral by 13.7% among non-cases when compared to the USPSTF2021 criteria [83].

In the context of risk-stratification of indeterminate nodules the PANOPTIC trial evaluated the impact of two plasma proteins (LG3BP and C163A) in 685 patients with 8–30 mm lung nodules and a pre-test probability of cancer of $\leq 50\%$. The proteins were integrated with a clinical risk prediction model to identify likely benign nodules. This approach reached a sensitivity of 97% with a specificity of 44% (NPV of 98%). This classifier performed better than PET-CT, validated lung nodule risk models and physician cancer probability estimates and could reduce 40% of procedures on benign nodules included 3% misclassification of malignant nodules [75]. Data on a direct comparison of this approach to, for example, VDT is not available.

The current data on protein markers suggest a potential role in LCS but the data is mainly based on retrospective selected cohorts. As a result, the performance in a true LCS population is unknown.

3.3. Auto-antibodies and inflammation

Auto-antibodies can develop as a result of abnormal tumor antigens. This reaction to the presence of a tumor was reported to be detectable 3–4 year before symptomatic presentation. A panel of seven autoantibodies (specificly for p53, NY-ESO-1, CAGE, GBU4-5, HuD, MAGE A4 and SOX2) has been evaluated in symptomatic lung cancer patients and a high-risk cohort, resulting in a specificity of 91% and a sensitivity of 34–37% for the detection of lung cancer [84–86]. This EarlyCDT-lung autoantibody test was evaluated in the Early diagnosis of lung cancer Scotland trial (ECLS) to select participants for any CT evaluation [87]. The ECLS enrolled 12,208 individuals at risk for lung cancer, randomizing the participants based on a positive EarlyCDT-lung test to an intervention arm with 6 monthly CT evaluation, while a participant with a negative result would follow standard of care as was

given to the control arm. Adding the EarlyCDT-lung test to any subsequent CT evaluation reduced the incidence of high-stage disease from 73.2% to 58.9% in a 2-year follow-up. In this setting the test reached a 90.8% specificity and a sensitivity of 32%. The absolute risk reduction in the intervention arm was 0.3%. At two years the study did not find a significant reduction in lung cancer mortality and in all-cause mortality. Evaluation of the psychological impact of a positive Early CDT-lung antibody in the selection process towards LDCT was short-lived and only small differences were observed between participants with a positive test vs a negative test [88].

In the German Lung Tumor screening and intervention study (LUSI) the Early CDT-lung antibody test was retrospectively evaluated and reported a limited sensitivity of 13% and a specificity of 88.9–91.1%, by analysing all participants with cancer and 180 selected cancer-free participants [89]. Other test specifications have been evaluated to use this test in nodule management [90]. More recent studies have explored the performance of this panel in a selected cohort of 329 LC patients and 202 non-LC controls in China, showing specificity of 86% and a sensitivity of 48% for the combined autoantibody panel [91]. Data on the performance and impact on a LCS program is only coming from the ECLS study showing a reduction in high stage disease. Although the trial does show promising results, it is difficult to compare to other screening studies due to the lack of a questionnaire-based risk-assessment, and a control arm without a biomarker intervention.

Next to autoantibodies, cancer can trigger a state of inflammation. Several studies have identified an association between inflammatory markers, including C-reactive protein [92], interferon gamma-induced cell immune activation [93], both especially in smokers, and an added value of interleukins in a risk-assessment for LC [94]. In an exploratory study, the predictive performance of a panel of 9 inflammatory proteins (CASP8, CCL11, CDCP1, CD8A, CD244, CXCL10, FGF19, MCP4 and SCF) in combination with pack-years reached AUCs of 0.811 and 0.798, and a specificity of 80% a sensitivity of 73 and 60% in the training and validation sets, respectively [95]. Although most results are coming from limited cohorts more general measurable consequences of cancer could provide information in the setting of an early lung cancer detection.

4. Challenges and opportunities

Lung cancer screening by LDCT has accelerated lung cancer research focusing on early detection. One of the areas of research is the discovery and development of biomarkers for early detection or screening. The presented data on liquid biomarkers underline the (high) potential of biomarkers to support pre-and post-LDCT risk-assessment and to improve overall efficacy of a LCS program. The main challenge of liquid biomarkers is the required sensitivity for small tumors. Several strategies have been developed and evaluated by a) increasing the number of detectable alterations (methylation, fragmentation, panels of proteins, miRNA), b) combining data from different sources, or c) exploiting more general consequences of cancer (inflammation, antibodies). These strategies have recently taken significant steps towards an improved sensitivity.

Liquid biomarkers could potentially improve the performance of an LCS at different steps in the screening program (Table 1). But, considering the data on different biomarkers, a highly fragmented picture arises. First of all, different biomarkers are at different stages of development, ranging from assessment of biomarker performance on retrospective training/validation cohorts to their impact in actual screening populations. The majority of biomarkers have not succeeded in surpassing the first steps of biomarker evaluation and implementation. Secondly, biomarker studies often differ in their intended use of the biomarker (detection, selection or improvement, multi-cancer vs single cancer), their intended screening population (general population vs high-risk population (based on variable criteria), or different populations within a screening program (participants with an indeterminate or

positive LDCT)). These aspects directly influence the required test specifications, while the cohort in which the biomarker is evaluated will influence the test performance. Further challenges are the lack of adequate validation of the pre- and analytic features of new markers. The last challenge is the lack of consensus on how to measure the success of a biomarker in a LCS program, since this can be based on the performance (sensitivity, specificity), reduction in overall cost, improvement of overall efficacy, improved detection of LC, improved survival, and so on. Still, the main determinant of the success of a biomarker will also depend on the specifics of a health-care system (for example US vs Europe). This has contributed to the fact that no biomarker has been implemented in a screening program, so far. A critical appraisal of the opportunities and limitations of the current biomarkers in LCS is difficult. Study design, the intended population, the intended use of a biomarker, the criteria for success vary between reports. This limits the comparability of the presented biomarker data and prevents a proper evaluation of the impact of a biomarker in a LCS program. As a result, a selection of one or more of the most optimal biomarkers is not feasible. From the available data, the image arises that implementation of biomarkers in LCS is not primarily limited by the performance of biomarkers but by the limitations in evaluating and selecting the most promising biomarkers. Taking the biomarker research, a step further will require large clinically annotated cohorts mirroring a true screening population. This will allow a more impactful evaluation of a biomarker and provide information required for implementation of biomarkers in LCS. Biobanking blood samples is crucial as a platform for the evaluation of available and future biomarkers to select the most impactful biomarker for further implementation. At this moment, several initiatives are ongoing to build such platforms. In addition, comparing different biomarkers on a single cohort will also allow further exploration of combinations of data to overcome the challenges of biomarkers in a screening population. This could also include other non-blood biomarkers such as volatile organic compounds in exhaled breath. In order to achieve such a comparison, blood collection and biomarker research should be an integral part of the design and set-up of screening studies.

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Author contributions

DB contributed to the conception, data curation, analysis of data, preparation of the manuscript, HG contributed to the analysis of data, preparation of the manuscript and revision for important intellectual content.

Conflict of interest

DB has no conflict of interest to report, HG has no conflict of interest to report.

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