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Impact of inspired substance concentrations on the results of breath analysis in mechanically ventilated patients

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Abstract

A well-defined relationship has to exist between substance concentrations in blood and in breath if blood-borne volatile organic compounds (VOCs) are to be used as breath markers of disease or health. In this study, the impact of inspired substances on this relationship was investigated systematically. VOCs were determined in inspired and expired air and in arterial and mixed venous blood of 46 mechanically ventilated patients by means of SPME, GC/MS. Mean inspired concentrations were 25% of expired concentrations for pentane, 7.5% for acetone, 0.7% for isoprene and 0.4% for isoflurane. Only if inspired concentrations were <5% did substance disappearance rates from blood and exhalation rates correlate well. Exhaled substance concentrations depended on venous and inspired concentrations. Patients with sepsis had higher *n*-pentane and lower acetone concentrations in mixed venous blood than patients without sepsis (2.27 (0.37–8.70) versus 0.65 (0.33–1.48) nmol L^{-1} and 69 (22–99) versus 18 (6.7–56) μmol L^{-1}). *n*-Pentane and acetone concentrations in breath showed no differences between the patient groups, regardless whether or not expired concentrations were corrected for inspired concentrations. In mechanically ventilated patients, concentration profiles of volatile substances in breath may considerably deviate from profiles in blood depending on the relative amount of inspired concentrations. A simple correction for inspired substance concentrations was not possible. Hence, substances having inspired concentrations >5% of expired concentrations should not be used as breath markers in these patients without knowledge of concentrations in blood and breath.

Keywords: *Acetone, breath analysis, carbon dioxide, inspired concentrations, gas chromatography, isoprene, mass spectrometry, pentane, solid-phase micro extraction*

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Introduction

Analysis of volatile organic compounds in exhaled air enables the observation of biochemical processes in the body in a non-invasive window. There is minimal risk for

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patients and for staff collecting exhaled air, and samples can be taken repeatedly and frequently during dynamically changing situations.

Despite a number of very promising results revealing interesting diagnostic properties of different markers (Kneepkens et al. 1994, Phillips 1997, Schubert et al. 1998, Risby & Sehnert 1999, Phillips et al. 2003a), analysis of volatile organic compounds in breath has not yet been introduced into clinical practice. The main obstacles are technical problems concerning sampling, preconcentration and analysis, as well as basic methodological issues such as normalization, expression of data and the effect of background contamination. A number of technical problems have been solved through new developments such as solid-phase micro-extraction (SPME) (Grote & Pawliszyn 1997), multi-bed adsorbent traps (Lärstad et al. 2002), and by improvements of separation and detection technologies. By contrast, the basic methodological issues are still a matter of debate and give rise to different interpretation and variation of results.

Volatile compounds can be produced anywhere in the body or in tissues lining the airway, and they may reflect physiological or pathological biochemical processes. Those substances not generated in the lung are transported via the blood stream and exhaled through the lung. If these substances are to be used as markers of physiological or pathological processes in the body, a clear relationship between substance concentrations in blood and in breath has to exist. Data exploring systematically parameters that may affect this relationship are lacking. In this context, the impact of inspired substance concentrations represents a fundamental issue since many breath biomarkers can also be present in inspired air. Different approaches to overcome this problem have been adopted. Substance concentrations in ambient or inspiratory air were measured and exhaled concentrations were corrected by subtracting inspiratory from expiratory concentrations (Phillips 1997, Phillips et al. 2003a). Others tried to eliminate ambient concentrations by having patients or volunteers breathe pure air for a certain time before measurement (Risby & Sehnert 1999). In any case, the methods could not be controlled since concentrations in blood were not measured.

In mechanically ventilated patients, these effects can be expected to be even more pronounced since shunt and dead space ventilation regularly occur, thus enhancing the effects of uptake and exhalation of volatile substances. Hence, this study was intended to investigate systematically the effects of inspired substance concentrations on the correlation between substance concentrations in blood and in breath. In a first step, the balance between substance elimination rates from blood and exhalation rates in breath was analysed in mechanically ventilated patients. In addition, it was investigated whether substance concentrations in blood or in breath were related to patients' clinical conditions.

For that purpose, the blood-borne volatile marker substances *n*-pentane (lipid peroxidation), isoprene (cholesterol biosynthesis), acetone (dextrose metabolism, lipolysis), and the volatile anaesthetic isoflurane were determined in the inspired and expired air and in mixed venous and arterial blood of mechanically ventilated critically ill patients.

Materials and methods

Patients

Following approval by the local ethics committee and after having obtained informed consent by the patient or his next of kin, 46 consecutive critically ill mechanically ventilated patients of an interdisciplinary intensive care unit (ICU) were enrolled into the study. Because of the severity of their underlying disease, all patients had indwelling arterial and pulmonary artery catheters (PAC, 7.5 F Arrow flow directed catheter; Arrow International, Erdingen, Germany). Twenty-one patients had sepsis (American College of Chest Physicians/Society of Critical Care Medicine Consensus Conference 1992); 25 did not. Seventeen of the septic patients had septic shock; two had severe sepsis. Sixteen patients without sepsis showed no inflammatory reaction at all; nine had systemic inflammatory reaction syndrome (SIRS). Nineteen patients had had open-heart surgery with extracorporeal circulation. SIRS was diagnosed according to the American College of Chest Physicians/Society of Critical Care Medicine Consensus Conference (1992) when two or more of the following criteria were fulfilled: temperature >38 or $<36^{\circ}$C; leukocytes $>12\,000$ or $<4000/\mu$l, or $>10\%$ of immature cells in the differential count, respectively; HR (heart rate) $>90\ \text{min}^{-1}$, RR (respiratory rate) $>20\ \text{min}^{-1}$. Sepsis was defined as SIRS plus a positive microbiological finding in a normally sterile specimen. Patients were classified as having severe sepsis when additional signs of organ dysfunction were present; and septic shock was diagnosed when blood pressure was <90 mmHg or when there was need for vasopressor support despite sufficient volume resuscitation.

Cardiac output (CO) was determined by means of thermodilution. A total of 10 ml sterile 0.9% NaCl ($T \leq 20^{\circ}$C) was injected into the upper caval vein through the corresponding lumen of the PAC. The consecutive change of blood temperature in the pulmonary artery was measured by the thermistor at the tip of the PAC and fed into a CO computer (CME-Monitor, Hewlett Packard, Böblingen, Germany). CO was calculated from these data. Each measurement was taken in triplicate.

Breath and blood sampling

Breath samples (10 mL) were drawn from the respiratory circuits into a gas-tight syringe and transferred immediately into evacuated 20-mL glass vials. Alveolar gas was taken near the endotracheal tube (ETT) under control of expired CO_2 using a simplified application of a technique described by Schubert et al. (2001). For that purpose, the sensor of a fast-responding mainstream CO_2 analyser (Capnogard, Novametrix Medical Systems, Inc., Wallingford, CT, USA) was inserted into the respiratory circuit near the ETT. Alveolar gas samples were withdrawn from the circuit under visual control of expired CO_2 in the way that gas collection only took place during the alveolar phase of expiration. Inspiratory samples were taken near the ventilator inlet. The access to the respiratory circuit was realized through sterilized stainless steel T-pieces. At least two samples for each measurement were collected.

To determine reliability and reproducibility of the visually CO_2 controlled alveolar sampling technique, ten gaseous samples were taken consecutively from one patient. In these samples, PCO_2 was determined in a blood gas analyser (ABL 615,

Radiometer, Copenhagen, Denmark) and isoprene concentrations were analysed via SPME, GC/MS. Reliability was assessed by the correlation between end tidal PCO_2 measured by the capnometer and PCO_2 in the samples. Variation was calculated from CO_2 and isoprene concentrations in the samples.

A total of 3 mL arterial and mixed venous blood was taken from each patient at exactly the same time as breath samples were collected. The whole blood was transferred immediately from the NH_4-ammonium tubes into sealed 20-mL headspace vials containing 6.9 ml aqueous solution of NaCl (30 g L^{-1}) and 0.1 mL 500 g L^{-1} phosphate buffer (pH 7.0).

Preconcentration and analysis of volatile substances

Volatile substances in breath were preconcentrated by means of SPME (Grote and Pawliszyn 1997). Preconcentration and desorption of the volatile organic compounds were done automatically by means of an SPME autosampler (CTC Multi Purpose Sampler; PAL, Zwingen, Switzerland). Substances in blood were determined via SPME headspace analysis as described previously (Miekisch et al. 2001) using identical fibres (polydimethylsiloxane (PDMS)/Carboxen, Supelco/Sigma Aldrich, Taufkirchen, Germany) and the same autosampling device as for the gaseous samples.

Before the SPME procedure, the PDMS/carboxen-coated fibre was pre-treated in the injection port of a gas chromatograph at 285°C for 30 min. Internal standard (2, 3-dimethylbutadiene) was added through the septum by means of a micro-syringe. The vial was vortexed for 5 min at 40°C in the heating block of the CTC autosampler. Then, the syringe needle of the SPME device was inserted into the headspace of the vial for 5 min. The needle containing the SPME fibre was withdrawn and introduced into the port of a capillary gas chromatograph (Varian, Walnut Creek, CA, USA). Port temperature was 280°C. The SPME device was held in the port for 2 min to allow complete desorption of the components.

Substances were separated on a 30-m (0.32 mm i.d.) Poraplot Q-HT column (Varian/Chrompack, Middleburg, The Netherlands), detected and identified by mass spectrometry (Saturn 2000, Varian) and quantified via calibration curves. Precision, variation and limits of detection of SPME procedures have already been extensively studied (Pawliszyn 1995, Eisert and Pawliszyn 1997, Grote and Pawliszyn 1997). Limits of detection (LOD) and precision (per cent relative standard deviation, RSD) were 0.05 (4.4), 0.10 (3.8), 0.02 (2.5) and 0.08 (5.9) nmol L^{-1} for the determination of isoprene, pentane, isoflurane and acetone in blood (Miekisch et al. 2001). LODs for the analysis of isoprene, pentane, isoflurane and acetone in exhaled gas were 0.01, 0.02, 0.02 and 0.1 nmol L^{-1} (Schubert et al. 2002). Intraday variation was $<5\%$ RSD ($n=8$) for all substances.

Estimation of shunt and dead space ventilation

Shunt was calculated according to the standard formula:

$$Q_s/Q_t = (C_c - C_a)/(C_c - C_v)$$

where Q_s/Q_t is the shunt fraction, C_c is the content of oxygen in blood flow to idealized ventilated alveolar units, C_a is the arterial oxygen content and C_v is the mixed venous oxygen content. C_c was approximated by the maximum alveolar oxygen content

computed from F_iO_2, PH_2O and P_aCO_2. Dead space ventilation (V_d/V_t) was assessed via the Bohr formula:

$$V_d/V_t = ([P_ACO_2] - [P_{ex}CO_2])/[P_ACO_2]$$

where P_ACO_2 is the alveolar PCO_2, $P_{ex}CO_2$ is the mixed expiratory PCO_2. P_ACO_2 was approximated by arterial PCO_2.

Statistical analysis

Exhaled substance concentrations and concentrations in blood were non-normally distributed. Statistical analysis was performed on three levels. (1) Correlation between different variables was assessed by means of Spearman correlation coefficients for non-parametric data. (2) Quantitative relationships between variables were investigated by means of linear regression analysis. Regression analysis of non-normally distributed variables was done following rank transformation of the dependant variables. Log transformation of the variables did not improve the distribution of standardized residuals. In addition, pentane and isoflurane concentrations could not reasonably be log transformed since many of the values were zero. Reliability of regression analysis was controlled by means of the standardized residuals, i.e. if standardized residuals were not approximately normally distributed after rank transformation regression analysis could not be applied. (3) Comparison between patient groups was done by a Mann–Whitney *U*-test.

For each substance, the correlation and quantitative relationship between disappearance rates (($C_{venous} - C_{arterial}$)*CO) and exhalation rates (C_{gas}*[minute ventilation (MV)]) were determined. In addition, the quantitative relationship between exhaled concentrations and venous substance concentrations (C_{venous}), inspired concentrations (C_{insp}), CO, minute ventilation, shunt and dead space ventilation (DSV) was calculated. Results are given as medians and 25–75 percentiles or as means and 95% confidence intervals, as appropriate. A $p < 0.05$ was considered to be statistically significant. For statistical testing, the computer program SPSS 11.0 for Windows was used.

Results

PCO_2 in the samples and end tidal PCO_2 were strongly correlated to each other. As expected, PCO_2 in the samples systematically was lower than end tidal PCO_2, as the former represents a mean alveolar concentration, whereas end tidal PCO_2 is the maximum alveolar concentration. Variation of the CO_2 controlled alveolar sampling technique was $<4\%$ ($n = 10$). Patients' demographic data, main diagnoses, ventilator type, mode of mechanical ventilation, shunt and dead space ventilation are listed in Table I. In the group without sepsis, there were more women than in the group with sepsis ($p = 0.02$). There was a small, but statistically significant ($p = 0.02$) difference of inspired oxygen concentrations between the groups (0.4 (0.4–0.4) no sepsis versus 0.4 (0.4–0.55) sepsis). The median age of patients was 64 (59–69) versus 57 (51–64) years ($p = 0.23$) for patients without and with sepsis, respectively. Shunt was greater in patients with sepsis (0.23 (0.15–0.28)) than in patients without sepsis (0.12 (0.08–0.16), $p = 0.003$). Dead space ventilation, the types of ventilators used and the mode of ventilation were not different between the patient groups.

Table I. Patients' demographics and inflammatory status.

Number	Gender	Age (years)	Sepsis	SIRS	EC	Ventilator	Mode	F_iO_2	Q_s/Q_t	V_d/V_t
1	F	75	0	0	EC	SV 900	PCV	0.4	0.12	0.27
2	M	62	0	0		Evita 4	BIPAP	0.35	0.07	0.70
3	M	61	0	0	EC	SV 300	PCV	0.4	0.07	0.61
4	M	64	0	0	EC	SV 900	PCV	0.4	0.15	0.45
5	M	52	0	0	EC	SV 900	PCV	0.4	0.11	0.62
6	F	75	0	0	EC	SV 900	PCV	0.38	0.10	0.41
7	M	72	0	0	EC	SV 300	PCV	0.42	0.05	0.57
8	F	63	0	0	EC	Evita 4	BIPAP	0.5	0.20	0.71
9	M	68	0	0	EC	SV 900	PCV	0.5	0.09	0.78
10	M	65	0	0	EC	SV 300	PCV	0.4	0.14	0.74
11	M	63	0	0		SV 300	ASB	0.4		
12	M	67	0	0	EC	SV 300	PCV	0.4	0.12	0.56
13	M	52	0	0		Evita 4	BIPAP	0.5	0.28	0.61
14	M	44	0	0		SV 900	PCV	0.5	0.13	0.74
15	M	63	0	0	EC	SV 300	PCV	0.41	0.08	0.52
16	M	65	0	0	EC	Evita 4	BIPAP	0.4	0.15	0.50
17	F	49	0	1		Evita 4	BIPAP	0.4	0.33	0.76
18	F	54	0	1	EC	SV 900	PCV	0.4	0.10	0.48
19	M	69	0	1		SV 300	ASB	0.4	0.17	0.67
20	F	76	0	1	EC	SV 300	PCV	0.4	0.12	0.64
21	F	77	0	1	EC	SV 300	PCV	0.4	0.07	0.50
22	F	69	0	1	EC	Evita 4	BIPAP	0.4	0.12	0.64
23	F	72	0	1	EC	SV 300	PCV	0.4	0.08	0.52
24	M	59	0	1	EC	SV 300	PCV	0.4	0.25	0.41
25	F	56	0	1	EC	Evita 4	BIPAP	0.4	0.16	0.56
26	M	43	1	2		Evita 4	ASB	0.4	0.17	0.69
27	M	23	1	2		Evita 4	BIPAP	0.4	0.04	
28	M	44	1	3		SV 900	PCV	0.4	0.31	0.43
29	M	79	1	3		SV 900	PCV	0.4	0.08	0.52
30	M	56	1	4		SV 900	PCV	0.4	0.29	0.36
31	M	56	1	4		SV 900	SIMV	0.4	0.23	0.56
32	M	61	1	4		SV 900	PCV	0.6	0.11	0.40
33	M	61	1	4		SV 900	PCV	0.5	0.13	0.40

Table I (*Continued*)

Number	Gender	Age (years)	Sepsis	SIRS	EC	Ventilator	Mode	F_iO_2	Q_s/Q_t	V_d/V_t
34	F	45	1	4		SV 900	PCV	0.5	0.23	0.77
35	M	78	1	4		Evita 4	BIPAP	0.4	0.27	0.75
36	M	69	1	4		SV 300	PCV	0.4	0.12	0.68
37	M	56	1	4		Evita 4	BIPAP	0.4	0.32	0.60
38	M	65	1	4		SV 900	PCV	0.6	0.30	0.48
39	F	65	1	4		SV 900	PCV	0.4	0.25	0.43
40	M	77	1	4		Evita 4	BIPAP	0.7	0.25	0.62
41	M	51	1	4		SV 300	PCV	0.4	0.20	0.58
42	M	73	1	4		Evita 4	ASB	0.44	0.18	0.52
43	M	72	1	4		SV 300	PCV	1.0	0.22	0.67
44	M	52	1	4		Evita 4	BIPAP	0.8	0.44	0.60
45	M	51	1	4		SV 300	PCV	0.5		
46	M	57	1	4		Evita 4	BIPAP	0.55		

Sepsis: 0, criteria of sepsis (American College of Chest Physicians/Society of Critical Care Medicine Consensus Conference 1992) not fulfilled; 1, criteria of sepsis fulfilled. SIRS (systemic inflammatory reaction syndrome) (American College of Chest Physicians/Society of Critical Care Medicine Consensus Conference 1992): 0, no SIRS; 1, SIRS; 2, sepsis; 3, severe sepsis; 4, septic shock; EC, extracorporeal circulation (<6 h before measurement); CABG, coronary artery bypass grafting; SV 900, Siemens ventilator 900 C (Siemens Elema, Solna Sweden); SV 300, Siemens ventilator 300 C; Evita 4, Evita 4 (Draeger, Luebeck, Germany); ASB, assisted spontaneous breathing; PCV, pressure controlled ventilation; BIPAP, biphasic airway pressure; SIMV, synchronized intermittent mandatory ventilation.

Shunt was calculated according to the formula $Q_s/Q_t = (C_c - C_a)/(C_c - C_v)$, where Q_s/Q_t is the shunt fraction, C_c is the content of oxygen in blood flow to idealized ventilated alveolar units, C_a is the arterial oxygen content, and C_v is the mixed venous oxygen content. C_c was approximated by the maximum alveolar oxygen content computed from F_iO_2, PH_2O and P_aCO_2. Dead space ventilation (V_d/V_t) was assessed via the Bohr formula: $V_d/V_t = ([P_ACO_2] - [P_{ex}CO_2])/[P_ACO_2]$, where P_ACO_2 is the alveolar PCO_2, $P_{ex}CO_2$ is the mixed expiratory PCO_2. P_ACO_2 was approximated by arterial PCO_2.

Table II. Inspired and expired concentration of volatile markers.

Substance	Inspiratory: Concentration (mmol L^{-1}), mean (CI), $n=46$	Expired: Concentration (mmol L^{-1}), mean (CI), $n=46$	(C_{insp}/C_{exp}) mean ± SEM
Acetone	6.20 (4.26–8.43)	328 (174–446)	7.5% ± 2.5%
Isoprene	0.20 (0.8–0.33)	29.2 (16.6–34.1)	0.7% ± 0.2%
n-Pentane	0.24 (0.9–0.39)	0.68 (0.27–1.09)	25% ± 10%
Isoflurane	0.26 (0.06–0.46)	59.1 (0–132)	0.4% ± 0.2%

CI, 95% confidence interval.

The ratios of inspired and expired substance concentrations are shown in Table II. The Spearman coefficient for the correlation between pentane delivery in blood (C_{venous}*CO) and pentane exhalation rate (C_{gas}*MV) was 0.11 when all measurements were considered, and 0.90 when only measurements with $C_{insp} < 0.05 * C_{exp}$ were taken into account. R^2 for linear regression (rank (C_{gas}*MV) = 18.994 + 0.0935*C_{venous}*CO) was 0.14 when all measurements were considered, and 0.48 when only measurements with $C_{insp} < 0.05 * C_{exp}$ were taken into account (Figure 1a, b).

The Spearman coefficient for the correlation between acetone delivery in the blood (C_{venous}*CO) and acetone exhalation rate was 0.63 when all measurements were taken into account and 0.89 when only measurements with $C_{insp} < 0.05 * C_{exsp}$ were considered. R^2 for linear regression (rank (C_{gas}*MV) = 14.596 + 1.91 × 10^{-5}* C_{venous}*CO) was 0.44 when all measurements were taken into account, and 0.59 when only measurements with $C_{insp} < 0.05 * C_{exsp}$ were considered.

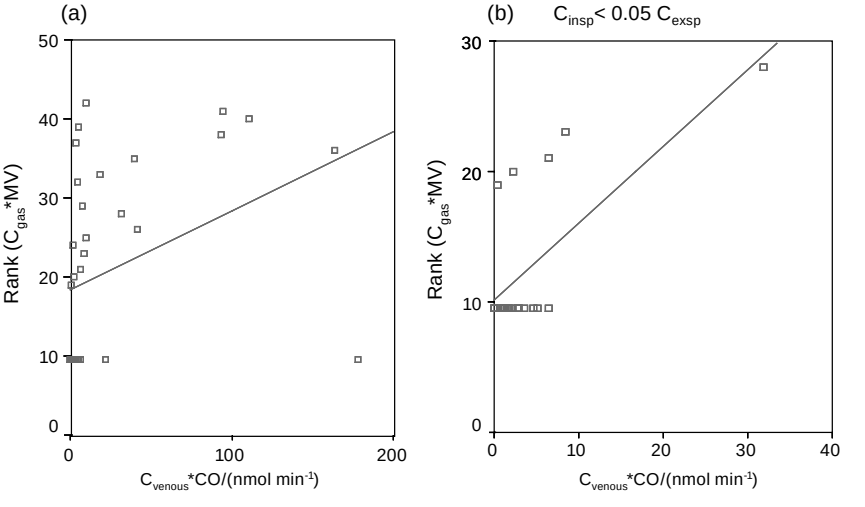

Figure 1. Pentane: scatter plot of rank transformed substance production in breath as a function of substance delivery in the blood. The solid line indicates linear regression fit: (a) all measurements, $R^2 = 0.14$ ($n = 38$), (b) only measurements where $C_{insp} < 0.05 * C_{exp}$, $R^2 = 0.48$ ($n = 20$). MV, minute ventilation (L min^{-1}); CO, cardiac output (L min^{-1}).

The Spearman coefficients for the correlation between substance clearance from the blood ($(C_{venous} - C_{arterial})$*CO) and the exhalation rate in breath (C_{gas}*MV) were 0.80 for isoprene and 0.94 for isoflurane.

Since arterial concentrations were highly affected by inspired substance concentrations, delivery rates (C_{venous}*CO) in blood were used instead of disappearance rates ($(C_{venous} - C_{arterial})$*CO) for the substances having high inspired concentrations (acetone, *n*-pentane).

Multiple linear regression analysis revealed that pentane concentrations in breath only depended on inspired concentrations ($C_{gas} = 16.943 + 18.837 * C_{insp}$, p for the coefficients was <0.001). Exhaled acetone concentrations depended on venous and inspired concentrations, cardiac output and dead space ventilation ($C_{gas} = 15.721 + 1.3\ 10^{-4} * C_{venous} + 0.689 * C_{insp} + 0.996 * CO - 20.724 * DSV$, p for the coefficients were 0.016, <0.001, 0.007, 0.012 and 0.026). Exhaled isoprene concentrations depended on venous concentrations and on cardiac output ($C_{gas} = 8.007 + 0.322 * C_{venous} + 1.036 * CO$, p for the coefficients was 0.032, <0.001, 0.03). Isoflurane concentrations in breath could be predicted from venous concentrations ($C_{gas} = 29.127 + 0.0104 * C_{venous}$, p for the coefficients were <0.001, 0.01)

Patients with sepsis had significantly higher *n*-pentane concentrations in mixed venous blood than patients without sepsis (2.27 (0.37–8.70) versus 0.65 (0.33–1.48) nmol L^{-1}, $p = 0.033$) (Figure 2). In arterial blood, the difference between septic and non-septic patients was still visible (1.16 (0.65–5.41) versus 0.55 (0.21–1.67) nmol L^{-1}, $p = 0.10$), but had lost significance (Figure 2). *n*-Pentane concentrations in exhaled air (0.33 (0.00–0.97) versus 0.028 (0.00–0.72) nmol L^{-1}, $p = 0.53$) showed no difference between the patient groups (Figure 2), regardless of whether or

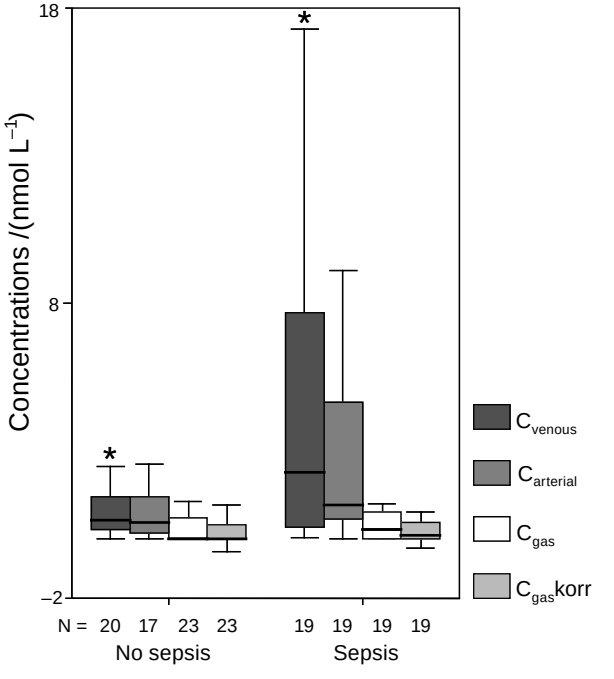

Figure 2. Box-plot of *n*-pentane concentrations in mixed venous (C_{venous}), arterial ($C_{arterial}$) blood and in exhaled air (C_{gas})·C_{gas}korr $= C_{gas} - C_{insp}$. *Statistically significant differences of medians.

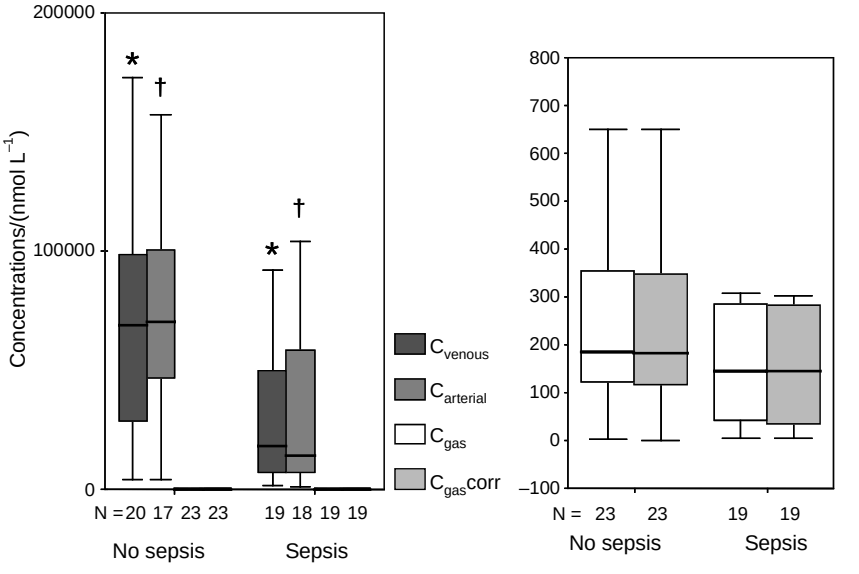

Figure 3. Box-plot of acetone concentrations in mixed venous (C_{venous}), arterial ($C_{arterial}$) blood and in exhaled air C_{gas}. C_{gas}korr $= C_{gas} - C_{insp}$. *,†Statistically significant differences of medians.

not expired concentrations were corrected for inspired concentrations. Results were identical when delivery rates in blood (C_{venous}⋆CO) and exhalation rates (C_{gas}⋆MV) were regarded instead of the corresponding concentrations. Acetone concentrations in mixed venous (18.0 (6.73–55.8) versus 69.1 (22.2–98.5) μmol L^{-1}, $p = 0.013$), and arterial blood (14.1 (7.11–63.6) versus 70.3 (44.7–102) μmol L^{-1}, $p = 0.028$) were significantly lower in septic patients than in non-septic patients (Figure 3). There was no significant difference of exhaled acetone concentrations between the patient groups (145.0 (40.9–307.7) versus 183.8 (119.7–361.6) μmol L^{-1}, $p = 0.38$), regardless of whether or not expired concentrations were corrected for inspired concentrations. Results were identical when delivery rates in blood (C_{venous}⋆CO) and exhalation rates (C_{gas}⋆MV) were regarded instead of the corresponding concentrations. Isoprene concentrations in blood and breath did not depend on patients' inflammatory status. Isoflurane concentrations were not analysed with respect to differences between septic and non-septic patients as the substance is of pure exogenous origin. Therefore, only patients having had anaesthesia shortly before measurement had a chance to have isoflurane in blood or breath which is obviously not linked to patients' inflammatory status.

Discussion

Volatile biomarkers may be generated in the body, transported via the bloodstream and exhaled through the lung. A well-defined relationship has to exist between substance concentrations in blood and in breath if these molecules are to be used as breath markers of disease or health in the body.

To assess the influence of inspired substances on the correlation between substance concentrations in blood and in breath, concentrations of volatile markers were

determined in blood, in inspired and in expired air of mechanically ventilated patients. Experimental data showed considerable deviation from the expected relationship between blood and breath concentrations when inspired substance concentrations were high. In addition, correlations between substance concentrations and patients' clinical conditions were different depending on whether concentrations were determined in blood or in breath. The effects of inspired substance concentrations could not be accounted for by subtracting inspired concentrations from expired concentrations.

Volatile substances can be generated anywhere in the body or may have been inhaled historically and stored in the body. Substances are transported via the blood stream and can then be excreted in exhaled air, in urine or faeces or can be metabolized in the body. Renal or intestinal excretion as well as metabolism is slow in comparison with exhalation through the lung. Hence, for reasons of mass conservation, substance disappearance rates from the blood have to be equal to exhalation rates if substance concentrations in blood and in breath are determined simultaneously. Verifying this condition of instantaneous mass balance appears to be an easy and reliable method to test the consistency of data and to assess the effects of experimental errors in breath analysis. As we used highly reproducible and precise analytical methods (Grote & Pawliszyn 1997, Miekisch et al. 2001), deviations of experimental data from the above-mentioned mass balance were, therefore, due to systematic errors rather than to random variation of the analytical method.

Low inspired substance concentrations yielded good correlation, high inspired concentrations yielded poor correlation between disappearance rates from the blood and exhalation rates in breath. Correlation was not improved by subtracting inspired from expired substance concentrations. Correlation improved remarkably, however, when only measurements were considered where inspired concentrations were $<5\%$ of expired concentrations. Multiple regression analysis was used to identify the factors determining exhaled substance concentrations. High inspired concentrations of pentane and acetone were identified as principal determinants of exhaled concentrations of these compounds. Low inspired concentrations of isoprene and isoflurane did not contribute to the composition of exhaled substance concentrations.

Further evidence for the crucial role of inspired substance concentrations was found when pentane and acetone concentrations were analysed in blood and breath and linked to patients' clinical conditions. Acetone and pentane concentrations in mixed venous blood of patients with sepsis were different from concentrations in the blood of patients without sepsis. This difference could still be seen in arterial blood but surprisingly disappeared completely in exhaled breath.

These findings may be explained as follows. (1) Exhaled substance concentrations are considerably affected by inspired substance concentrations in mechanically ventilated patients. Addition of inhaled concentrations to exhaled concentrations blurs the concentration differences present in the mixed venous samples. Expired samples may be diluted or contaminated by inspired and/or dead space gas depending on the ratio of alveolar and dead space ventilation, which itself depends on the breathing pattern (Cope et al. 2004). (2) The influence of inspired substance concentrations on the correlation between concentrations in blood and in breath is not purely linear (Van den Aardweg et al. 2001) and, therefore, cannot be accounted for by simple subtraction of inspired concentrations from expired concentrations. Alveolar concentration gradients may be altered through inspired substances and

substance elimination is affected accordingly, i.e. less substance is eliminated from the blood into the alveoli than venous concentrations would induce without the presence of inspired substances. The extent of this influence is controlled by the ventilation/perfusion ratio in the lung (Wagner et al. 1974, West & Wagner 1998). (3) The non-linear effects of inspired substance concentrations on concentrations in exhaled air may have been enhanced by the fact that at the time of measurement, inspired substance concentrations and concentrations in blood were not at equilibrium.

These results are in contrast to the findings of other researchers who use subtraction methods to account for inspired substance concentrations. The recognition pattern used by Phillips et al. (2003a) to detect lung cancer was based on the difference between expired and inspired concentration normalized to a GC internal standard (Phillips 1997) regardless of the relative amount of inspired concentrations. The patients Phillips et al. investigated substantially differed from the patients of the present study in that they were spontaneously breathing. Hence, the likelihood of severe deviation from normal breathing patterns and the presence of ventilation/perfusion mismatch were much lower. The non-linear effects of inspired substance concentrations we observed were most probably linked to the occurrence of dead space ventilation and shunt perfusion, which is a common and frequent problem in mechanically ventilated patients (Feihl et al. 2000, Dembinski et al. 2002). This may explain why Phillips et al. could obtain clinically relevant results by using a subtraction method. In addition, no information on the ratio of substance concentrations in blood and breath in his patients is available since Phillips et al. did not determine marker concentrations in blood. This bears the risk that results might change if for any reason patients' breathing patterns (Cope et al. 2004) or the composition of room air were modified.

Since arterial concentrations may have been altered through substance intake delivery rates (C_{venous}*CO) were calculated instead of disappearance rates (($C_{venous} - C_{arterial}$)*CO) when inspired concentrations were high. This explains why pentane and acetone production rates in blood seem to be much higher than exhalation rates in breath. In the case of low (isoprene) or very low (isoflurane) inspired substance concentrations, disappearance rates and exhalation rates were almost identical.

The *n*-alkanes pentane and ethane are looked upon as markers of lipid peroxidation (Van Rij & Wade 1985, Morita et al. 1986, Kneepkens et al. 1994, Aghdassi & Allard 2000). Hence, *n*-pentane production can be expected to increase in clinical conditions where radical mediated inflammatory processes are involved (Weitz et al. 1991, Mendis et al. 1995, Miller et al. 1997, Aghdassi et al. 2003). For that reason, mixed venous pentane concentrations were higher in septic patients than in non-septic patients. Acetone is generated via decarboxylation of acetoacetate, which is produced mainly during lipolysis (Nelson et al. 1998). All septic patients were fed enterally and subject to a strict control of blood dextrose levels (Van den Berghe et al. 2001). By contrast, the majority of patients without sepsis had had open-heart surgery involving extracorporeal circulation. Lipolytic effects of preoperative fasting and operative stress (Bagchi et al. 1994, De Zwart et al. 1997), therefore, increased acetone concentrations. Hence, higher acetone concentrations in patients without sepsis than in septic patients were to be expected.

Due to the design of this study, the results are subject to some limitations. In the patient groups studied, there were no clinical parameters linked to isoprene or isoflurane concentrations. Hence, we could not positively demonstrate that in the case

of low inspired substance concentrations, the relationships between concentrations of volatile markers and clinical conditions were identical regardless whether substance concentrations were determined in blood or in breath. Nevertheless, we feel that the results sufficiently support the view that inspired substance concentrations can have an important impact on results in breath analysis.

As only mechanically ventilated patients were studied, we cannot conclusively answer the question whether there is a fundamental difference between spontaneously breathing individuals and mechanically ventilated patients concerning the impact of inspired concentrations.

Gender, F_iO_2 and shunt were not equally distributed between septic and non-septic patients. Gender is not known to have any influence on the concentration of exhaled ethane or pentane (Phillips et al. 2000). Pentane concentrations may have been increased in septic patients through higher F_iO_2 (Phillips et al. 2003b). Since the difference of F_iO_2 between the patient groups was rather small, the amount of additional pentane due to higher inspired oxygen can be neglected. In addition, as each patient served as his own control, the principal results concerning mass balance and correlations between substance concentrations and patients' clinical conditions were not affected. Higher shunt fractions in septic patients could have induced relatively higher pentane concentrations in the blood, since smaller amounts of low soluble substances are exhaled when shunt is high (Wagner et al. 1974). However, regression analysis did not reveal any shunt dependency of pentane exhalation and mass balance between blood and breath cannot be influenced by shunt.

As theory predicts (West & Wagner 1998) for well soluble volatiles, exhaled acetone concentration were reciprocally related to dead space ventilation.

In conclusion, the results demonstrate that inspired substance concentrations can seriously affect the accuracy and reliability of breath analysis in mechanically ventilated patients. Concentration profiles in breath may considerably deviate from profiles in blood depending on the relative amount of inspired substance concentrations. Due to the non-linearity of these effects in mechanically ventilated patients, a simple correction for inspired substance concentration was not possible. Hence, substances with inspired concentrations $>5\%$ of expired concentrations should not be used as breath markers without knowledge of the concentrations in blood and breath.

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