Determining the clinical utility of a breath test for screening an asbestos-exposed population for pleural mesothelioma : baseline results

Determining the Clinical Utility of a Breath Test for Screening an Asbestos-Exposed Population for Pleural Mesothelioma: Baseline Results

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Abstract

Background: pleural mesothelioma (PM) is an aggressive cancer of the serosal lining of the thoracic cavity, predominantly caused by asbestos exposure. Due to nonspecific symptoms, PM is characterized by an advanced-stage diagnosis, resulting in a 5-year survival rate of <5%. However, early diagnosis is believed to improve patient outcome. Currently, no diagnostic biomarkers or screening tools are available. Therefore, exhaled breath was explored as this can easily be obtained and contains volatile organic compounds (VOCs), which are considered biomarkers for multiple (patho)physiological processes. A breath test, which differentiates asbestos-exposed (AEx) individuals from PM patients with 87% accuracy, was developed. However, before being implemented as a screening tool, the clinical utility of the test must be determined.

Methods: Occupational asbestos-exposed individuals who agreed to be subjected to an annual breath test using multicapillary column/ion mobility spectrometry (MCC/IMS) were included. A baseline breath test was taken and their individual risk of PM was estimated. PM patients were included as controls.

Results: In total, 112 AEx individuals and six PM patients were included in the first of four screening rounds. All six PM patients were correctly classified as having mesothelioma (100% sensitivity) and out of 112 AEx individuals 78 were classified by the breath-based model as PM patients (30% specificity).

Discussion: Given the large false positive outcome, the breath test will be repeated annually for three more consecutive years to adhere to the 'test, re-test' principle and improve the false positivity rate. A low-dose computed tomography (CT) scan in those with two consecutive positive tests will correlate test positives with radiological findings and the possible growth of a pleural tumor. Finally, the evaluation of the clinical value of a breath-based prediction model may lead to the initiation of a screening program for early detection of PM in asbestos-exposed individuals, which is currently lacking.

Keywords: Pleural mesothelioma, Screening, Biomarker, Early detection, Breathomics, Volatomics, Asbestos

1. Introduction

Pleural mesothelioma (PM) is an aggressive cancer of the serosal lining of the lungs and chest cavity, predominantly caused by asbestos exposure [1]. Although the use of asbestos was prohibited in most western countries by the end of the 20th century, the long average latency period of up to 50 years between first exposure and diagnosis will extend the present high plateau of mesothelioma incidence in Western Europe to the following decades [2]. Moreover, no safe threshold for asbestos exposure has been established below which PM will not occur [3, 4]. Additionally, 2 million tons of asbestos are still produced and utilized globally each year, highlighting that PM will remain relevant in populations worldwide [2]. Despite promising advances in treatment [5], PM remains an incurable disease with a poor prognosis. This is partly attributed to nonspecific symptoms that manifest late in the disease's course [6]. Consequently, PM is typically detected in the late stages of the disease, resulting in a five-year survival rate of 12% [7]. Furthermore, while the presence of pleural abnormalities on low-dose computed tomography (CT) imaging may be indicative of disease and warrant further investigation, a definitive diagnosis of PM currently requires an invasive procedure, which involves the collection of either a core needle aspiration of a surgical biopsy [8].

Hence, it is critical to investigate innovative methods for early detection, assuming that therapy is more effective if initiated earlier [1], and ultimately resulting in a better prognosis of PM. Notwithstanding the search for PM biomarkers has been of great interest for many decades [9-12], none have been found sensitive enough for the early detection of mesothelioma.

Therefore, volatile organic compounds (VOCs) in exhaled breath were investigated to serve as non-invasive biomarkers for pleural mesothelioma, as shown in Figure 1. Previously, a breath test based upon multicapillary column/ion mobility spectrometry (MCC/IMS) was developed by our research group, differentiating PM patients from former occupational asbestos-exposed (AEx) and non-exposed individuals (healthy controls) with 87% accuracy [13].

The findings of this proof of principle study were subsequently replicated by our research group in a follow-up study including additional participant groups such as patients with benign asbestos-related diseases (ARD), benign lung diseases unrelated to asbestos exposure (BLD) and primary lung cancer (LC) next to PM patients and AEx individuals [14]. The model differentiated PM patients from AEx controls with 87% accuracy, as estimated by Leave-One-Out Cross Validation (LOOCV). When combining AEx and ARD patients in one group, a model with 94% sensitivity and 96% negative predictive value (NPV) was obtained [14]. The high sensitivity and NPV of this model showed the potential of breath analysis to screen individuals at risk for developing PM. Eleven VOCs were selected by the regression to be included in this prediction model, namely VOC1, VOC7, VOC9, VOC15, VOC21, VOC26, VOC84, VOC88, VOC101, VOC122, and VOC236.

The clinical validity of the IMS-based breath model was recently assessed by externally validating and updating the model that has been developed in previous studies [15]. Therefore, a multicenter study was set up where PM patients, lung cancer patients, asbestos-exposed individuals and patients with benign asbestos-related diseases were included. The model was updated by fitting a new lasso regression to the external validation cohort using the subset of 11 VOCs as input variables. By updating the model, 4 VOCs were retained as important predictors (P9, P88, P101 and P122), which improved the model's performance and led to simplification of the model by reducing the number of features. Moreover, this reduction in predictors aids in reducing the risk of overfitting. The retention times (RT) and inverse reduced ion mobility ($1/K_0$) values of these 4 predicting VOCs have previously been published by our research group [14, 15]. The improved model performance led to the differentiation between PM patients and AEx controls with 73% accuracy, 92% sensitivity and 92% NPV [15].

However, before the updated VOC-based model can be used as a diagnostic tool, it needs to be externally validated in turn and, additionally, its clinical utility of detecting early-stage PM is yet to be prospectively determined. Therefore, over the course of a 4-year follow-up, individuals exposed to asbestos will undergo yearly breath sampling, as shown in Figure 2, in orde to perform external validation of the model.

The trained lasso model, which was previously updated by our research group [15], will be applied on this new group of asbestos exposed individuals and will predict the probability of each particpant to be developing a pleural tumor. A correlation between the breath profiles and paired low-dose CT will be done to match test outcomes with radiological findings. This study aims to cross-sectionally externally validate the model at baseline.

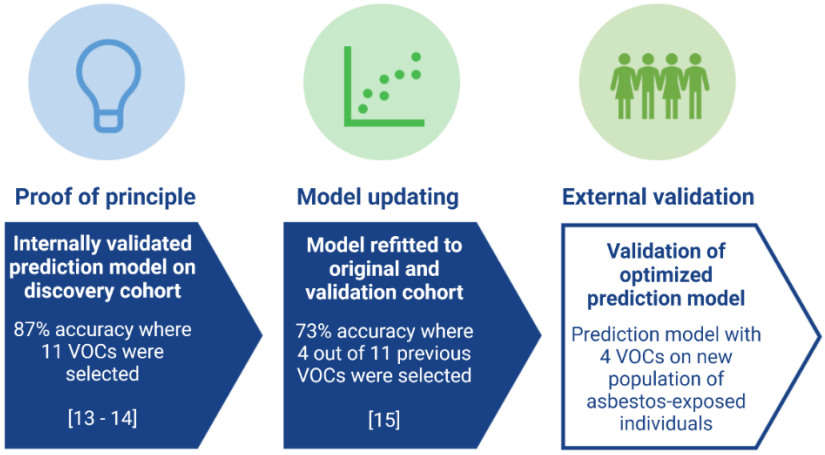

Figure 1. Overview of earlier performed research. A previously internally validated prediction model, based upon 11 volatile organic compounds (VOCs) was updated subsequently, where the amount of predictors was reduced to 4, and will be externally validated in the current research.

2. Materials and Methods

2.1 Study Design and Participants

A prospective, multicenter study was set up and carried out in compliance with the Helsinki Convention. The study was authorized by the ethical committee of the Antwerp University Hospital (Belgian registration number B300201837007). PM patients were included after referral through the Thoracic Oncology department of the Antwerp University Hospital (Belgium). Patients were treatment-naive at the time of inclusion and PM diagnosis needed to be histologically verified. AEx individuals were recruited through collaboration with the departments of occupational medicine of three Belgian companies that previously worked with asbestos until 1997. Due to the long latency period between first asbestos exposure and PM development, we included an at-risk population of AEx individuals with substantial professional asbestos exposure with first exposure starting at least 25 years or longer ago.

In order to determine whether the inclusion criteria were satisfied and to gather participant information about demographics and asbestos exposure, participants were asked to provide written informed consent and complete two questionnaires.

2.2 Exhaled Breath Sampling

According to a previously validated protocol [13, 14], breath was collected and analyzed using a BioScout system, consisting of an MCC/IMS (BreathDiscovery 2nd Generation, B&S Analytik, Dortmund, Germany) with an integrated breath sampler (SpiroScout; Ganshorn Medizin Electronic, Niederlauer, Germany) connected by a sample loop. Briefly, volatiles are pre-separated by a non-polar OV-5 MCC column before being ionized by a 95MBq ^{63}Ni-radiation source. Subsequently, under the influence of an electrical field and a counter gas (α1-nitrogen gas (99.999% pure); Air Liquide, Belgium), the ionized breath components enter a 12 cm drift tube where a second separation occurs based on their ion mobility. The VOCs finally collide on a Faraday plate detector, causing an electrical current generating a peak that is proportional to the concentration of the VOC.

Participants were instructed to fast and refrain from smoking for at least two hours prior to sampling to comply to the European recommendations to sample exhaled VOCs [16]. Participants were additionally requested to rinse their mouths with distilled water and put on a nose clip and rubber gloves. Participants must breathe normally through the mouthpiece of the SpiroScout, which is attached to a bacterial filter, a viral filter and the MCC/IMS sample loop, while seated upright and without the use of any forced breathing techniques. Utilizing capno-volumetry, the SpiroScout device detects CO_2 in exhaled breath and initiates breath sampling upon reaching a plateau in CO_2 levels, signifying the collection of alveolar air. Using this technique, 10 mL of alveolar air will be collected and transferred to the MCC/IMS for analysis after three minutes. A background sample was taken by sampling 10 mL of room air after each breath sample.

Disposable mouthpieces and filters were used to reduce external contamination, and the MCC/IMS equipment was flushed with humid air to remove any potential pollutants between samplings of various participants.

2.3 Data Processing and statistical analysis

Breath and background samples were analyzed using VisualNow software (B&S Analytik, Dortmund, Germany). Firstly the raw 2D chromatograms were de-noised through baseline correction and aligned. Subsequently, the data was normalized to the reactant ion peak (RIP) and RIP-tailing was compensated. After smoothing the data, VOCs were then manually selected and a list with peak intensities for each

VOC in each sample was generated. The alveolar gradient was calculated for each selected VOC by subtracting the peak intensity in the background sample from the peak intensity in the corresponding breath sample in order to limit external confounding.

To externally validate the previously updated model [15], which utilized four VOCs to distinguish between PM patients and AEx participants, the calculated alveolar gradient values of these VOCs were employed to the prediction model to predict the probabilities of PM in an independent AEx and PM population. This validation process involved assessing various performance metrics, including sensitivity, specificity, NPV, positive predictive value (PPV), and accuracy, to evaluate the accuracy and reliability of the model's predictions. By comparing the predicted probabilities with the actual outcomes of participants, we were able to quantify the model's accordance and assess its discrimination characteristics. This comprehensive validation approach provides robust evidence supporting the reliability and clinical utility of the updated VOC-based model.

3. Results

3.1 Participant Characteristics

The validation group included 118 participants: six PM patients and 112 AEx controls (Table 1). The groups were matched regarding smoking status and packyears. In comparison to the AEx controls, PM patients were significantly older and had a significantly lower BMI. Additionally, there were significant differences in sex between PM patients and AEx participants.

Table 1. Overview of the baseline clinical characteristics of the two participant classes.

	PM	AEx	*p*-value
Subjects (N)	6	112	
Sex			
Male (%)	4 (67%)	112 (100%)	0.002 [a]
Female (%)	2 (33%)	0 (0%)	
Smoking status			
Never (%)	3 (50%)	50 (45%)	
Current (%)	1 (17%)	8 (7%)	0.613 [a]
Ex (%)	2 (33%)	54 (48%)	
Packyears	13.35 (0.00–55.00)	9.21 (0.00–87.00)	0.807 [b]
BMI (kg/m²)	24.23 ± 2.74	27.42 ± 3.71	0.042 [c]
Age (years)	69.59 ± 10.16	60.61 ± 6.60	0.002 [c]

Values are presented as n, mean ± SD or median (Q1–Q3). AEx: asbestos-exposed; PM : pleural mesothelioma. a: Fisher's exact test; b: Mann–Whitney U test; c: T-test.

3.2 Assessment of clinical utility

The previously updated model [15] was applied to the breath samples of 112 asbestos-exposed individuals in order to assess its clinical validity, the classification outcome and model characteristics were determined (Tables 2 and 3). At baseline, all six PM patients were correctly classified by the model as having PM, meaning the model has 100% sensitivity. This is accompanied by a 100% NPV, making sure that a negative test rules out PM, enriching those at risk for further follow-up. 78 out of 112 AEx were additionally classified by the model as PM patients, leading to a specificity of 30% and a PPV of 7%.

Table 2. Classification after applying the breath-based prediction model after the first measurement of the 4-year external validation.

		True outcome		
		PM	**AEx**	**Total**
Test outcome	**PM**	6	78	84
	AEx	0	34	34
	Total	6	112	118

AEx: asbestos-exposed; PM : pleural mesothelioma.

Table 3. Performance characteristics of the breath-based prediction model after the first measurement of the 4-year external validation.

	Values (95% confidence interval)
Sensitivity	1.000 (0.652 – 1.000)
Specificity	0.301 (0.222 - 0.390)
PPV	0.081 (0.037 - 0.154)
NPV	1.000 (0.916 – 1.000)
Accuracy	0.342 (0.261 - 0.430)

NPV: negative predictive value; PPV: positive predictive value.

4. Discussion

The field of breathomics has yielded numerous studies using VOCs as an innovative and non-invasive early detection technique for pleural mesothelioma [13-15, 17-20].

In 2010, de Gennaro et al. [19] used GC-MS to analyze breath samples from MPM patients, asbestos-exposed individuals, and healthy controls, finding dominant compounds like cyclopentane and cyclohexane. Dragonieri et al. [21] validated breathprint analysis for MPM screening using eNoses and GC-MS. Subsequently, Chapman et al. [22] achieved 95% accuracy in differentiating MPM patients from control subjects using electronic nose technology. Lamote et al. [13, 14] successfully distinguished MPM patients from asbestos-exposed individuals. Lastly, Di Gilio et al. [20] identified 10 diagnostic VOCs using GC-MS to discriminate between MPM patients and healthy controls.

However, the lack of comprehensive validation studies of these small-scale findings in larger populations has hindered

the implementation of VOC-based prediction models in clinical practice. This oversight represents a significant waste of research resources and time, considering the potential of VOCs as non-invasive biomarkers. Therefore, we aim to perform a long-term follow-up study to externally validate a previously internally validated [14] and optimized [15] prediction model by our research group, by applying it to an external asbestos-exposed validation cohort.

Following the analysis of the baseline measurement, it was observed that all patients diagnosed with PM were accurately classified as having the disease. Consequently, the breath-based prediction model exhibited 100% sensitivity, indicating its ability to correctly identify all individuals with PM. Moreover, the NPV of the model was also determined to be 100%, suggesting that none of the MPM patients were misclassified as disease-free based on the breath-based prediction model. Due to the breath test's high sensitivity and NPV, the breath test lends itself to be used in screening programs based upon a ruling-out principle, namely to omit AEx people who are not yet developing disease from further invasive and high-cost follow-up, such as CT scan. This ruling-out method is of high relevance in diseases where a large at-risk population is clearly defined and the lifetime risk of developing the disease is low, such as seen in AEx populations developing PM. This real-world expectation is reflected in our small sample size of PM patients.

Additionally, 78 out of 112 AEx were classified by the model as PM patients, leading to a specificity of 30% and a PPV of 7%. The occurrence of these false positives could be attributed to a multitude of reasons, such as the presence of pleural deviations, (asbestos-related) inflammation, development of other types of thoracic cancers or the actual early development of PM in some of the AEx participants in an asymptomatic phase. In order to lower the false positive rate, the 'test, re-test' approach will be applied [23]. According to this 'test, re-test' principle, we will optimize the current ruling-out characteristics of the breath test: the chance of being false positive after two positive consecutive tests drops to 48%, while the chance of being false negative is 0%.

When a patient has a positive test result twice in a row, we will perform a low-dose CT scan to evaluate the clinical value of the breath test. This will link test positives with radiological findings and the possible growth of a pleural tumor, helping to determine whether the breath test can be used as an enrichment tool for those with increased risk for PM before imaging. Considering that 78 out of 112 AEx individuals had positive test results, we anticipate that about half of the study population will have successive positive results in the second screening round, meaning 54 individuals are expected to undergo a CT scan. A chest radiologist and radiology resident, with expertise in the field of thoracic oncology, will review the radiographic data on a Picture Archiving and Communication System (PACS) in consensus.

Depending on the outcome of the CT scan, these individuals will then be referred for further diagnostic work-up when PM is suspected (thoracoscopic biopsy) or remain in the annual follow-up with breath tests. If PM is detected, staging will be done according to the 8th TNM staging criteria of the International Association for the Study of Lung Cancer (IASLC) [24]. Although research has been conducted on the use of (low dose) CT imaging as a screening tool for mesothelioma in an asbestos-exposed population [25-27], none have found serial imaging alone to have an effect on early diagnosis of participants. Moreover, some found lung cancer to be more prevalent in a population of asbestos-exposed individuals than PM. However, in combination with a validated VOC profile, there may be a use for CT screening in a population exposed to asbestos.

The breath test will be repeated for 3 more consecutive years. Conducting annual screenings over a 4-year period was justified due to the long latency period of PM, the lifetime risk of developing pleural mesothelioma of approximately 5-10% [28] and in accordance with empirically determined screening intervals observed in occupational medicine for other types of cancer. Moreover, annual screenings will provide an opportunity to track potential disease progression, and consequently the changes in breath pattern. Choosing this annual screening frequency strikes a balance between timely detection and resource optimization, as more frequent screenings may be burdensome, less practical and increase costs, while still allowing for an effective clinical design. The specific time window between consecutive tests may be subject to refinement based on the outcomes of our study as the potential rapid growth of PM may warrant a smaller window-of-opportunity to detect PM in an early stage of disease.

Even though we found promising results in the external validation of the breath-based model, some limitations should be adressed.

Firstly, although AEx and PM groups were matched for smoking status and pack years, significant differences were observed between the groups in age (p-value: 0.002), sex (p-value: 0.002) and BMI (p-value: 0.042). Although our previous research concluded that none of the 4 predictors of the current optimized model were correlated with sex, BMI or age, we cannot exclude the possibility that these confounding factors may affect the breath patterns of the included individuals, as some studies have found certain VOCs to be age-specific [29] or associated with sex [30]. Moreover, other confounding factors, such as air pollution [31], medications [32], and cigarette smoke [33], have been shown to adjust the VOC composition of exhaled breath. Therefore, we followed European guidelines [16] to standardize our breath sampling method, asking participants to adjust their dietary and smoking patterns at least 2 hours before sampling their breath. However, whether the elimination of these factors will

improve reliability of the exhaled VOC patterns, is still to be determined [16].

Additionally, variations in VOC pharmacokinetics due to ambient air contaminants may introduce a confounding factor [34, 35]. While no standardized method fully excludes this factor, we have implemented a literature-supported methodology [2]. However, we mitigate confounding by excluding low-threshold VOCs and those at background levels, focusing on group differences rather than individual variations to reduce bias.

Within the context of limitations, it is important to acknowledge the specific clinical setting of the breath test screening as a ruling-out tool for an asbestos-exposed population. The test's sensitivity of 100% and NPV of 100% offer valuable reassurance in ruling out malignant asbestos-related disease. Combining the breath test with potential follow-up through CT scans can further enhance the screening process and improve diagnostic accuracy over time. The longitudinal nature of the screening, with repeated breath tests, allows for tracking changes and potential disease progression, ultimately enabling timely interventions while minimizing unnecessary CT scans.

Lastly, MCC/IMS, which does not allow the determination of the exact chemical identity of the discriminating VOCs, as it provides a "pseudo-identification", was used as a breath analysis technique. MCC/IMS offers advantages such as fast analysis, high sensitivity, portability, and simplicity in clinical settings [36]. Furthermore, it allows direct breath sampling and is user-friendly. These benefits make MCC/IMS an appealing method for clinical use, enabling potential illness diagnosis based on peak pattern recognition without the need for additional chemical identification.

Despite these limitations of our research, our study demonstrates the potential of breathomics and VOC analysis as a non-invasive and promising approach for the early detection of pleural mesothelioma in asbestos-exposed populations. By externally validating our previously optimized breath-based prediction model, we have achieved a high sensitivity and NPV, offering valuable reassurance for ruling out disease and reducing the need for invasive and costly follow-up procedures.

5. Conclusion

A breath-based model independently detected PM patients with 100% sensitivity. Given the high NPV, a negative test allows us to rule out disease in those at risk and exclude these persons for further (invasive) diagnostic procedures. However, the false positive rate is high, which enriches those persons who may be at increased risk for developing PM. In order to assess this, the breath test needs to be repeated over time to a population exposed to asbestos and the results of these breath samples need to be correlated to the corresponding CT scans. This can potentially allow early-stage diagnosis of a (pleural) cancer and initiate a screening program for PM in asbestos-exposed individuals, which is currently lacking.

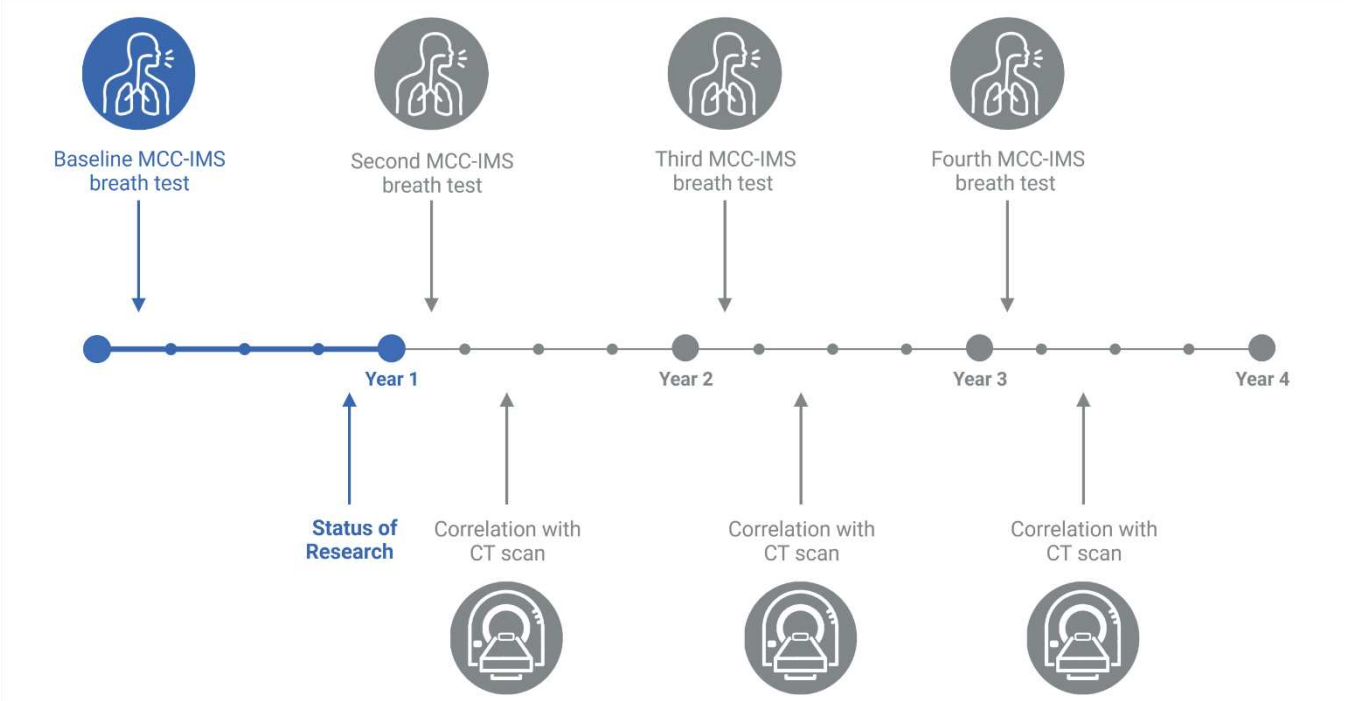

Figure 2. Timeline of study protocol of the current research. Baseline measurements with multicapillary column/ion mobility spectrometry (MCC/IMS) of the asbestos-exposed group have been performed once and will be repeated yearly three

consecutive times. Additionally, correlation with low-dose computed tomography (CT) scan will be performed after the second sampling round.

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Informed Consent Statement

Written informed consent was obtained from all participants included in the study.

Institutional Review Board Statement

The study was conducted in accordance with the Declaration of Helsinki and approved by the Ethics Committee of the Antwerp University Hospital (Belgian registration number B300201837007) on 12 April 2022.

Conflicts of interest

The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript; or in the decision to publish the results.

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