UNIVERSITEIT GENT · Stichting tegen Kanker · UZ Universitair Ziekenhuis Gent

Volatile Organic Compounds as a Diagnostic Tool for Malignant Pleural Mesothelioma.

Kevin Lamote[1]; Joris Van Cleemput[2]; Kristiaan Nackaerts[3]; Jan P. van Meerbeeck[4]

[1]Dpt of Respiratory Medicine, Ghent University Hospital, Ghent, Belgium, [2]Occupational Health Service, Eternit NV, Kapelle-op-Den-Bos, Belgium, [3]Dpt of Respiratory Medicine, University Hospital Gasthuisberg, Leuven, Belgium, [4]Thoracic Oncology/MOCA, Antwerp University Hospital, Antwerp, Belgium.

Background

- Malignant pleural mesothelioma (MPM) is a disease with a long latency period and a dismal prognosis. Early diagnosis of MPM can improve patients' outcome but is hampered by non-specific symptoms and investigations, which delay diagnosis and result in advanced stage disease [van Meerbeeck JP, 2011]. An accurate non-invasive test allowing early stage diagnosis in asbestos-exposed persons is currently lacking and blood biomarkers have not proven to be useful.

- Breathomics aims at a non-invasive analysis of volatile organic compounds (VOCs) in breath reflecting the cells' metabolism. Recently, it was possible to discriminate MPM from controls using an electronic nose [Chapman EA 2009, Dragonieri S 2011]. However, the breathogram of MPM obtained by this eNose does not allow identification of MPM-related VOCs. Ion mobility spectrometry (IMS) combines the advantages of online direct sampling with the possibility of VOC identification and linking to MPM pathogenesis [Baumbach JI 2009].

- With a non-targeted approach, we investigated which VOCs could play a role in MPM pathogenesis in order to build a possible diagnostic MPM tool using IMS.

Methods

- ***Participants:*** 10 MPM patients, 10 healthy asbestos-exposed individuals (mean asbestos fiber year count 14,6 (5,5) fibre.years/cc) and 10 healthy non-exposed individuals were included after refraining from eating, drinking and smoking for at least 2 hours before sampling.

- ***Breath sampling:*** Subjects breathed tidally with a nose clip for 3 minutes through a mouthpiece connected to a bacteria filter. Ten ml alveolar air was sampled via a CO_2-controlled ultrasonic sensor and subsequently analyzed using the BioScout Multicapillary Column/Ion Mobility Spectrometer (MCC/IMS, B&S Analytik, Dortmund, Germany, Figure 2) [Westhoff M 2009], by using N_2 as a carrier and drift gas. Per subject a background sample was taken to correct for contamination.

- ***Breath analysis:*** Preprocessing of the data was done by base correction, normalization to the reactant ion peak (RIP), compensating RIP-tailing and smoothening techniques. Peaks of interest were visually selected in breath and background samples and their intensity (V) was analyzed and compared via on-board VisualNow 3.2 software and SPSS v21 (IBM) using Mann-Whitney-U tests. Further selection of interesting peaks was done by looking at the alveolar gradient. MPM diagnostic accuracy was obtained by ROC-analysis.

Results

Table 1: Baseline characteristics.

	MPM Patients	AEx Individuals	Healthy Individuals	p-value
N	10	10	10	
Gender (Male/Female)	8/2	9/1	8/2	1,00[a]
Age (year)[b]	65,0 (59,0 – 67,0)	55,0 (54,0 – 56,0)	55,5 (49,0 – 61,0)	**<0,01**
Weight (kg)[c]	73,0 (11,8)	84,1 (12,7)	79,2 (9,8)	0,12
Length (m)[c]	1,73 (0,06)	1,77 (0,06)	1,77 (0,08)	0,33
BMI (kg/m²)[c]	24,3 (3,6)	26,9 (3,6)	25,1 (2,1)	0,20
Smoking status (current/ex/non)	3/3/4	3/4/3	1/0/9	0,05[a]

[a]Fisher's exact test, [b]Median (IQR), [c]Mean (SD). AEx: Asbestos-exposed. MPM: Malignant Pleural Mesothelioma.

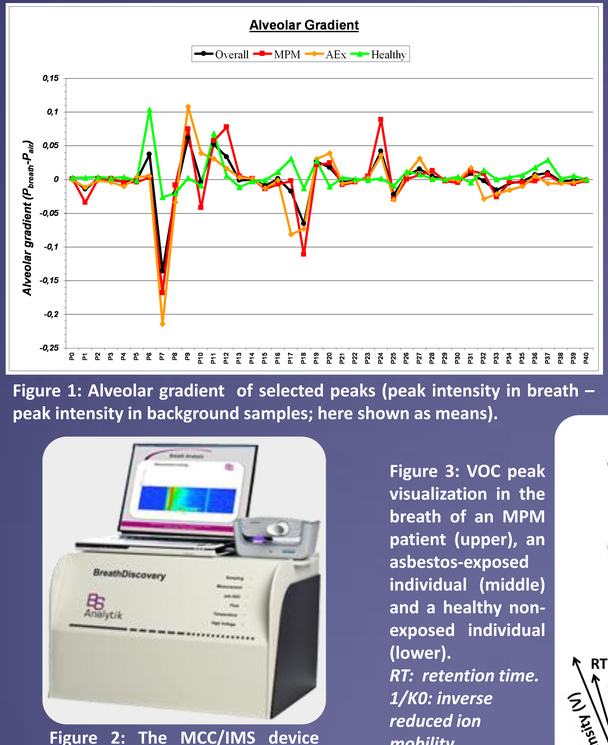


Figure 1: Alveolar gradient of selected peaks (peak intensity in breath – peak intensity in background samples; here shown as means).

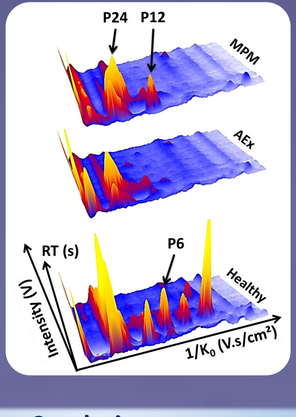
Table 2: VOC peak comparison.

Peak	MPM Intensity (V)*	AEx Intensity (V)*	Healthy Intensity (V)*	p-value	Between group significance[a]	AUC_{ROC}
P6	0,001 [-0,008 – 0,006]	0,010 [-0,011 – 0,022]	0,082 [0,049 – 0,168]	**<0,01**	†, #	0,300
P9	0,087 [0,045 – 0,111]	0,102 [0,088 – 0,138]	0,011 [-0,018 – 0,025]	**<0,01**	†, #	0,565
P11	0,050 [0,025 – 0,058]	0,045 [0,032 – 0,054]	0,056 [0,045 – 0,071]	0,35		0,450
P12	0,043 [0,024 – 0,077]	0,015 [0,011 – 0,022]	0,002 [-0,001 – 0,003]	**<0,01**	‡, †	**0,865**
P16	-0,006 [-0,012 – -0,001]	-0,003 [-0,006 – 0,003]	0,003 [-0,001 – 0,028]	**0,03**	†	0,235
P19	0,021 [0,007 – 0,023]	0,034 [0,017 – 0,041]	0,031 [0,007 – 0,044]	0,63		0,350
P20	0,023 [0,004 – 0,044]	0,047 [0,022 – 0,069]	-0,002 [-0,009 – 0,005]	**0,01**	†, #	0,555
P24	0,058 [0,031 – 0,089]	0,037 [0,013 – 0,067]	-0,003 [-0,015 – 0,026]	**0,01**	†, #	**0,770**
P26	0,000 [-0,002 – 0,007]	0,005 [-0,002 – 0,009]	0,008 [0,001 – 0,024]	0,33		0,355
P27	0,002 [-0,002 – 0,010]	0,011 [-0,002 – 0,044]	0,009 [0,001 – 0,014]	0,47		0,360
P28	0,002 [0,001 – 0,015]	0,001 [0,000 – 0,003]	0,000 [-0,001 – 0,002]	0,29		0,670
P31	0,008 [0,000 – 0,022]	0,020 [-0,016 – 0,036]	0,007 [-0,016 – 0,013]	0,46		0,515
P36	-0,001 [-0,010 – 0,001]	0,004 [-0,006 – 0,013]	0,018 [0,002 – 0,038]	**0,05**	†	0,265
P37	0,005 [0,003 – 0,010]	0,002 [-0,014 – 0,004]	0,029 [0,007 – 0,039]	**<0,01**	‡, †, #	0,515

*Median [IQR]. $1/K_0$: inversed reduced ion mobility. AEx: healthy asbestos exposed individual. AUC_{ROC}: Area under the receiver operator characteristic curve (accuracy in diagnosing MPM). MPM: Malignant Pleural Mesothelioma patient. RT: retention time. [a]p<0,05 for MPM vs. AEx (‡), MPM vs. Healthy (†) and AEx vs. Healthy (#).

Figure 2: The MCC/IMS device (BioScout) with sampling unit (SpiroScout).

Figure 3: VOC peak visualization in the breath of an MPM patient (upper), an asbestos-exposed individual (middle) and a healthy non-exposed individual (lower).
RT: retention time. 1/K0: inverse reduced ion mobility.



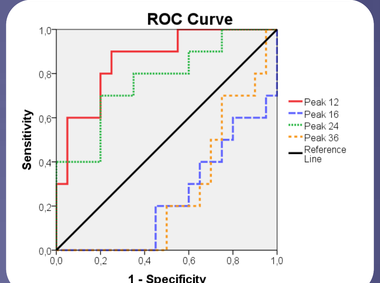


Figure 4: ROC curves displaying the diagnostic accuracy of four selected peaks in discriminating MPM patients from asbestos-exposed and non-exposed controls.

Conclusions

- Several VOCs of interest were derived from the breath according to the alveolar gradient. Four peaks (P12, P16, P24 and P36) had a significant effect in discriminating MPM patients from controls. Only P12 and P24 have a relevant AUC_{ROC} to positively diagnose MPM.
- The intensity of P12 was found to be significantly higher in MPM patients. Hence, this could be linked to MPM development and serve as an early diagnostic marker for MPM. P24 was significantly lower in non-exposed persons and could serve as a marker for asbestos-exposure.
- GC-MS analysis and further large cohort studies including healthy unexposed individuals are ongoing in order to validate the accuracy of IMS as a diagnostic tool for MPM. Results need to be validated in an independent test set.

- Malignant pleural mesothelioma (MPM) is a disease with a long latency period and a dismal prognosis. Early diagnosis of MPM can improve patients' outcome but is hampered by non-specific symptoms and investigations, which delay diagnosis and result in advanced stage disease [van Meerbeeck JP, 2011]. An accurate non-invasive test allowing early stage diagnosis in asbestos-exposed persons is currently lacking and blood biomarkers have not proven to be useful.

- Breathomics aims at a non-invasive analysis of volatile organic compounds (VOCs) in breath reflecting the cells' metabolism. Recently, it was possible to discriminate MPM from controls using an electronic nose [Chapman EA 2009, Dragonieri S 2011]. However, the breathogram of MPM obtained by this eNose does not allow identification of MPM-related VOCs. Ion mobility spectrometry (IMS) combines the advantages of online direct sampling with the possibility of VOC identification and linking to MPM pathogenesis [Baumbach JI 2009].

- With a non-targeted approach, we investigated which VOCs could play a role in MPM pathogenesis in order to build a possible diagnostic MPM tool using IMS.

- *Participants:* 10 MPM patients, 10 healthy asbestos-exposed individuals (mean asbestos fiber year count 14,6 (5,5) fibre.years/cc) and 10 healthy non-exposed individuals were included after refraining from eating, drinking and smoking for at least 2 hours before sampling.

- *Breath sampling:* Subjects breathed tidally with a nose clip for 3 minutes through a mouthpiece connected to a bacteria filter. Ten ml alveolar air was sampled via a CO_2-controlled ultrasonic sensor and analyzed using the BioScout Multicapillary Column/Ion Mobility Spectrometer (MCC/IMS, B&S Analytik, Dortmund, Germany, Figure 2) [Westhoff M 2009], by using N_2 as a carrier and drift gas. Per subject a background sample was taken to correct for contamination.

- *Breath analysis:* Preprocessing of the data was done by base correction, normalization to the reactant ion peak (RIP), compensating RIP-tailing and smoothening techniques. Peaks of interest were visually selected in breath and background samples and their intensity (V) was analyzed and compared via on-board VisualNow 3.2 software and SPSS v21 (IBM) using Mann-Whitney-U tests. Further selection of interesting peaks was done by looking at the alveolar gradient.

- Several VOCs of interest were derived from the breath according to the alveolar gradient. Four peaks (P12, P16, P24 and P36) were discriminating MPM patients from controls with only P12 and P24 having a relevant AUC_{ROC}.
- The intensity of P12 was found to be significantly higher in MPM patients. Hence, this could be linked to MPM development and serve as an early diagnostic marker for MPM. P24 could serve as a marker for asbestos-exposure.
- GC-MS analysis and further large cohort studies including healthy unexposed individuals are ongoing in order to validate the accuracy of IMS as a diagnostic tool for MPM. Results need to be validated in an independent test set.

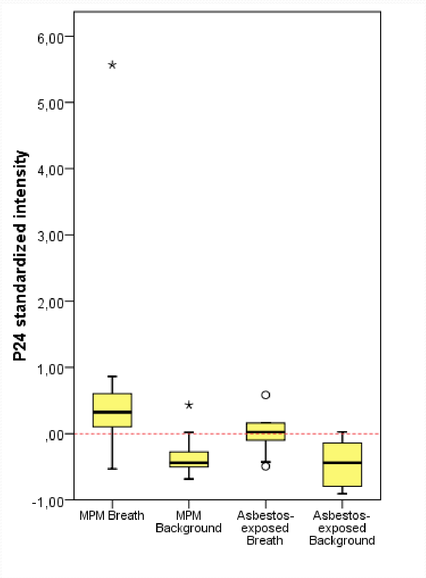
Results

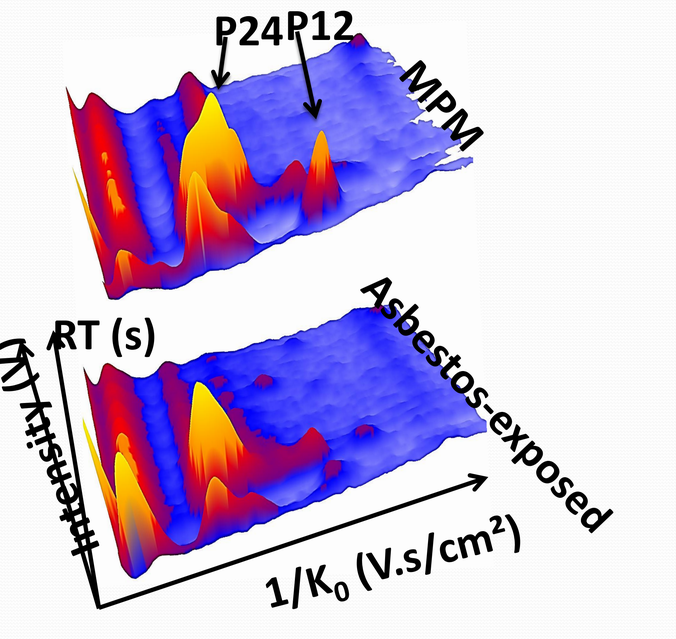
	MPM Patients	Asbestos-Exposed Individuals	P-value
N	10	10	
Gender (Male/Female)	8/2	9/1	1,00[a]
Age (year)[b]	65,0 (59,0 – 67,0)	55,0 (54,0 – 56,0)	**<0,01**
Weight (kg)[c]	73,0 (11,8)	84,1 (12,7)	0,06
Length (m)[c]	1,7 (0,06)	1,8 (0,06)	0,21
BMI (kg/m²)[c]	24,3 (3,6)	26,9 (3,6)	0,13
Smoking status (current/ex/non)	3/3/4	3/4/3	0,87[a]
CO (ppm)[b]	2,5 (2,0 – 5,0)	4,0 (2,0 – 13,0)	0,14
COHb (%)[b]	1,1 (1,0 – 1,4)	1,3 (1,0 – 2,7)	0,14
FeNO (ppb)[c]	19,8 (9,7)	20,2 (10,0)	0,94

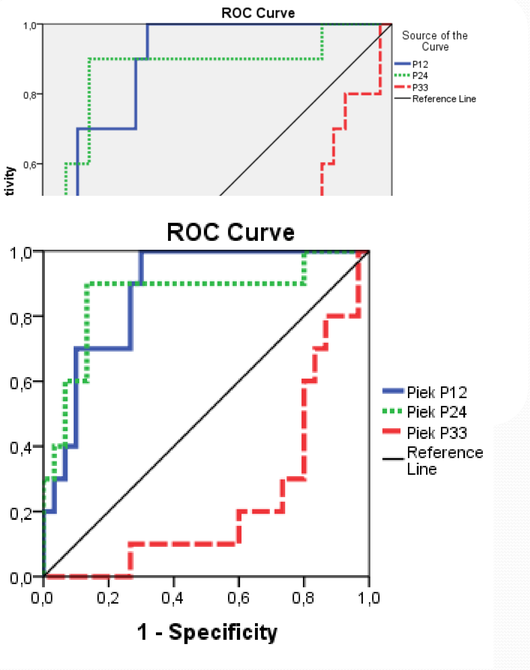
[a]Fisher's exact test
[b]Median (IQR)
[c]Mean (SD)

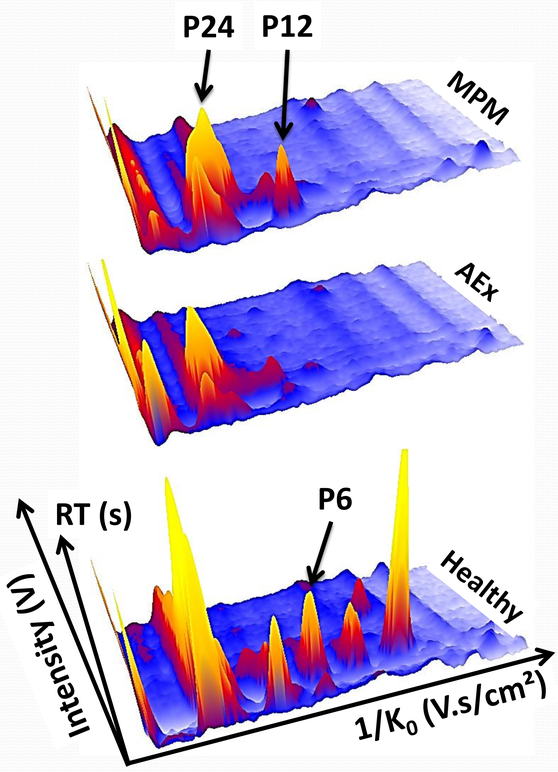
Peak	MPM Intensity (V)*	AEx Intensity (V)*	p-value	AUC_{ROC}
P6	0,018 [0,013 – 0,027]	0,026 [0,019 – 0,031]	0,41	0,490
P9	0,106 [0,085 – 0,124]	0,116 [0,104 – 0,145]	0,17	0,763
P11	0,089 [0,068 – 0,120]	0,082 [0,064 – 0,093]	0,45	0,750
P12	0,078 [0,054 – 0,168]	0,027 [0,022 – 0,060]	<0,01	0,877
P19	0,044 [0,038 – 0,048]	0,045 [0,042 – 0,055]	0,76	0,777
P20	0,044 [0,029 – 0,059]	0,072 [0,043 – 0,078]	0,17	0,620
P24	0,102 [0,085 – 0,123]	0,079 [0,069 – 0,089]	0,03	0,863
P26	0,010 [0,007 – 0,016]	0,011 [0,006 – 0,013]	0,76	0,567
P27	0,012 [0,008 – 0,021]	0,013 [0,010 – 0,052]	0,60	0,618
P33	0,006 [0,004 – 0,007]	0,010 [0,007 – 0,019]	0,03	0,237
P36	0,012 [0,009 – 0,016]	0,012 [0,009 – 0,016]	0,82	0,517

*Median [IQR]. $1/K_0$: inversed reduced ion mobility. AEx: healthy asbestos exposed individual. AUC_{ROC}: Area under the receiver operator characteristic curve (accuracy in diagnosing MPM). MPM: Malignant Pleural Mesothelioma patient. RT: retention time.








P24
P12
MPM
AEx
P6
Healthy
RT (s)
Intensity (V)
1/K0 (V.s/cm2)