

Breath analysis in non small cell lung cancer patients after surgical tumour resection

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Abstract. Exhaled volatile organic compounds (VOCs), mainly aliphatic and aromatic hydrocarbons, have been proposed as a diagnostic test for early lung cancer detection, but the effect of lung cancer surgical removal on exhaled VOCs pattern has never been specifically addressed. The aim of this study was to compare VOC levels measured in non small cell lung cancer (NSCLC) patients before surgery (T0), one month (T1) and 3 years (T2) after surgical removal of tumour. In order to better understand the pathophysiological meaning of exhaled aromatic hydrocarbons, the same exhaled biomarkers were also assessed in cancerous and macroscopically unaffected lung tissue samples collected during surgical operation. Exhaled breath was collected in a specially designed Teflon® bulb trapping the last 150 ml of a single slow vital capacity. After solid phase micro-extraction, VOCs were analysed in gas chromatography-mass spectrometry. VOC levels were unaffected by surgical removal, except for isoprene, whose concentration was significantly reduced. Three years after surgical operation, some VOCs significantly changed from baseline: in particular, we noted a decrease in isoprene and benzene concentrations, whereas the levels of pentane, toluene and ethylbenzene were increased in comparison with baseline values. Finally, lung tissue analysis showed that all aromatic hydrocarbons, except xylenes, were significantly higher in cancerous than in unaffected tissue. This study showed that surgical operation can influence the concentration of some exhaled VOCs opening a new scenario in the use of exhaled VOCs in lung cancer patients, not only for diagnostic but also for follow up purposes. (www.actabiomedica.it)

Key words: Breath analysis, VOCs, NSCLC, lung resection, follow up

Introduction

Lung is the target organ for several toxic substances introduced into the body by inhalation and is particularly subjected to oxidative stress processes that can impair its functionality and induce lung diseases, including cancer (1-3). Non invasive diagnostic strategies, such as breath analysis, aimed at identifying biomarkers of lung status, are of great interest.

The use of exhaled VOCs for the early diagnosis of lung cancer has been recently proposed and an increasing body of evidence suggests that exhaled breath

of lung cancer patients contains a certain specific pattern of volatile substances (4-6). In a previous paper published by our group, we selected a pattern of VOCs (aliphatic and aromatic hydrocarbon) which was able to correctly classify about 80% of NSCLC patients before surgery, considering chronic obstructive pulmonary disease patients, asymptomatic smokers and non smokers as control groups (7).

In order to confirm and to extend our previous observation, the aim of this study was to measure VOC levels in exhaled breath of operated lung patients, one month and three years after surgical re-

removal of the tumour. Furthermore, in order to improve the pathophysiological meaning of exhaled VOCs, the concentration of selected VOCs was measured in cancerous and macroscopically unaffected lung tissue samples collected during surgical operation.

Materials and Methods

Study design

The design of the present study was focused on an extension and subsequent follow-up study of NSCLC patients as previously described (7). Exhaled breath samples from NSCLC patients were collected before surgery (T0) and at different times after lung resection (T1= after one month, T2= after 3 years).

Subjects

NSCLC patients who underwent tumour resection were enrolled at the Section of Thoracic Surgery of the University Hospital of Parma. The assessment of tumour size and node was based on the International Unit Against Cancer TNM staging system (8). All patients involved were classified as having stage Ia, Ib and IIa lung cancer. None of the patients received radiation or chemotherapy before surgery.

Among the 36 patients who agreed to perform sampling before surgery, only 21 and 10 patients were willing or in good clinical conditions to repeat breath collection 1 month (21 patients) and 3 years after surgery (10 patients). None of them showed radiological signs of recurrence.

For comparison purposes, 50 asymptomatic non-smokers were selected as control group. None of them

referred pulmonary symptoms or a history of clinically significant pulmonary disease and had normal lung spirometry tests (data not shown). The characteristic of the subjects are reported in table 1.

Breath collection and analysis

Breath collections and analyses were performed as previously described (7). Briefly, subjects were asked to perform a single slow vital capacity breath in a one-way Teflon® bulb (Bio-VOC® sampler, Markers International Ltd, Rhondda Cynon Taff, UK), which traps the last portion of exhaled breath. After adding internal standard (I.S, n-heptane-d₁₆ and styrene-d₈ methanolic solution), exhaled VOCs were extracted using 75 µm-Carboxen-PDMS SPME fibers (Supelco, Bellefonte, PA, USA). Analyses were carried out in gas chromatography mass spectrometry technique operating both in *full scan* (ion range 40–350 m/z) and in *sim* (selected ion monitoring) mode.

Lung Tissue analysis

In 27 NSCLC patients during surgery, 50–100 mg of cancerous and macroscopically unaffected lung tissue samples were collected and stored until analysis at -20°C in RNA-later™ solution (Ambion, USA). Before analysis, each sample was weighed and placed in a 4ml SPME-vial containing 0.5 g of NaCl and buffer solution. Styrene -d₈ was added as Internal Standard.

Extraction was performed using 75 µm-Carboxen-PDMS fiber sampling at 70°C for 20 min. Fiber was desorbed into gas chromatography injection port at 280°C for 5 mins. Under this operative condition no memory effect was observed.

Analyses were carried out in gas chromatography mass spectrometry technique operating in *sim* mode.

Table 1. Demographic characteristic of the groups

	NSCLC (T0)	NSCLC (T1)	NSCLC (T2)	Controls
Subjects (n)	36	21	10	50
Age (median, years)	67.2	66.52	68.56	55.7
Sex (male/female)	28/8	15/6	9/1	27/23
Current smokers	2	4	1	0
Ex smokers	28	16	9	0
Never smokers	6	1	0	50

Table 2. Exhaled VOC levels in studied groups*

(10 ⁻¹² M	NSCLC (T0)	NSCLC (T1)	NSCLC (T2)	Controls
Pentane	647.5 (388.5-1013)	529.5 (329.6-960.0)	1569.0 (497.9-3214)	268.0 (107.7-462.7)
Isoprene	6121 (4069-9031)	4125 (2415-7407)	678.9 (359.8-1111.0)	3789 (1399-6589)
2-methylpentane	139.5 (68.8-291.6)	123.5 (81.1-227.6)	87.9 (35.5-278.9)	27.7 (3.4-50.3)
Benzene	95.7 (62.9-132.2)	99.6 (60.0-119.2)	46.0 (34.6-76.0)	44.7 (27.7-68.6)
Heptane	15.1 (0.9-34.6)	18.7 (9.5-39.5)	10.8 (6.1-105.5)	8.4 (5.0-15.3)
Toluene	161.9 (118.7-232.5)	160.3 (119.0-232.7)	297 (202.6-297.0)	80.8 (58.9-140.0)
Octane	65.7 (45.8-131.4)	49.7 (28.5-102.5)	44.3 (32.6-60.4)	20.2 (4.0-50.8)
Ethylbenzene	24.0 (14.8-28.0)	19.7 (15.7-34.5)	46.4 (38.6-90.9)	13.6 (10.8-15.1)
Styrene	22.1 (11.5-38.1)	18.0 (12.1-43.1)	21.3 (11.8-26.4)	12.3 (5.3-21.8)
Xylenes	69.0 (45.8-105.6)	67.8 (51.2-129.4)	56.2 (38.9-80.4)	31.1 (21.1-56.4)
Trimethylbenzene	15.2 (10.1-22.3)	13.2 (10.2-22.5)	15.3 (11.7-22.3)	6.2 (4.7-11.0)
Pentamethylheptane	2.6 (1.7-10.0)	2.5 (1.1-8.8)	8.8 (2.2-15.2)	0.9 (0.1-2.6)

* Concentrations are expressed as median values (25°-75° percentile). These values have been partially described in a previous study (7)

Statistical analysis

The comparisons between NSCLC patients at time T0, T1, and controls were already described in our previous study (7). Due to the low number of NSCLC patients enrolled at time T2, instead of repeated measure analogue, non parametric test for independent measures (Kruskal-Wallis test followed by Dunn's post-hoc test) was used to compare NSCLC patients at time T0, T1, T2, and controls. In addition, Wilcoxon test was used to compare VOC levels in cancerous and unaffected tissue. The relationships between different VOCs at all times of the follow-up study were evaluated by means of non-parametric Spearman's rho test.

Results

Table 2 summarizes exhaled VOC levels in NSCLC patients before and after surgery and in controls; Table 3 shows statistically significant differences between groups in pairs. At T1, isoprene concentrations were significantly decreased in comparison with T0 levels, whereas the levels of other VOCs were similar to those measured at T0. Nevertheless, comparing VOC levels before (T0) and 3 years after surgery (T2) five substances were significantly different: in particular, isoprene and benzene concentrations were decreased while pentane, toluene and ethyl benzene levels were increased compared to values sampled at T0.

Table 3. Statistical differences between groups*

	T0 vs. T1	T0 vs. T2	T1 vs. T2	Controls vs. T0	Controls vs. T1	Controls vs. T2
Pentane	n.s.	p<0.05	p<0.01	p<0.001	p<0.05	p<0.001
Isoprene	p<0.05	p<0.01	p<0.01	n.s.	n.s.	p<0.01
2-methylpentane	n.s.	n.s.	n.s.	p<0.001	p<0.001	p<0.05
Benzene	n.s.	p<0.05	p<0.05	p<0.001	p<0.01	n.s.
Heptane	n.s.	n.s.	n.s.	n.s.	p<0.05	n.s.
Toluene	n.s.	p<0.01	p<0.01	p<0.001	p<0.01	p<0.001
Octane	n.s.	n.s.	n.s.	p<0.001	p<0.05	n.s.
Ethylbenzene	n.s.	p<0.05	p<0.01	p<0.01	p<0.001	p<0.001
Styrene	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Xylenes	n.s.	n.s.	n.s.	p<0.001	p<0.01	p<0.05
Trimethylbenzene	n.s.	n.s.	n.s.	p<0.01	p<0.001	p<0.001
Pentamethylheptane	n.s.	n.s.	n.s.	p<0.001	p<0.05	p<0.001

* Non parametric test for independent measures (Kruskal-Wallis test followed by Dunn's post-hoc test) was used to compare NSCLC patients at time T0, T1, T2, and controls

Figure 1 shows the distribution for the substances and the observed differences. Considering controls *versus* NSCLC subjects, the same substances were significantly different at time T0 and T1. At time T2, benzene, heptanes, and octane levels became similar to those measured in the control subjects. On the other hand, isoprene concentrations that were similar to the controls both at time T0 and T1, 3 years after surgery (T2) had significantly decreased.

Correlations between substances in NSCLC both before and after surgery were also evaluated. Pentane correlated with its methylated form, 2-methylpentane, at time T0 ($r=0.59$, $p<0.001$) while at time T2 the correlation was negative and near significance ($r=-0.59$; $p=0.074$). Pentane correlated also with isoprene both at time T0 ($r=0.48$, $p<0.01$) and T2 ($r=-0.71$, $p<0.05$) with opposite slopes as shown in Figure 2.

Heptane and octane, whose levels became similar to the controls at time T2, did not correlate before surgery but at time T1 ($r=0.80$; $p<0.001$). At time T2 the correlation was close to statistical significance ($r=0.55$, $p=0.08$).

Regarding aromatic hydrocarbons, benzene correlated at time T0 with the other BTEX, in particular with toluene ($r=0.68$, $p<0.001$), ethyl benzene ($r=0.49$, $p<0.01$), xylenes ($r=0.62$, $p<0.001$), trimethylbenzene ($r=0.51$, $p<0.01$). At time T1, benzene correlated only with trimethylbenzene and at time T2 correlation was near significance only with toluene ($r=0.58$, $p=0.08$).

Ethylbenzene and toluene correlated at time T0 ($r=0.36$, $p<0.05$) and strongly at time T1 ($r=0.92$, $p<0.001$).

During surgery, cancerous and unaffected lung tissue samples were also collected from 27 NSCLC patients. In these specimens, it was possible to measure only aromatic hydrocarbon concentrations, in particular BTEX (benzene, toluene, ethylbenzene and xylenes), styrene and trimethylbenzene because the other VOCs had been lost during collection and storing. Their distribution and relatively statistic differences are reported in Figure 3. All aromatic hydrocarbons, except xylenes, were significantly higher in cancerous than in unaffected tissues.

Discussion

This is the first study aimed at monitoring VOC pattern in exhaled breath of lung cancer patients over a 3-year period after surgical removal of the tumour. The main finding is that all selected VOCs (with the exception of isoprene) did not show significant differences one month after surgery in comparison with baseline values, whereas VOC variations were observed when sampling was done 3 years after surgical removal of the tumour. Of note, most of the studied VOCs in lung cancer patients were higher at baseline in comparison with control values and this difference was still evident either one month after surgery and 3 years later.

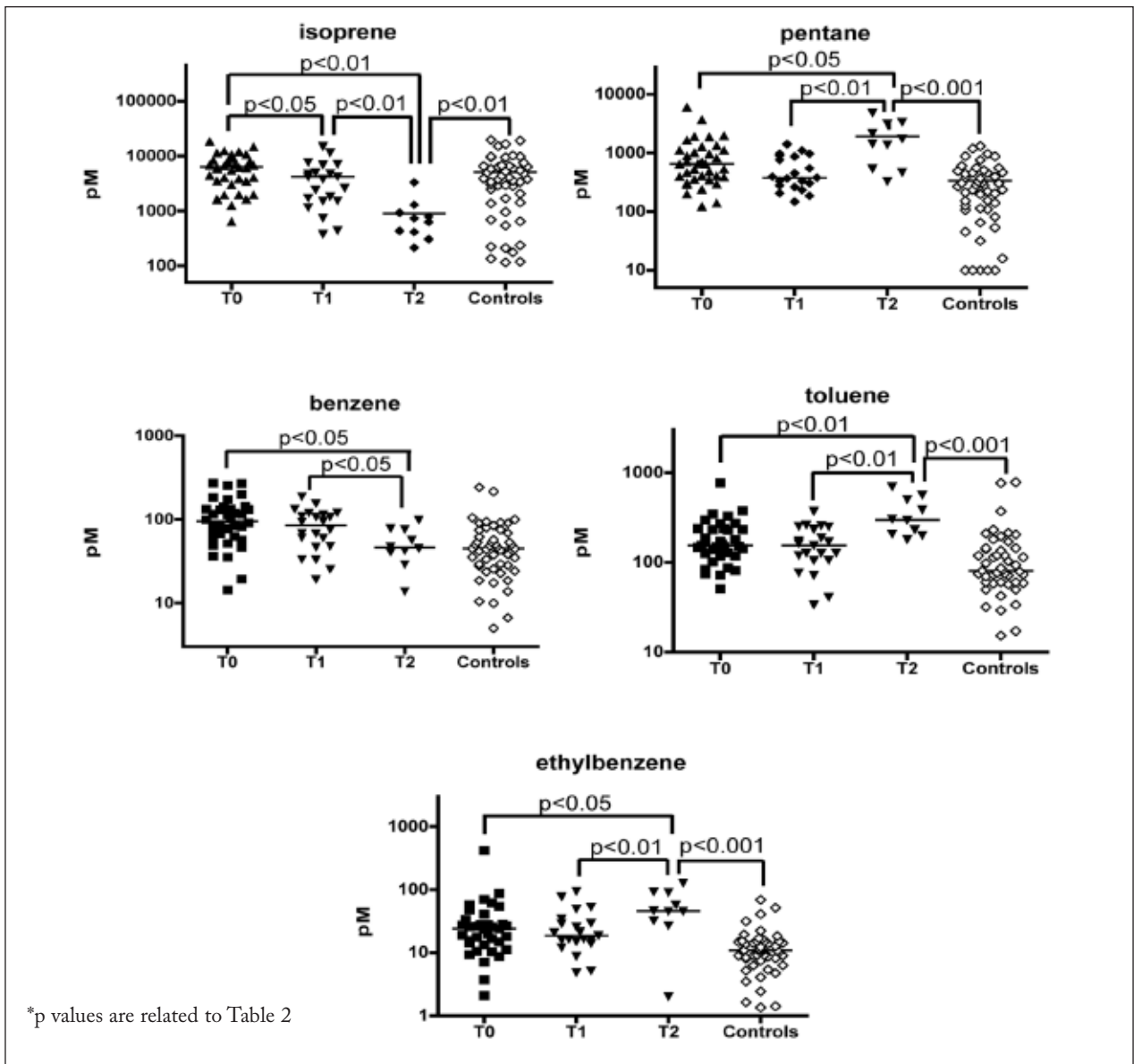


Figure 1. Distribution of the substances in studied group where significant differences were observed before and after surgery and at time T2 with respect to the controls. The differences between controls and NCLC at time T0 and T1 have been described in our previous study (7)*

Lung cancer surgical removal did not change VOC levels (except for isoprene) at T1: this points out an important issue related to the pathophysiological meaning of VOC measurement if exhaled air. In fact, our data shows that exhaled VOCs do not, or only in part, derive from cancerogenous tissue, being their concentration is unaffected by surgical removal. In ad-

dition, post surgical VOC levels remained higher than control values. A possible explanation of this data is that exhaled VOCs cannot be considered biomarkers of lung cancer *per se*, rather epiphenomena which accompany lung cancer development, probably due to the chronic load and burden of VOCs in overall lung tissue. Therefore, the majority of studied VOCs can

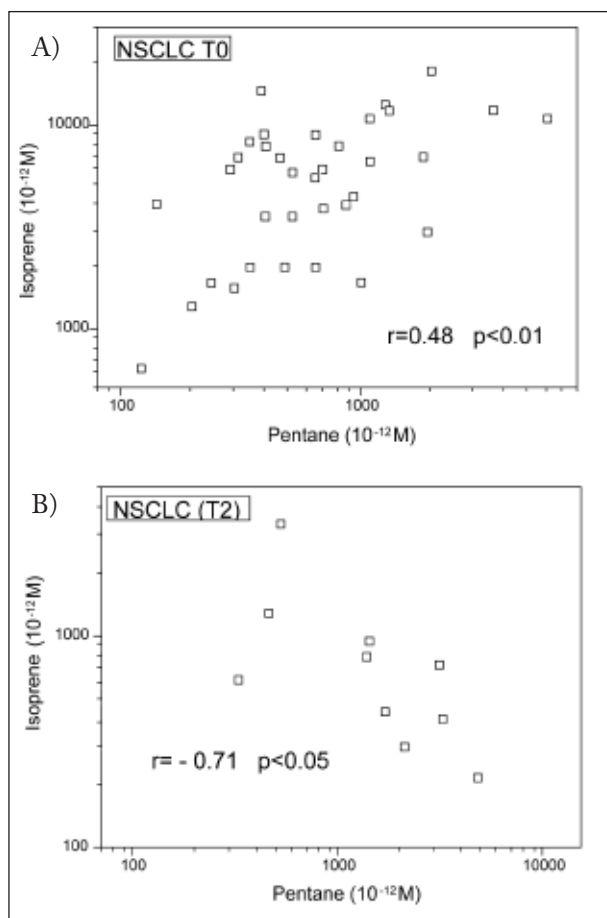


Figure 2. Correlation between pentane and isoprene in NSCLC patients at time T0 (A) and T1 (B)

not be proposed as specific biomarkers of lung cancer, as for blood biomarker frequently used in other cancerous lesions (i. e. PSA in prostatic cancer).

On the contrary, among studied biomarkers, isoprene gradually reduced after surgical removal, thus suggesting a possible role as prognostic biomarker, after we had excluded the effect of known confounding factor being able to modify isoprene levels (i.e. drugs). The gradual reduction in isoprene levels is somehow difficult to interpret. The same lobe or segmental lung removal might be able to reduce isoprene levels, but this can hardly explain its further reduction three years after surgical intervention. A further possible, but still speculative interpretation could be a chronic oxidative stress role occurring in the lung after its partial resection, causing a reduction in isoprene levels. In this re-

gard, isoprene, being an unsaturated molecule with conjugated double bonds, could be the target of free radicals in oxidative stress process, both a short and long term after surgery. In fact, the role of isoprene in oxidative stress is confirmed by its capacity to scavenge OH radicals in plants, in particular to protect photosynthetic apparatus against ozone and singlet oxygen damage and therefore reduce lipid peroxidation of cellular membranes (9-11).

Low levels of isoprene have been reported in chronic conditions associated with oxidative stress (12-15). These results are in lines with oxidative stress conditions described after lung resection, even if only short term post-operative investigations were described (16, 17). In literature, it is reported that lung equilibrium can be modified by lung resection due to the considerable tissue manipulation and the following lung re-expansion reperfusion, conditions that can induce oxidative stress. In fact, although lung tissue is resistant to hypoxia condition because of its dual blood flow and the use of reserves in alveolar spaces, re-oxygenation can significantly increase the reactive oxygen species (ROS) through a well known mechanism (18, 19). In this regard, a prospective randomized study has shown that lung resection in NSCLC patients can induce an increase in lipidic peroxidation, monitored through malondialdehyde (MDA) plasma levels, after the first postoperative hours (20). Additionally, the important role of one-lung ventilation (OLV) as a powerful free radical generator has been demonstrated, and cellular damage following a hypoxic insult is biphasic, considering the initial lack of oxygen and exacerbation during re-oxygenation (21). In particular, free radicals mainly generated in mitochondria can cause oxidative damage to a wide variety of biological molecules including DNA, proteins and polyunsaturated fatty acids (PUFAs) (22, 23). It has been also described that hyperoxia induces an increase in methylated alkanes produced by lipid peroxidation, and that these biomarkers of oxidative stress can be monitored in exhaled breath (24).

According to this hypothesis, after surgical operation, even though only at 3 years (T2), pentane levels increased significantly respect to time T0. Also in this case, this could be related to chronic oxidative stress processes, since it is known that pentane is a final

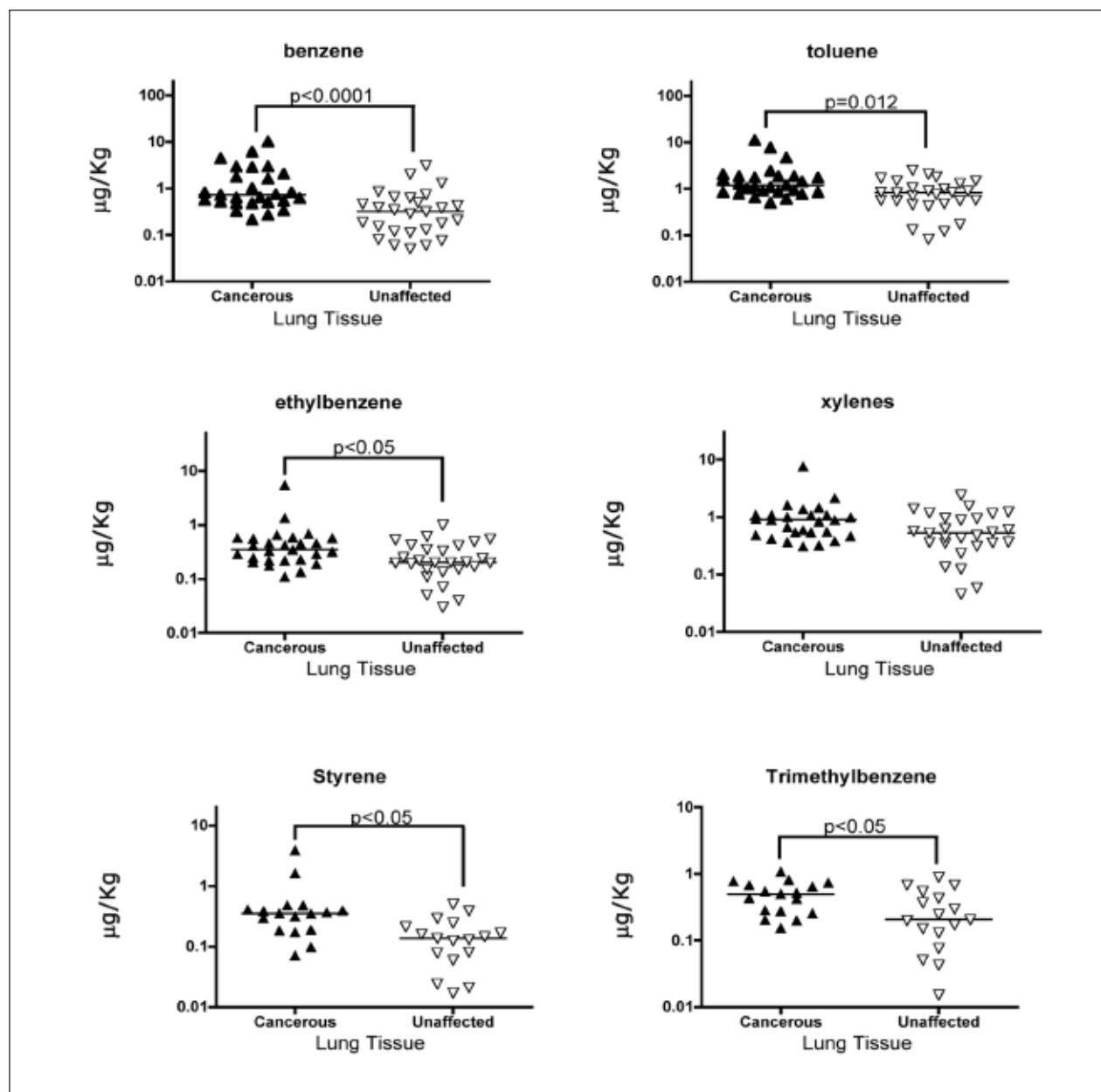


Figure 3. Distribution of aromatic hydrocarbons measured in cancerous and macroscopically unaffected lung tissue samples and their statistical differences evaluated using Wilcoxon test

product of lipid peroxidation. Therefore, the contrary behaviour of isoprene and pentane at T2 might have had the same origin and been related to the same free radical induction process in the airways. Pentane correlated also at time T0 with its methylated form, 2-methylpentane, while at time T2 this correlation was near significance, confirming their similar origin. In

fact, in literature, it has also been reported that methylation of alkanes is a secondary process of oxidative stress (25). On the other hand, pentane formation process was probably more notable with respect to 2-methylpentane, whose levels were similar both before and after surgery, but always higher than the controls. These results confirmed once again the endogenous

origin for isoprene, pentane and methylpentane (7).

In contrast, heptane and octane can be considered as mainly exogenous substances, deriving from environmental pollution and/or related to smoking habits. This origin was in accordance with their values which decreased after surgery, and at time T2, the levels became similar compared to controls. Therefore, their trend was contrary compared to that of the endogenous substances mentioned above. Additionally, it has to be considered that also these substances, being alkanes, can undergo reactions of methylation and thus decrease in concentration. Unfortunately, it was not possible to measure their corresponding methylated forms, their concentrations being lower than the LODs in almost all subjects. In fact, it was possible to monitor only pentamethylheptane levels that at time T2 were generally higher than T0, even if the limited number of cases did not permit a complete statistical analysis to achieve significance.

With regard to aromatic compounds, whose origin was related mainly to smoking habits or environmental pollution, a different trend was observed. In fact, at T2, while benzene significantly decreased and became similar to the controls, toluene and ethylbenzene significantly increased as compared to the levels measured before surgery. This behaviour is extremely difficult to explain because BTEX, like exogenous substances, have a similar origin as demonstrated by their correlation at T0. According to this hypothesis, analyses performed on tissue sample confirmed that all BTEX, except xylenes, were always significantly higher in cancerous and unaffected lung tissue. This result is in line with the unexpected higher concentrations of aromatic hydrocarbons in exhaled breath of NSCLC patients with respect to the controls. These substances, characteristic of cigarette smoke but also environmental pollutants, accumulated in the lung tissues, especially in the portion where cancer had developed. In fact, it is important to bear in mind that almost all NSCLC patient enrolled were ex or current heavy smokers.

The decrease in benzene in exhaled breath after surgery could be related to lung resection, but in this case even other BTEX would be expected to show the same behaviour. Therefore, the anomalous increase in toluene and ethylene breath concentrations and their

high correlation at time T1 suggested that surgery could have influenced both of them in a similar way.

T2 correlation between benzene and toluene was near significance and this result can be explained due to the fact that toluene represents benzene methylated form. On the other hand, in literature no methylation process on aromatic hydrocarbon related to oxidative stress have not yet been described.

We previously related BTEX origin in exhaled breath to smoking habits, these aromatic hydrocarbons being characteristic compounds of the cigarette smoke. However, the literature has described that benzene has a lower solution-phase activity coefficient if dissolved in the particular matter phase arising from cigarette smoke as compared to toluene and ethylbenzene (26). This evidence could suggest a higher deposition and therefore a slower release of the last two compounds as compared to benzene. In fact, it is important to note that NSCLC patients did not change their smoking habits after surgery. However, further investigations are necessary, mainly considering the low number of subjects enrolled at time T2.

Conclusion

The main result of this study was that two endogenous substances, i.e. isoprene and pentane, showed an opposite trend 3 years after surgery. A further possible, but still speculative interpretation, could be that induced long-term oxidative stress generates free radicals that react with isoprene and at the same time leads to pentane formation as a secondary product of oxidative stress. Methylation of pentane remains however more difficult to interpret and additional studies are needed to better understand the role of methylation of endogenous VOCs in oxidative stress in general. On the other hand, the behaviour of those VOCs that have been classified as exogenous/related to smoking habits or of mixed origin is complex and substance-dependent. In particular among BTEX, known to have similar origin, benzene on one side and toluene and ethyl benzene on the other showed an opposite trend after surgery and therefore a differential release by lungs. In conclusion, this study showed that surgical operation can influence exhaled

VOC levels, opening a possible new scenario in interpreting the relationship between lung status and VOC exhalation in NSCLC patients.

References

- Grace PA. Ischaemia-reperfusion injury. *Br J Surg* 1994; 81: 637-47.
- de Perrot M, Liu M, Waddell TK, Keshavjee S. Ischemia-reperfusion-induced lung injury. *Am J Respir Crit Care Med* 2003; 167: 490-511.
- Li C, Jackson RM. Reactive species mechanisms of cellular hypoxia-reoxygenation injury. *Am J Physiol Cell Physiol* 2002; 282: C227-41.
- Phillips M, Gleeson K, Hughes JM, et al. Volatile organic compounds in breath as markers of lung cancer: a cross-sectional study. *Lancet* 1999; 353: 1930-3.
- Phillips M, Cataneo RN, Cummin AR, et al. Detection of lung cancer with volatile markers in the breath. *Chest* 2003; 123: 2115-23.
- Gordon SM, Szidon JP, Krotoszynski BK, Gibbons RD, O'Neill HJ. Volatile organic compounds in exhaled air from patients with lung cancer. *Clin Chem* 1985; 31: 1278-82.
- Poli D, Carbognani P, Corradi M, et al. Exhaled volatile organic compounds in patients with non-small cell lung cancer: cross sectional and nested short-term follow-up study. *Respir Res* 2005; 6: 71.
- Wittekind C, Compton CC, Greene FL, Sobin LH. TNM residual tumour classification revisited. *Cancer* 2002; 94: 2511-6.
- Loreto F, Mannozi M, Maris C, Nascetti P, Ferranti F, Pasqualini S. Ozone quenching properties of isoprene and its antioxidant role in leaves. *Plant Physiol* 2001; 126: 993-1000.
- Loreto F, Velikova V. Isoprene produced by leaves protects the photosynthetic apparatus against ozone damage, quenches ozone products, and reduces lipid peroxidation of cellular membranes. *Plant Physiol* 2001; 127: 1781-7.
- Affek HP, Yakir D. Protection by isoprene against singlet oxygen in leaves. *Plant Physiol* 2002; 129: 269-77.
- McGrath LT, Patrick R, Mallon P, et al. Breath isoprene during acute respiratory exacerbation in cystic fibrosis. *Eur Respir J* 2000; 16: 1065-9.
- McGrath LT, Patrick R, Silke B. Breath isoprene in patients with heart failure. *Eur J Heart Fail* 2001; 3: 423-7.
- Miekisch W, Schubert JK, Vagts DA, Geiger K. Analysis of volatile disease markers in blood. *Clin Chem* 2001; 47: 1053-60.
- Kietzmann D, Kahl R, Muller M, Burchardi H, Kettler D. Hydrogen peroxide in expired breath condensate of patients with acute respiratory failure and with ARDS. *Intensive Care Med* 1993; 19: 78-81.
- Zieba M, Suwalski M, Kwiatkowska S, et al. Comparison of hydrogen peroxide generation and the content of lipid peroxidation products in lung cancer tissue and pulmonary parenchyma. *Respir Med* 2000; 94: 800-5.
- Waller DA, Keavey P, Woodfine L, Dark JH. Pulmonary endothelial permeability changes after major lung resection. *Ann Thorac Surg* 1996; 61: 1435-40.
- Lang JD, McArdle PJ, O'Reilly PJ, Matalon S. Oxidant-antioxidant balance in acute lung injury. *Chest* 2002; 122: 314S-20S.
- Lases EC, Duurkens VA, Gerritsen WB, Haas FJ. Oxidative stress after lung resection therapy: A pilot study. *Chest* 2000; 117: 999-1003.
- Misthos P, Katsaragakis S, Theodorou D, Milingos N, Skottis I. The degree of oxidative stress is associated with major adverse effects after lung resection: a prospective study. *Eur J Cardiothorac Surg* 2006; 29: 591-5.
- Misthos P, Katsaragakis S, Milingos N, et al. Postresectional pulmonary oxidative stress in lung cancer patients. The role of one-lung ventilation. *Eur J Cardiothorac Surg* 2005; 27: 379-82; discussion 82-3.
- Kneepkens CM, Ferreira C, Lepage G, Roy CC. The hydrocarbon breath test in the study of lipid peroxidation: principles and practice. *Clin Invest Med* 1992; 15: 163-86.
- Kneepkens CM, Lepage G, Roy CC. The potential of the hydrocarbon breath test as a measure of lipid peroxidation. *Free Radic Biol Med* 1994; 17: 127-60.
- Phillips M, Cataneo RN, Greenberg J, Grodman R, Gunawardena R, Naidu A. Effect of oxygen on breath markers of oxidative stress. *Eur Respir J* 2003; 21: 48-51.
- Phillips M, Cataneo RN, Greenberg J, Gunawardena R, Naidu A, Rahbari-Oskoui F. Effect of age on the breath methylated alkane contour, a display of apparent new markers of oxidative stress. *J Lab Clin Med* 2000; 136: 243-9.
- Pankow JF, Luo W, Tavakoli AD, Chen C, Isabelle LM. Delivery levels and behavior of 1,3-butadiene, acrylonitrile, benzene, and other toxic volatile organic compounds in mainstream tobacco smoke from two brands of commercial cigarettes. *Chem Res Toxicol* 2004; 17: 805-13.

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