Exhaled breath to screen for malignant pleural mesothelioma : a validation study

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Take Home message: Breath analysis can be used to screen for malignant pleural mesothelioma in high-risk asbestos-exposed persons.

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Abbreviations list:

AEx	persons with previous professional asbestos-exposure
ANOVA	analysis of variance
ARD	patients with benign asbestos-related diseases
AUC_{ROC}	area under the receiver operating characteristic curve
BLD	patients with benign, non-asbestos related diseases
GC-MS	gas chromatography – mass spectrometry
HC	healthy control
LC	lung cancer patient
MCC-IMS	multicapillary column – ion mobility spectrometry
MPM	malignant pleural mesothelioma
NPV	negative predictive value
PPV	positive predictive value
PTFE	polytetrafluoroethylene
RIP	reactant ion peak
VOCs	volatile organic compounds

Abstract

Malignant Pleural Mesothelioma (MPM) is predominantly caused by previous asbestos exposure and is characterised by a poor prognosis. Breath contains volatile organic compounds (VOCs) and can be explored as early detection tool. Using multi-capillary column/ion mobility spectrometry (MCC/IMS), we previously discriminated with large accuracy MPM patients from asymptomatic high-risk persons. Our aim is to validate these findings in different control groups.

Breath and background samples were obtained for analysis from 52 MPM patients, 59 asymptomatic former asbestos (AEx) workers, 52 healthy non-asbestos exposed (HC) persons, 41 patients with benign asbestos-related diseases (ARD), 70 patients with benign non-asbestos-related lung diseases (BLD) and 56 lung cancer (LC) patients. After background correction, logistic lasso regression and ROC analysis, MPM patients could be discriminated from HC, AEx, ARD, BLD and LC patients with 65%, 88%, 82%, 80% and 72% accuracy, respectively. Combining AEx and ARD patients resulted in 94% sensitivity and 96% negative predictive value (NPV). The most important VOCs selected were P1, P3, P7, P9, P21, and P26.

We discriminated MPM patients with great accuracy from at risk subjects. The high sensitivity and NPV allow to use breath analysis as a screening tool for ruling out MPM.

Introduction

Malignant pleural mesothelioma (MPM) is a tumour from the serosal lining of the thorax, predominantly linked to previous asbestos exposure[1, 2]. Despite the European ban on the use of asbestos in 2005[3], asbestos is still being mined and imported in countries in need for industrial growth and, hence, remains an important worldwide health issue[4]. The incidence of MPM is expected to further increase fuelled by the large amount of asbestos consumed in the past and the long mean latency period of 40-50 years between first exposure and incidence date. MPM is usually diagnosed in an advanced stage and requires an adequate tissue sample, often to be obtained through an invasive biopsy[5, 6]. This delayed diagnosis results in a poor outcome with a best-observed median overall survival in highly selected patients of up to 18 months with standard of care platinum-based chemotherapy in combination with bevacizumab[7, 8]. Taken together, this stresses the importance for screening tools for early detection, where treatment options are believed not to be restricted to a palliative setting[9].

There are currently no uniformly agreed guidelines on screening for MPM in asbestos-exposed persons[10], and this lack results in an inappropriate and 'blind' use of different imaging techniques and blood tests. Present research efforts have not yet resulted in a validated diagnostic blood biomarker like soluble mesothelin-related peptide (SMRP) or fibulin-3[11-13]. So, there is an unmet need for a first pass 'rule out' test which can help to separate asbestos-exposed persons with low risk and no need for further screening studies from those with an early stage MPM, requiring further work up and treatment. The analysis of volatile organic compounds (VOCs) in breath is a promising tool in this prospect[14]. A proof-of-concept study using gas chromatography-mass spectrometry (GC-MS) has identified cyclohexane as discriminator of breath samples of MPM patients from occupational asbestos-exposed (AEx) and healthy non-exposed (HC) controls with 97.4% accuracy[15]. Other series using pattern recognition with cross-reactive sensor technology discriminated MPM patients from

asymptomatic AEx persons with acceptable accuracy[16, 17]. Using ion mobility spectrometry (IMS), patients with benign asbestos-related diseases (ARD) were discriminated from healthy controls with 99.9% accuracy[18] and our research group discriminated MPM patients from HC controls and AEx patients with 82% and 87% accuracy, respectively[19]. The goal of the present study was to extend previous research, validate these earlier findings in a larger population and to determine the specificity of VOC analysis for the detection of MPM compared to lung cancer.

Materials and Methods

Study design and participants

This is a multicentre, cross-sectional, case-control study, approved by the Institutional Review Board of Ghent University Hospital (Belgian registration number B670201111954) and conducted in accordance with the Helsinki Convention. Before inclusion, participants had to give their written informed consent. Healthy controls (HC), patients with benign asbestos-related diseases (ARD), patients with benign lung diseases (BLD) unrelated to asbestos exposure, primary lung cancer (LC) patients and pleural mesothelioma (MPM) patients were randomly recruited via the departments of respiratory medicine of the University Hospitals of Ghent, Leuven and Antwerp (Belgium) and the OLV Hospital in Aalst (Belgium). Other ARD patients and asymptomatic persons formerly exposed to asbestos fibres (AEx) were recruited via the occupational health service of a company using asbestos until 1997. MPM cases needed to be confirmed by the Belgian Mesothelioma Pathology Panel. MPM and LC patients had to be treatment-naïve and were included in between diagnosis and start of treatment. Benign asbestos-related diseases were not allowed to be present in any of the control groups except for the ARD patients. A recent CT-scan or chest X-ray (<12 months) was mandatory to confirm the medical condition. Participants had to refrain from eating, drinking and smoking for at least 2 hours before the breath sampling. Participants had to complete questionnaires to check the inclusion criteria and to collect demographical and previous asbestos-exposure data. For patients, a detailed patient record about their medical condition had to be available.

Breath Sampling and Analysis

For sampling, a SpiroScout breath sampler (Ganshorn Medizin Electronic, Niederlauer, Germany) was connected to the sample loop of a BioScout multicapillary column/ion mobility spectrometer (MCC/IMS; B&S Analytik, Dortmund, Germany)[14, 19]. Breath samples were

obtained between January, 2012 and December, 2014. After resting at least 10 minutes, all participants were asked to rinse their mouth with distilled water and put on rubber gloves and a nose clip. While sitting in an upright position and without any forced breathing manoeuvres, they breathed normally through the SpiroScout's mouthpiece, connected to a bacteria filter and the MCC/IMS sample loop. After 3 minutes, 10 ml of alveolar air was sampled and sent to the MCC/IMS for analysis. Details about the breath analysis protocol are described elsewhere[14, 19]. In short, the breath analytes were pre-separated by a non-polar OV-5 MCC column (Multichrom Ltd, Novosibirsk, Russia), after which they became ionised by a 95MBq ^{63}Ni β-radiation source. Subsequently, the ionised breath compounds entered a 12 cm drift tube where a second separation takes place based upon their ion mobility characteristics under the influence of an electrical field and a counter gas (α_1-nitrogen gas; 99.999% pure; Air Liquide Medical, Schelle, Belgium). Finally, the VOCs collided on a Faraday plate detector, evoking an electrical current, which resulted in a VOC peak intensity (Volt, V) that correlates to the VOC's concentration. MCC/IMS is a technique that allows a 'pseudo-identification' of VOCs based upon their retention time and ion mobility and cross-checking with an MCC/IMS database. However, for definite identification, the MCC/IMS data needs to be crosschecked with GC-MS analysis of VOCs. After breath sampling, a background sample was taken using the same materials and sampling conditions. In order to rule out external contamination or sampling artefacts, we used disposable mouthpieces and filters and the MCC/IMS was constantly flushed with α_1-nitrogen gas. All unheated sample lines are made of Teflon (PTFE), known not to retain compounds[20]. Between the breath sampling of different participants, the MCC/IMS was flushed with humid air to remove contaminants and to make sure that IMS-chromatograms were clean.

Statistics

VOC analysis was done with VisualNow v3.7 software (B&S Analytik, Dortmund, Germany) as previously described[19]. In short, the raw IMS-chromatograms were denoised through baseline correction using a 5x3 low pass filter and aligned[19, 21]. The data was subsequently normalized to the reactant ion peak (RIP) and compensated for RIP-tailing by subtracting a median spectrum from each chromatogram within the data set[19, 21]. Next, the data was smoothed and the chromatograms were inspected visually for the presence or absence of VOCs. If a VOC was present in either breath or background sample, these were manually selected and analysed (N=250), resulting in a list of VOC-peak intensities (maximum peak height in the selected peak area).

To remove the effect of environmental chemical confounders, the alveolar gradient was calculated for every VOC by subtracting the standardized peak intensity in the background samples from the standardized peak intensity in the corresponding breath samples[22]. These alveolar gradient intensities (Volt, V) were used in R (v3.3.1) as predictors[23]. Because of the high dimensionality setting (large number of variables/low number of samples), penalized logistic regression (lasso) was used to discriminate MPM patients from AEx and HC controls. We used the *glmnet* R-package (v2.0-2) for fitting binomial lasso logistic models[24]. Using the predicted outcomes of all of the patients, we then constructed a ROC curve and estimated sensitivity, specificity, positive (PPV) and negative predictive value (NPV) and the diagnostic accuracy of the final model and their 95% confidence intervals. Furthermore, we had a look at the number of times (folds) a VOC was selected by the lasso regressions. We opted to consider variables selected in a large proportion of folds (>50%) as important, as previously described[19]. Furthermore, since asbestos-exposed persons are at risk for MPM, we examined if MPM patients could be discriminated from ARD and pooled AEx and ARD participants. We also compared MPM patients to BLD and LC patients and LC patients to HC, AEx and BLD controls.

Summary statistics of the continuous variables were calculated. A Fisher's exact test was used to test whether the categorical outcomes were equally likely. For continuous variables, a Kolmogorov-Smirnov test was performed to assess normality and, subsequently, an ANOVA or Kruskal-Wallis test was performed to assess differences of means or distributions, respectively. Bonferroni-adjusted p-values below 5% were considered statistically significant.

Results

Patient characteristics

In total, 330 participants were included in the study: 52 HC controls, 59 asymptomatic AEx persons, 41 ARD patients, 70 BLD patients, 56 primary LC patients and 52 MPM patients (Table 1). The BLD patients consisted mostly of patients with COPD (40%), cystic fibrosis (21%) and pneumonia (14%). There were significantly more males in the MPM, AEx and ARD groups compared to the other groups and the patient groups were significantly older than the HC and AEx group. There were more current smokers in the AEx, ARD, BLD and LC groups.

Breath analysis

MPM patients could be discriminated from HC controls with 65% accuracy, 89% sensitivity and 79% NPV (Table 2, Figure 1). We discriminated MPM from AEx patients with 88% accuracy, 87% sensitivity, 90% specificity, 88% PPV and 88% NPV. The AUC_{ROC} was 0.879. MPM patients could be discriminated from ARD patients with 82% accuracy. The sensitivity, specificity, PPV and NPV were 89%, 73%, 81% and 83%, respectively. The AUC_{ROC} was 0.850. Furthermore, pooling both groups allowed to discriminate MPM patients with 85% accuracy, 94% sensitivity, 80% specificity, 71% PPV and 96% NPV. The AUC_{ROC} was 0.890. MPM patients were also nicely discriminated from BLD patients with 80% accuracy. The MPM

patients were discriminated from LC patients showing 72% accuracy, 73% sensitivity, 71% specificity, 70% PPV and 74% NPV. The AUC_{ROC} was 0.770 (Table 2, Figure 1).

Furthermore, we were not able to discriminate AEx from ARD controls, even when many VOCs were included in the models (Table 3, Figure 2). BLD patients were discriminated from AEx and ARD patients with 90% and 85% accuracy, respectively.

LC patients were discriminated from HC controls with 71% accuracy, 77% sensitivity, 65% specificity, 71% PPV and 72% NPV. Furthermore, LC patients were also discriminated from AEx and BLD participants with 90% and 71% accuracy, respectively (Table 3, Figure 2).

By lasso regression, the most important VOCs selected to discriminate MPM from the at risk groups and BLD patients were P1, P3, P7, P9, P21, and P26 (Table 3, Table 4, Supplementary figure S1). These were not selected when discriminating MPM from HC controls (Table 2), stressing their importance as markers for MPM. These VOCs were also used to discriminate MPM from LC patients, but this did not allow to discriminate both patient groups with large accuracy, suggesting a more common VOC signature.

Discussion

In this multicentre, cross-sectional, case-control study, we showed that breath analysis by MCC/IMS discriminated MPM patients from AEx persons, and ARD and BLD patients with clinically relevant accuracy. The lifetime risk for MPM in persons with occupational asbestos exposure is approximately 10% and can be higher in persons who directly processed asbestos fibres [25]. We examined if MPM patients could be discriminated from people with known past asbestos-exposure (healthy or with benign asbestos-related stigmata) and confirmed that MPM patients were discriminated from these AEx and ARD patients with 88% and 82% accuracy, respectively. Strengthened by the fact that we could not separate both groups, we examined the screening capability of the breath test in the combined AEx and ARD groups. The resulting accuracy was 85%, sensitivity 94% and NPV 96%. Although screening studies for diagnostic purposes typically require a large specificity[26], the PPV and NPV are more clinically meaningful because their interpretation is more straightforward[27]. Therefore, the high sensitivity and NPV from our comparisons make them a powerful tool for ruling out the disease in a true negative population. This will exclude persons from further investigations and could help to diagnose MPM in a more cost-effective manner. Compared to blood biomarkers, our results outperform those of mesothelin (SMRP) and fibulin-3[11, 13]. A meta-analysis showed that, at 95% sensitivity, the specificity of SMRP was only 22%, limiting its property to rule out disease, and, when using a high specificity of 95% to rule in the diagnosis in high-risk individuals, its sensitivity of 33% falls clearly too short[11]. Furthermore, a recent meta-analysis of fibulin-3 showed only a modest value for discriminating MPM from cancer-free controls with a 62% sensitivity and 82% specificity[13].

The strength of the present study lies within its inclusion and comparison of multiple control groups from a large number of participants. It hence serves as the final proof-of-concept by confirming and validating all previous research on breath analysis for MPM screening in small

sample sizes. With GC-MS, de Gennaro *et al.* discriminated MPM patients from AEx and HC persons with 97.4% accuracy and identified cyclohexane as a marker for MPM[15]. Using pattern recognition, Dragonieri *et al.* distinguished the same groups with 80.8% and 84.6% accuracy, respectively[16] and Chapman *et al.* discriminated MPM patients from HC subjects and ARD patients with 88% accuracy[17]. However, these studies using pattern recognition did not identify VOCs, a technical drawback of electronic noses. Using MCC/IMS, Cakir *et al.* discriminated ARD patients from HC with 96% sensitivity and 50% specificity and identified alpha-pinene and 4-ethyltoluol as markers for asbestos-related diseases[18], where we previously discriminated MPM patients from AEx and HC subjects with 87% and 82% accuracy, respectively[19]. We found P3, P5, P50 and P71 as most important VOCs in these discriminations, of which P3 is confirmed in this present study. However, MPM patients were modestly discriminated from LC patients, a characteristic also observed with SMRP[11]. This could be due to the fact that VOCs reflect underlying inflammation. Since inflammation is one of the hallmarks in cancer[28], these VOCs could be more general cancer markers rather than be tumour-specific. However, the observation of a modest discrimination between MPM and LC patients and that the VOCs used for this discrimination are mostly different from those used to discriminate MPM from the AEx and ARD groups, suggests that at least some VOCs might be able to discriminate between the different tumour types.

Despite these satisfying results, we acknowledge some limitations. First, since this is no randomized study, and the groups were not matched for age, gender and smoking status. MPM and LC patients were significantly older which could be explained by the latency period between first exposure to the causal agent and the diagnosis of these diseases and by the fact that age-matched healthy controls without significant comorbidities are hard to find. Although some studies suggest that aging has an effect on human metabolism and VOCs[29-31], other

groups did not find this correlation[32-34]. Furthermore, a higher incidence of males was seen in the groups with asbestos exposure. This can be explained by the fact that the asbestos industry had a male predominance. This industry also explains the difference in smoking status since this blue-collar industry is known to have a higher incidence of current smokers[35, 36]. Furthermore, since smoking is the main causal agent of lung cancer, it was expected to have the highest incidence of current smokers in our study. However, we do not believe smoking had any impact on the modelling considering MPM pathogenesis is independent of smoking. This is further strengthened by the fact we could not satisfactorily discriminate MPM patients from LC patients. Furthermore, we did not include patients with secondary malignant pleural effusions, which can impede the differential diagnosis of MPM. Hence, important compounds to differentiate patients with MPM from those with secondary malignant effusions could be missed. Future validation research should include this group of patients in order to optimize the screening test.

Secondly, we cannot fully exclude the possibility that external VOCs could have influenced the breath samples, despite the fact that we took background samples for correction. Dependent on their kinetics, inhaled VOCs can be stored in the body's fat compartments and slowly released over time[37, 38]. Although we have tried to counteract environmental contamination as much as possible by using inert sampling materials and by calculating the alveolar gradient of the VOCs[22], this may not be sufficient to completely remove the impact of environmental confounders. However, since patients and controls were randomly sampled and, hence, also the background samples, the effects of contamination were excluded as much as possible and can be expected to be minimal.

Lastly, our selected VOCs have not yet been identified and are not included in the MCC/IMS VOC-library. For definite identification of the VOCs, the MCC/IMS data needs to be crosschecked with an additional GC-MS analysis of the suspected VOCs. This has no impact

on the actual discriminating accuracy but further identification of the VOCs should allow us to link these to MPM pathogenesis and serve as additional proof. Therefore, GC-MS analysis of VOCs in breath and in the headspace of MPM cell lines is advocated.

In summary, we here show that MCC/IMS allows adequate discrimination of MPM patients from at-risk individuals. Given the fact that the present study describes an extension cohort of our previous proof-of-principle study, future research should now focus on the external validation in a prospective, case-control series in independent patient cohorts, with blinding of the investigator for the underlying pathology, and with follow-up of these at-risk subjects over time. This will ultimately identify VOCs that reflect the transition from chronic inflammation towards malignant transformation and allow to assess the clinical utility of the breath test[39].

Conclusion

Using breath analysis with MCC/IMS, we discriminated MPM patients from high risk subjects previously exposed to asbestos, and patients with benign asbestos-related and non-related lung diseases. Validating these results in an independent, blinded prospective study will allow to assess the clinical utility of breath analysis for MPM screening in persons previously exposed to asbestos. Its high observed sensitivity and NPV allow for a step-up tool in the screening for MPM, whereby only asbestos-exposed individuals with an aberrant VOC signature should be further investigated for the possible presence of MPM with appropriate imaging techniques and downstream investigations.

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KL had full access to the data in the study and takes responsibility for the integrity of the data. KL, MV and OT take responsibility for the accuracy of the data analysis. JVC, KN and JPvM helped with the inclusion of the participants. KL drafted the manuscript. All authors contributed to the design of the manuscript and gave their approval for final submission. KL is the guarantor of the content of the manuscript.

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Tables

Table 1: Baseline patient characteristics.

	HC	AEx	ARD	BLD	LC	MPM	p-value
N	52	59	41	70	56	52	
Gender							
Male	34 (65%)	58 (98%)	40 (98%)	47 (67%)	37 (66%)	43 (83%)	<0.001
Female	18 (35%)	1 (2%)	1 (2%)	23 (33%)	19 (34%)	9 (17%)	
Age (years)							
Median	51.2	53.2	58.3	58.8	69.9	67.3	<0.001
(Q1-Q3)	(34.5-56.7)	(50.2-55.3)	(55.3-62.2)	(40.6-68.0)	(64.3-72.7)	(61.6-72.9)	
Weight (kg)							
Mean	78.3	85.6	84.5	71.3	70.7	74.5	<0.001
SD	17.1	11.4	14.6	15.1	14.4	10.3	
Length (m)							
Mean	1.76	1.77	1.74	1.71	1.68	1.72	<0.001
SD	0.09	0.06	0.05	0.09	0.08	0.08	
BMI (kg/m²)							
Median	25.2	26.9	26.8	24.4	24.0	25.4	<0.001
(Q1-Q3)	(22.2-27.7)	(24.9-28.9)	(24.5-31.5)	(20.8-25.9)	(21.6-27.8)	(23.6-27.2)	
Smoking status							
Never	35 (67%)	19 (32%)	15 (37%)	24 (35%)	6 (10%)	19 (37%)	
Current	1 (2%)	14 (24%)	8 (20%)	14 (20%)	25 (45%)	5 (9%)	<0.001
Ex	16 (31%)	26 (44%)	18 (43%)	31 (45%)	25 (45%)	28 (54%)	
Pack years							
Median	0.0	6.0	5.3	7.5	30.0	2.65	<0.001
(Q1-Q3)	(0.0-1.61)	(0.0-21.5)	(0.0-24.8)	(0.0-36.0)	(14.4-45.0)	(0.0-14.7)	
Subgroups							
Lung embolism				3			
COPD				28			
Fibrous tumour				1			
Allergy				1			
Cystic fibrosis				15			
Asthma				6			
Pneumonia				10			
Emphysema				3			
Pleuritis				1			
Pulmonary hypertension				1			
Wegener disease				1			
Epithelioid						36	
Sarcomatoid						3	
Bifasic/mixed						3	
Unknown						10	

AEx: asymptomatic former asbestos-exposed individual. ARD: patients with benign asbestos-related diseases. BLD: patients with benign non-asbestos related lung diseases. HC: Healthy control. LC: primary lung cancer patients. MPM: malignant pleural mesothelioma patients. Q1: Quartile 1. Q2: Quartile 2.

Table 2: Model characteristics for discriminating mesothelioma

	MPM vs. HC	**MPM vs AEx**	**MPM vs ARD**	**MPM vs AEx+ARD**	**MPM vs BLD**	**MPM vs LC**
N	*52 vs 52*	*52 vs 59*	*52 vs 41*	*52 vs 100*	*52 vs 70*	*52 vs 56*
Sensitivity	88.5% (77.6%-95.2%)	86.5% (75.2%-93.9%)	88.5% (77.6%-95.2%)	94.2% (85.1%-98.5%)	71.2% (57.8%-82.2%)	73.1% (59.9%-83.8%)
Specificity	42.3% (29.5%-56.0%)	89.8% (80.1%-95.8%)	73.2% (58.2%-85.0%)	80.0% (71.3%-87.0%)	87.1% (77.8%-93.5%)	71.4% (58.7%-82.1%)
PPV	60.5% (49.3%-71.0%)	88.2% (77.2%-95.1%)	80.7% (69.0%-89.4%)	71.0% (59.6%-80.8%)	80.4% (67.2%-90.0%)	70.4% (57.3%-81.4%)
NPV	78.7% (60.7%-90.8%)	88.3% (78.3%-94.7%)	83.3% (68.6%-92.9%)	96.4% (90.5%-99.1%)	80.3% (70.2%-88.1%)	74.1% (61.2%-84.4%)
Accuracy	65.4% (55.9%-74.0%)	88.3% (81.3%-93.3%)	81.7% (72.9%-88.6%)	84.9% (78.5%-89.9%)	80.3% (72.6%-86.7%)	72.2% (63.3%-80.0%)
AUC_{ROC}	0.612 (0.502-0.724)[#]	0.879 (0.799-0.948)[#]	0.850 (0.764-0.927)[#]	0.890 (0.832-0.942)[#]	0.837 (0.759-0.907)[#]	0.770 (0.678-0.855)[#]
VOCs (>50% of times selected)	**P0**, **P4**, **P10**, **P15**, P66, P85, P88, P92, **P99**, **P103**, **P104**, **P108**, **P114**, **P119**, P170, P189, P192, P196, **P203**, **P207**, **P208**, **P212**, **P218**, **P223**	**P1**, **P3**, P7, **P9**, **P15**, **P21**, P22, **P26**, P65, **P66**, P73, P75, **P84**, P99, **P101**, **P110**, **P112**, **P114**, P118, **P120**, **P126**, **P132**, P133, **P137**, P176, **P177**, P184, P186, **P195**, P210, **P212**, **P221**, **P223**, **P225**, P229, **P231**, **P237**, **P243**, P244, **P248**	**P1**, **P9**, P15, **P21**, **P26**, **P34**, **P83**, P88, **P92**, **P94**, **P102**, P108, **P114**, **P119**, **P127**, **P176**, P181, **P185**, **P187**, **P195**, P201, **P207**, **P212**, **P220**	**P1**, **P7**, **P9**, **P15**, **P21**, **P26**, P70, P83, **P84**, **P88**, **P101**, P110, P118, **P122**, P123, P142, **P151**, **P153**, **P159**, **P161**, **P167**, **P173**, **P178**, P222, P235, **P236**, **P240**	**P1**, **P8**, P9, P15, **P42**, P98, P115, **P121**, **P123**, **P130**, P131, P137, **P164**, P220, P237, **P243**, **P245**	**P0**, **P7**, **P8**, **P9**, P15, **P21**, **P28**, **P37**, **P42**, **P43**, **P48**, P64, **P73**, **P78**, **P107**, **P108**, **P115**, **P116**, **P117**, P123, **P129**, **P136**, **P145**, **P150**, **P151**, **P156**, P172, **P181**, **P186**, **P215**, **P216**, **P223**, **P224**, **P225**, P231, **P237**, **P244**

[#]AUC_{ROC} significantly different from 0.5.

AEx: asymptomatic former asbestos-exposed controls. ARD: patients with benign asbestos related diseases. AUC_{ROC}: area under the receiver operator characteristic curve. HC: healthy controls. MPM: malignant pleural mesothelioma. NPV: negative predictive value. PPV: positive predictive value. VOC: volatile organic compound. VOCs in bold are selected in >80% of folds.

Table 3: Model characteristics for discriminating lung cancer.

	LC vs. HC	**LC vs AEx**	**LC vs BLD**	**AEx vs ARD**	**AEx vs BLD**	**ARD vs BLD**
N	*56 vs 52*	*56 vs 59*	*56 vs 70*	*59 vs 41*	*59 vs 70*	*41 vs 70*
Sensitivity	76.8% (64.5%-86.4%)	89.3% (79.1%-95.5%)	64.3% (51.2%-76.0%)	82.9% (69.2%-92.2%)	88.6% (79.5%-94.5%)	88.6% (79.5%-94.5%)
Specificity	65.4% (51.8%-77.3%)	89.8% (80.1%-95.8%)	77.1% (66.3%-85.8%)	35.6% (24.2%-48.4%)	91.5% (82.3%-96.8%)	78.0% (63.6%-88.7%)
PPV	70.5% (58.2%-80.9%)	89.3% (79.1%-95.5%)	69.2% (55.8%-80.6%)	47.2% (36.0%-58.7%)	92.5% (84.3%-97.2%)	87.3% (78.1%-93.6%)
NPV	72.3% (58.4%-83.7%)	89.8% (80.1%-95.8%)	73.0% (62.1%-82.1%)	75.0% (56.7%-88.3%)	87.1% (77.0%-93.8%)	80.0% (65.6%-90.2%)
Accuracy	71.3% (62.3%-79.2%)	89.6% (83.0%-94.2%)	71.4% (63.1%-78.8%)	55.0% (45.2%-64.5%)	89.9% (83.8%-94.3%)	84.7% (77.1%-90.5%)
AUC_{ROC}	0.752 (0.659-0.839)[#]	0.936 (0.884-0.976)[#]	0.724 (0.630-0.813)[#]	0.522 (0.366-0.591)	0.957 (0.917-0.988)[#]	0.855 (0.766-0.930)[#]
VOCs (>50% of times selected)	**P4**, P7, **P8**, **P10**, P23, **P28**, P43, **P55**, P59, **P76**, **P83**, **P107**, **P112**, P115, **P116**, **P118**, P131, **P136**, P151, **P163**, P167, **P184**, **P191**, **P215**, **P220**, P223, **P224**, **P226**, P239, **P244**	P0, **P1**, **P3**, P14, **P21**, **P26**, P43, **P61**, **P65**, **P66**, P72, P84, **P88**, P90, **P101**, P112, **P114**, P115, **P116**, **P118**, P129, **P136**, **P141**, P158, **P176**, **P180**, **P181**, P187, P199, **P203**, P205, **P216**, P227, **P229**, P230, **P231**, **P233**, P244	**P0**, P1, **P42**, P44, P107, **P125**, **P126**, P127, **P168**, P170, P233	P1, **P3**, P20, P23, P26, P34, P37, P44, P66, P69, P70, P80, P83, P84, **P90**, P92, **P99**, P101, **P103**, P120, P123, P126, P134, P137, P144, P166, P169, P170, P180, P183, P184, **P190**, P192, **P199**, P201, **P203**, P223, P226, P234, P237, P244	**P1**, **P3**, **P21**, **P42**, P50, **P84**, **P87**, **P88**, P97, **P101**, P104, **P128**, **P130**, **P132**, **P150**, **P171**, P179, **P213**, **P216**, **P217**, P226, **P230**, P233	**P21**, P25, **P42**, **P87**, **P88**, **P101**, **P110**, **P132**, P136, **P153**, **P198**, **P199**, **P212**, **P221**, **P243**, P247

[#]AUC_{ROC} significantly different from 0.5.

AEx: asymptomatic former asbestos-exposed controls. ARD: patients with benign asbestos related diseases. AUC_{ROC}: area under the receiver operator characteristic curve. HC: healthy controls. MPM: malignant pleural mesothelioma. NPV: negative predictive value. PPV: positive predictive value. VOC: volatile organic compound. VOCs in bold are selected in >80% of folds.

Table 4: Peak characteristics

	Peak characteristics		Alveolar gradient (V)[a]						
Peak	**RT (s)**	**1/K₀ (V/cm²)**	**HC**	**AEx**	**ARD**	**BLD**	**LC**	**MPM**	**p-value[b]**
P0	282.1	0.668	0.0019 (-0.0003;0.0066)	0.0002 (-0.0008;0.0012)	0.0000 (-0.0022;0.0017)	-0.0005 (-0.0019;0.0010)	0.0007 (-0.0002;0.0031)	-0.0005 (-0.0018;0.0016)	<0.001
P1	5.9	0.503	0.0354 (0.0070;0.0597)	0.0948 (0.669;0.1277)	0.0904 (0.0405;0.1274)	0.0746 (0.0482;0.1043)	0.0507 (0.0261;0.0665)	0.0350 (0.0179;0.0688)	<0.001
P3	6.7	0.544	0.0333 (0.0003;0.0786)	0.0766 (0.0622;0.1026)	0.0588 (0.0370;0.0922)	0.0416 (0.0073;0.0660)	0.0383 (0.0030;0.0669)	0.0363 (0.0039;0.0672)	<0.001
P7	6.6	0.578	0.0158 (0.0064;0.0265)	0.0168 (0.0093;0.0323)	0.0202 (0.0109;0.0288)	0.0108 (0.0015;0.0275)	0.0068 (-0.0012;0.0272)	0.0083 (-0.0038;0.0297)	NS
P9	1.6	0.601	0.0083 (0.0005;0.0270)	0.0032 (-0.0002;0.0067)	0.0045 (0.0005;0.0068)	-0.0007 (-0.0121;0.0044)	0.0018 (-0.0052;0.0052)	0.0070 (0.0021;0.0192)	<0.001
P15	4.5	0.715	-0.0012 (-0.0036;0.0027)	-0.0027 (-0.0050;0.0015)	-0.0022 (-0.0061;0.007)	0.0020 (-0.0015;0.0040)	-0.0042 (-0.0091;-0.0001)	-0.0053 (-0.0090;-0.0017)	<0.001
P21	1.2	0.514	-0.0250 (-0.0661;-0.032)	-0.0039 (-0.0481;0.0420)	-0.0341 (-0.0540;0.0201)	-0.0518 (-0.0768;-0.0201)	-0.0464 (-0.0679;-0.0175)	-0.0499 (-0.0853;-0.0258)	NS
P26	4.2	0.689	0.0040 (-0.0024;0.0121)	0.0045 (0.0007;0.0186)	0.0063 (0.0016;0.0245)	0.0055 (0.0028;0.0073)	0.0036 (-0.0005;0.0068)	0.0024 (0.0001;0.0117)	<0.001
P42	4.5	0.457	-0.1371 (-0.2189;-0.0841)	-0.2069 (-0.2878;-0.0951)	-0.2364 (-0.3166;-0.0887)	0.0035 (-0.0543;0.0485)	-0.1551 (-0.2243;-0.0387)	-0.2212 (-0.2969;-0.0912)	<0.001
P66	66.5	0.733	-0.0057 (-0.0125;0.0003)	-0.0149 (-0.0206;-0.0080)	-0.0111 (-0.0201;-0.0066)	-0.0052 (-0.0114;-0.0024)	-0.0124 (-0.0178;-0.0070)	-0.0100 (-0.0209;-0.0039)	<0.001
P83	160.6	0.764	-0.0009 (-0.0026;0.0003)	-0.0028 (-0.0044;-0.0016)	-0.0041 (-0.0048;-0.0015)	-0.0011 (-0.0023;-0.0001)	-0.0027 (-0.0047;-0.0010)	-0.0022 (-0.0038;-1.0006)	<0.001
P84	116.1	0.742	0.0001 (-0.0006;0.0008)	-0.0010 (-0.0024;-0.0004)	-0.0002 (-0.0015;0.0004)	0.0003 (-0.0004;0.0009)	-0.0001 (-0.0009;0.0007)	-0.0002 (-0.0011;0.0007)	<0.001
P88	5.5	0.657	0.0013 (-0.0003;0.0037)	0.0011 (-0.0011;0.0027)	0.0014 (-0.0002;0.0032)	-0.0007 (-0.0025;0.0022)	0.0004 (-0.0049;0.0023)	0.0009 (-0.0030;0.0032)	NS
P101	20.0	0.716	-0.0007 (-0.0037;0.0025)	-0.0122 (-0.0216;-0.0028)	-0.0100 (-0.0166;-0.0025)	-0.0033 (-0.0062;-0.0001)	-0.0042 (-0.0072;-0.0010)	-0.0008 (-0.0041;0.0002)	<0.001
P114	99.8	0.728	-0.0001 (-0.0009;0.0007)	-0.0009 (-0.0023;-0.0002)	-0.0004 (-0.0017;0.0001)	-0.0001 (-0.0008;0.0004)	-0.0005 (-0.0011;0.0002)	-0.0003 (-0.0014;0.0004)	0.025
P115	98.7	0.665	0.0004 (-0.0005;0.0012)	-0.0009 (-0.0029;0.0002)	-0.0009 (-0.0031;-0.0002)	0.0000 (-0.0010;0.0007)	-0.0004 (-0.0012;0.0007)	-0.0006 (-0.0015;0.0005)	<0.001
P118	184.6	0.639	-0.0001 (-0.0006;0.0005)	-0.0006 (-0.0014;0.0000)	-0.0005 (-0.0011;0.0003)	-0.0003 (-0.0007;0.0003)	-0.0003 (-0.0010;0.0004)	-0.0001 (-0.0007;0.0007)	NS
P123	67.1	0.759	-0.0011 (-0.0030;0.0005)	-0.0049 (-0.0091;-0.0023)	-0.0044 (-0.0074;-0.0027)	-0.0019 (-0.0039;-0.0008)	-0.0042 (-0.0074;-0.0016)	-0.0033 (-0.0046;-0.0014)	<0.0001
P136	34.6	0.910	0.0002 (-0.0009;0.0009)	-0.0004 (-0.0007;0.0004)	-0.0002 (-0.0006;0.0003)	0.0001 (-0.0004;0.0006)	-0.0003 (-0.0009;0.0004)	-0.0004 (-0.0010;0.0003)	NS
P212	138.1	0.771	-0.0001 (-0.0005;0.0008)	-0.0003 (-0.0016;0.0007)	-0.0004 (-0.0014;0.0001)	-0.0002 (-0.0006;0.0003)	0.0001 (-0.0005;0.0005)	0.0002 (-0.0009;0.0009)	NS
P223	309.1	0.601	-0.0005 (-0.0012;0.0001)	0.0000 (-0.0005;0.0005)	0.0002 (-0.0006;0.0006)	0.0001 (-0.0005;0.0008)	-0.0001 (-0.0005;-0.0005)	-0.0001 (-0.0009;0.0005)	NS
P237	4.5	0.700	0.0020 (-0.0004;0.0046)	0.0018 (-0.0007;0.0038)	0.0025 (-0.0006;0.0055)	0.0012 (-0.0004;0.0034)	0.0019 (-0.0001;0.0043)	0.0015 (-0.0005;0.0061)	NS
P244	13.5	0.917	-0.0001 (-0.0009;0.0008)	-0.0004 (-0.0013;0.0003)	-0.0001 (-0.0008;0.0002)	-0.0004 (-0.0010;0.0002)	-0.0006 (-0.0017;0.0002)	-0.0004 (-0.0012;0.0009)	NS

[a]Median(Q1;Q3). [b]Kruskal-Wallis test.

1/K₀: inverse reduced ion mobility. AEx: asymptomatic former asbestos workers. ARD: patients with benign asbestos-related diseases. BLD: patients with benign, non asbestos-related diseases. HC: healthy non-asbestos exposed control persons. LC: lung cancer patients. MPM: malignant pleural mesothelioma patients. NS: not significant. RT: retention time (seconds). V: volt.

Figures

Figure 1: ROC Curves for MPM discrimination. AEx: asymptomatic persons with past asbestos exposure. ARD: patients with benign asbestos-related diseases. BLD: patients with benign non-asbestos-related lung diseases. HC: healthy controls without occupational asbestos exposure. LC: lung cancer patients. MPM: malignant pleural mesothelioma patients. ROC: receiver operating characteristic.

Figure 2: ROC Curves for lung cancer discrimination. AEx: asymptomatic persons with past asbestos exposure. ARD: patients with benign asbestos-related diseases. BLD: patients with benign non-asbestos-related lung diseases. HC: healthy controls without occupational asbestos exposure. LC: lung cancer patients. MPM: malignant pleural mesothelioma patients. ROC: receiver operating characteristic.