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Application of a new method for analysis of exhaled gas in critically ill patients

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Abstract *Objective:* Application of a new method for analysis of exhaled gas in critically ill patients.
Design: Open study.
Setting: Surgical intensive care unit of an university hospital.
Patients: Thirty-seven consecutive, critically ill, mechanically ventilated patients.
Interventions: None.
Measurements and results: Chemical analysis of the patient's exhaled gas was based upon substance adsorption and concentration onto activated charcoal, microwave desorption and gas chromatographic separation. Patients with acute respiratory distress syndrome (ARDS) exhaled less isoprene than those without ARDS [9.8 (8.2–21.6) vs 21.8 (13.9–41.4) nmol/m^2 per min [median (95 % confidence interval)], p = 0.04]. In patients who developed pulmonary infection, pentane elimination increased from 0.4 (0.0–5.4) to 2.7 (0.6–6.1, p = 0.05) nmol/m^2 per min and isoprene elimination decreased from 5.2 (0–33) to 5.0 (0–17, p = 0.05) nmol/m^2 per min, resulting in a significant increase in pentane/isoprene ratio from 0.1 (0–0.3) to 0.4 (0–15, p = 0.007) when compared to patients without pulmonary infection.
Conclusions: The new method allows quantitative analysis of human gas samples with low substance concentrations and is well suited for clinical studies which involve the investigation of metabolic processes in the lung and the body.

Key words Acetone · Gas chromatography · Isoprene · Lipid peroxidation · Microwave desorption · N-pentane

Introduction

Analysis of exhaled gas may provide useful insights into metabolic processes in certain diseases. The volatile constituents of human breath include acetone, n-pentane and isoprene [1, 2]. Acetone is formed by decarboxylation of acetoacetate which derives from lipolysis or lipid peroxidation. High concentrations of acetone are found in uncontrolled diabetes mellitus [3]. N-pentane and ethane are regarded as markers of lipid peroxidation and have been demonstrated in a variety of pathological conditions [4–15]. Isoprene (2-methylbutadiene-1,3) is thought to be formed along the mevalonic pathway of cholesterol synthesis [16]. There is experimental evidence that isoprene exhalation is related to oxidative damage of the fluid lining of the lung [17] and the body [18].

We therefore hypothesised that, in clinical conditions with high oxidative and inflammatory activity, metabolic changes should be mirrored in the composition of exhaled gas. To test this hypothesis, we studied patients with acute respiratory distress syndrome (ARDS) and with a pulmonary infection, employing a new gas chromatographic method.

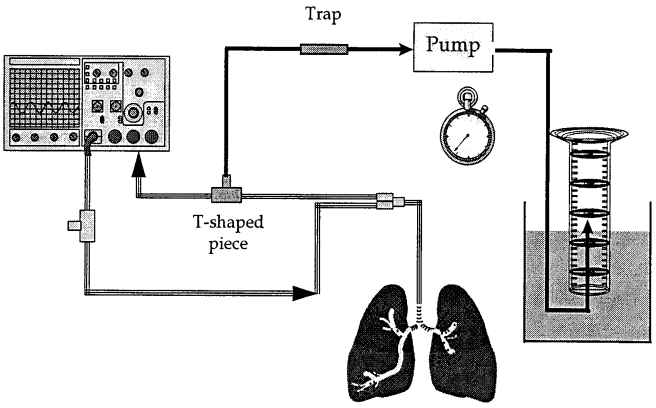

Fig. 1 Schematic drawing of the respiratory circuit and the incorporated sampling devices, T-pieces, adsorption trap and roller pump. Gas passing through the adsorption trap is collected in the calibrated water-filled cylinder. Total volume and flow can be derived from the sampling time

Methods and materials

Patients

After approval by the local Ethics Committee and after having obtained informed consent from the nearest relative, 37 consecutive critically ill, mechanically ventilated patients of a surgical intensive care unit (SICU) were enrolled in the study. Nineteen patients (12 men and 7 women) suffered from ARDS. The criteria for ARDS were PaO_2/FIO_2 of 200 mm Hg or less regardless of positive end-expiratory pressure (PEEP), bilateral infiltrates on anteroposterior chest radiograph and pulmonary artery wedge pressure (PCWP) of 18 mm Hg or less [19]. Eighteen patients (8 men and 10 women) did not meet the ARDS criteria. Six of the 19 ARDS patients and 3 of the 18 non-ARDS patients developed nosocomial pneumonia during their SICU stay, six ARDS patients and four non-ARDS patients did not. Seven ARDS patients and 11 non-ARDS patients already presented with pneumonia or positive bacteriological findings in other sites at SICU admission. Criteria for the diagnosis of pneumonia in ventilated patients were clinical signs of infection and a positive culture of bronchoalveolar lavage fluid ($> 10^4$ CFU/ml).

Measurements

During the collection of expired gas from the patient, no changes in ventilator settings were made. Measurements were performed between 8 a.m. and 6 p.m. All patients were studied at least 2 times during their SICU stay. The first analysis was performed within 48 h after SICU admission Without correcting for any other diagnosis, 19 ARDS patients under conventional respiratory therapy were compared to 18 patients without ARDS. In all patients who developed pneumonia during their SICU stay, the results of the last measurements prior to infection were compared to the results of the measurements after established infection. The mean interval between these measurements was 4 (range 1–9) days. In seven ARDS and three non-ARDS patients who did not develop nosocomial pneumonia, the mean interval between two consecutive measurements was 3 (range 1–6) days.

Gas sampling

Volatile substances in the inspired and expired gas were collected and concentrated by adsorption onto activated charcoal. Charcoal in 80 mg aliquots, was housed in ceramic traps (Analyt, Müllheim, Germany) [20, 21]. Approximately 1000 ml of gas were drawn through the trap within 3–5 min by means of a roller pump (Cole-Parmer Instruments Co., Niles, USA). Sample volume and gas flow were measured by collecting the gas under water and recording the time of measurement. The traps were mounted in parallel to the respiratory circuit (Fig. 1). The ports of the respiratory circuit consisted of stainless steel, autoclaved T-pieces, the connecting tubing of polytetrafluoroethylene. The inspiratory gas samples were collected 120 cm distal from the ventilator outlet, the expiratory samples at the expiratory inlet of the ventilator. At least two samples for each measurement were collected at the two sites in the respiratory circuit. Time of sampling, ventilator settings, diagnosis, medication and the patient's clinical status were recorded.

Gas chromatographic analysis

Substances were desorbed from the activated charcoal by means of microwave energy. The traps were heated to about 600 °C within 10 s in a microwave thermoinjector [20] (Model MW1, Analyt, Müllheim, Germany). The compounds were subsequently separated by gas chromatography (GC) (Hewlett Packard, 5980 Series A, Bad Homburg, Germany) and identified by flame ionisation (FID) or mass spectrometry (MS) (MSD, Hewlett Packard, Bad Homburg, Germany). For GC, a 50 m capillary column (Model CP Sil 8 CB, Chrompack, Middelburg, The Netherlands) was used. The GC temperature was held at 5 °C for 2 min after injection, subsequently raised to 40 °C by 2° increments per minute, thereafter increased to 280° by 10°/min where it was kept for another 5 min. The total GC run time was 43.5 min.

The unknown compounds were identified by comparing their retention times and their sequence of elution to known test substances. The identification of acetone, pentane and isoprene was confirmed by subsequent mass spectrometry. Substance concentrations were obtained from calibration curves. For that purpose, a gaseous calibration mixture was prepared from commercially available acetone, pentane and isoprene by mixing them in a 100 cm^3 glass vessel with a rotating Teflon-coated magnet for 15 min. Aliquots of 10 µl of the headspace gas were then transferred into the trap with a gas-tight syringe (Hamilton, Bonaduz, Switzerland). A calibration curve in the range 0–600 ng was established twice for each substance. The correlation between peak area and substance concentration was linear ($r^2 > 0.95$). Pulmonary substance elimination rates were obtained by multiplying expired substance concentrations and minute ventilation. Pentane concentrations had been corrected for background levels ($C_{Ex} - C_{In}$). To account for different sensitivity of the FID and for differences in the characteristics of the individual trap, 64.9 ng of 2,2-dimethylbutane, as internal standard, was injected into the trap after exposure to the expired gas. The internal standard was prepared the same way as was the calibration mixture. The mean peak area of internal standard was derived from 230 measurements. The ratio between the mean area and the area of the internal standard in the individual sample was calculated. If it differed from 1, the peak area of the substance under investigation was corrected by this factor.

Table 1 Demographic data of the patients, main diagnosis at SICU admission

Nº	Diagnosis	Sex	Age	FIO_2	ARDS	Pneumonia
1	Empyema of the pleura after lower lobe resection	m	52	0.96	–	
2	Head injury, intracerebral haemorrhage, epilepsy	f	42	0.40	–	0
3	Adrenalectomy for hyperaldosteronism	f	65	0.70	–	
4	Liver transplantation, cerebral haemorrhage	m	48	0.21	–	
5	Perforation of Zenker's diverticulum, mediastinitis	m	58	0.41	–	0
6	Blunt abdominal trauma	f	35	0.40	–	
7	Pneumonia	m	31	0.40	–	
8	Peritonitis	f	72	0.40	–	*
9	Pneumonia following caesarean section	f	25	0.40	–	
10	Head injury with intracerebral haemorrhage and oedema	m	71	0.40	–	
11	Head injury with intracerebral haemorrhage	f	29	0.40	–	
12	Colitis, peritonitis	f	74	0.88	–	
13	Septic shock after resection of sigmoid colon	f	71	1.0	–	
14	Pancreatic resection, pancreatitis	f	61	0.95	–	0
15	Peritonitis after hemicolectomy, hemihepatectomy	f	52	0.40	–	
16	Bleeding gastric ulcer, empyema of the knee	m	74	1.0	–	0
17	Head injury	m	38	1.0	–	*
18	Broncheolitis obliterans obstructive pneumonia (BOOP)	m	57	0.60	–	*
19	Pyosalpinx, sepsis	f	36	0.86	+	
20	Blunt thoracic and abdominal trauma	m	25	0.99	+	0
21	Tracheo-oesophageal fistula	m	42	0.46	+	
22	ARDS following viral pneumonia	m	44	0.50	+	0
23	Pulmonary embolism, right heart failure	m	47	0.56	+	
24	Pancreatitis, pneumonia	f	64	0.80	+	
25	Multiple trauma	m	39	0.70	+	
26	Perforation of gastric ulcer	f	40	0.60	+	0
27	ARDS following pneumonia	f	48	0.43	+	*
28	ARDS following aspiration	m	27	1.0	+	0
29	ARDS following pneumonia	f	41	0.50	+	*
30	ARDS following aspiration	m	21	0.80	+	0
31	ARDS following pneumonia, acute renal failure	m	41	0.44	+	*
32	Multiple trauma, acute renal failure	m	54	0.41	+	*
33	Ludwig's angina, pneumonia	m	45	0.74	+	
34	Mitral valve replacement, pneumonia	m	44	0.68	+	
35	Multiple trauma	m	32	0.87	+	*
36	Pancreatitis	f	48	1.0	+	*
37	ARDS following caesarean section	f	21	0.85	+	0

Pneumonia: * indicates patients who developed nosocomial pneumonia during SICU stay, 0 indicates patients who did not. Patients not designed with * or 0 presented with pneumonia or bacterial infection in other sites at SICU admission

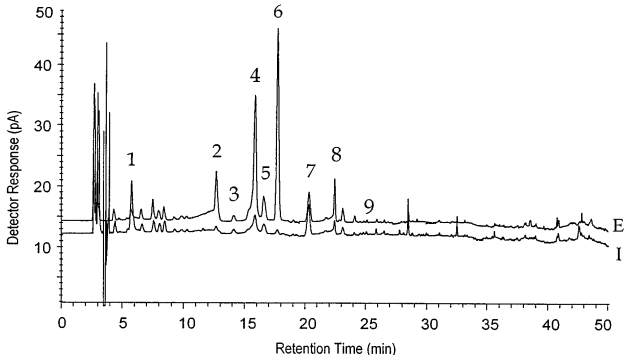

Fig. 2 Overlay of two chromatograms from a patient with head injury and pneumonia (*E* gas sample from the expiratory limb, *I* gas sample from the inspiratory limb, 1 = methanol, 2 = ethanol, 3 = 2-methylbutane, 4 = acetone, 5 = n-pentane, 6 = isoprene, 7 = 2,2-dimethylbutane (internal standard), 8 = 2,3-dimethylbutane, 9 = n-hexane)

Substance origin

Since any substance detected in the gas sample may come either from the patient or from the delivery system, it was necessary to sample from the two different sites of the respiratory circuit and to determine the inspiratory (C_I) and expiratory (C_E) substance concentrations. Subsequently, the ratio (Q) of the difference between expiratory and inspiratory concentrations and the expiratory concentration ($Q = (C_E\text{-}C_I)/C_E$) was calculated. For each substance the mean ratio of 40 measurements was calculated and interpreted as follows: a mean Q value between 0.9 and 1 represents a low inspiratory substance concentration ($C_E \geq 10{*}C_I$) and is therefore indicative of a substance coming from within the patient. A Q value between 0.5 and 0.9 represents an expiratory substance concentration considerably higher than the inspiratory concentration ($2{*}C_I \leq C_E < 10{*}C_I$), which means that the substance predominantly emanates from the patient. A Q value less than 0.5 represents expiratory substrate concentration comparable to ($0 \leq Q < 0.5$), or less than the inspiratory concentration ($Q < 0$), and is therefore indicative of a substance given off by the delivery system.

Table 2 Ratio of concentrations of constituents of exhaled human gas (C_E expiratory, C_I inspiratory concentration, n number of measurements)

Substance	$(C_E–C_I)/C_E$	n
Acetone	0.90 ± 0.15	45
2,3-Dimethylbutane	0.50 ± 0.06	32
2,4-Dimethylpentane	0.47 ± 0.18	23
N-hexane	–6.2 ± 5.0	41
Isoflurane	0.96 ± 0.15	31
Isoprene	0.90 ± 0.03	39
Methanol	0.16 ± 0.07	49
2-Methylbutane	–0.88 ± 0.56	35
N-Pentane	0.54 ± 0.08	40

Values are means ± SE

Variation of the measured values

In order to assess the reproducibility of the method, the coefficients of variation for acetone, pentane and isoprene elimination rates were calculated. For this purpose, in the first 25 patients each sample was collected in triplicate in different traps. The mean coefficient of variation for acetone and isoprene elimination rates was 11 %, the corresponding value for pentane elimination rates was 21 %.

Statistical analysis

Since substance elimination rates were not normally distributed, for unpaired samples the Mann-Whitney U-Wilcoxon rank sum test, and for paired samples the Wilcoxon matched-pairs signed-ranks test, was used to compare the medians of the samples. Results are reported as medians and 95 % confidence intervals. Since the Q values [i.e., $Q = (C_E - C_I)/C_E$] were normally distributed, the results are reported as the mean ± standard error (SE). A p value of less than 0.05 was considered statistically significant.

Results

Patients' clinical characteristics are listed in Table 1. Figure 2 shows the chromatograms of two samples, one collected at the expiratory site (E), the other at the inspiratory site (I). The substances (labeled 2, 4–6, 8) exhaled by the patient can be unequivocally identified from the overlay of the two chromatograms.

Substance origin

The Q values [$Q = (C_E - C_I)/C_E$] for isoflurane, acetone and isoprene were 0.90 or more (Table 2) indicating that these substances are coming from the patient. The corresponding values for n-pentane and 2,3-dimethylbutane were 0.50 or more (Table 2), which means both compounds are predominantly originating in the patient. The Q values for n-hexane and 2-methylbutane were below 0 (Table 2), indicating that they emanate from the respiratory delivery system.

Correlation with clinical parameters

Patients with ARDS produced over 50 % less isoprene than those without ARDS. No difference in the pentane elimination was observed. Acetone elimination rates were slightly higher in ARDS, than in non-ARDS patients. The difference, however, was not significant because of the large variation of the values (Table 3).

Patients without pulmonary infection showed unchanged elimination rates for acetone, pentane and isoprene at both observations (Table 4). In patients developing pneumonia, however, the pentane elimination rate increased 6-fold, while the isoprene elimination rate decreased (4 %). The pentane/isoprene ratio increased 3-fold (Table 5). Acetone elimination rates remained unchanged in these patients.

Discussion

A non-invasive method for chemical analysis of inspired and expired gas has been applied in critically ill, mechanically ventilated patients. The method allows quantitative analysis of gas samples with low substance concentrations and overcomes the problems associated with the gas collection and high water content in expired breath. It differentiates between substances produced by the patient and those introduced by the respiratory delivery system.

Table 3 Substance elimination (nmol/m^2 per min) in the presence and absence of ARDS (n = number of patients). Acetone could not be determined in one patient of the ARDS group, and in four patients of the non-ARDS group for technical reasons. Non-ARDS: patients 1–18, ARDS: patients 19–37 as listed in Table1

Variable	Non-ARDS		ARDS		p
	Median (95 % CI)	n	Median (95 % CI)	n	
Acetone	119 (52–270)	14	149 (113–485)	18	0.25
Pentane	5.1 (1.4–18.6)	18	4.15 (3.7–9.3)	19	0.37
Isoprene	21.8 (13.9–41.4)	18	9.8 (8.2–21.6)	19	0.04

Values are medians and 95 % confidence intervals (95 % CI)

Table 4 Substance elimination rates (nmol/m^2 per min) in patients without pulmonary infection (n = number of patients). Relative changes are given in percent of the value at observation 1. Patients 2, 5, 14, 16, 20, 22, 26, 28, 30, 37 as listed in Table 1

Variable	Observation 1 without pneumonia Median (95 % CI)	Observation 2 without pneumonia Median (95 % CI)	n	p	Relative changes Median [%]
Acetone	80.6 (0.0–263)	26.6 (0.0–207)	10	0.58	–10
Pentane	2.2 (0.0–12.7)	3.6 (1.4–10.4)	10	0.80	54
Isoprene	10.6 (5.1–25.8)	11.4 (5.1–19.1)	10	0.58	–4
Pentane/isoprene	0.53 (0.0–2.5)	0.36 (0.10–1.74)	10	0.58	0.2

Values are medians and 95 % confidence intervals (95 % CI)

Table 5 Substance elimination rates (nmol/m^2 per min) in patients having a newly diagnosed pulmonary infection at observation 2 (n = number of patients). Relative changes are given in percent of the value at observation 1. Patients 8, 17, 18, 27, 29, 31, 32, 35, 36 as listed in Table 1

Variable	Observation 1 without pneumonia Median (95 % CI)	Observation 2 with pneumonia Median (95 % CI)	n	p	Relative changes Mean [%]
Acetone	69.2 (6.5–366)	79.6 (24–192)	9	0.44	0.2
Pentane	0.4 (0.0–5.4)	2.7 (0.6–6.0)	9	0.05	450
Isoprene	5.2 (0.0–32.9)	5.0 (0.0–17.8)	9	0.05	–32
Pentane/isoprene	0.14 (0.0–0.3)	0.43 (0.0–15.0)	9	0.007	260

Values are medians and 95 % confidence intervals (95 % CI)

The expired gas sample consists of alveolar gas and dead space gas. The reported expired substance concentrations, therefore, do not represent true alveolar substance concentrations. During mechanical ventilation, however, the dead space mainly depends on the geometry of the respiratory circuit, which was basically the same for all patients. For inter- and intra- patient comparison substance elimination rates were related to body surface area, because this parameter is more likely to be independent of minute ventilation than the substance concentration. Ethane, which has also been described as a marker of lipid peroxidation [4, 6, 8, 9, 11], was not determined because ethane analysis requires cooled traps and there is evidence that ethane may be produced during microwave desorption. After the use of isoflurane during anaesthesia, this substance was found in patients' exhaled gas in abundant concentrations sometimes. Since isoflurane was well separated from all other substances on the column we used, there was no interference with the determination of acetone, pentane and isoprene. Patients apparently absorb n-hexane and 2-methylbutane from the delivery system. Since expiratory concentrations are lower than inspiratory ones, it has to be supposed that these substances were metabolised or stored in the organism.

The substances under investigation in this study were acetone, pentane and isoprene. The intra-assay variability of acetone and isoprene elimination rates was low. Higher background concentrations may be responsible for the higher variation in the pentane elimination rates. Since pentane may come from different sources [22], the net pulmonary elimination rate was calculated by correcting for background concentrations. Acetone and isoprene elimination rates were calculated from the expiratory concentrations without correction, because inspiratory concentrations of these substances were negligible.

The elimination rates of isoprene and acetone we measured are in agreement with the work of others [2, 23, 24]. Our pentane elimination rates, however, were more than 10 times higher than those reported by Euler [24], Kohlmüller [25] and Cailleux [26] in spontaneously breathing volunteers. Pentane elimination may increase in critically ill, mechanically ventilated patients due to the elevated level of oxidative and metabolic stress. Furthermore, the pentane concentration present in the respiratory delivery system may have contributed to the difference.

Elimination rates of acetone, pentane and isoprene vary within patients with ARDS and pneumonia. Isoprene is thought to be generated along the mevalonic pathway of cholesterol synthesis [16]. Foster [17] observed an increase in isoprene elimination in humans 19 h after exposure to ozone. An activation of cholesterol synthesis at the onset of repair processes following oxidative damage to fluid linings in the lung is assumed to be the underlying mechanism. Mendis et al. [18] reported an increase in isoprene elimination in patients with acute myocardial infarction. They considered a relationship between isoprene elimination and the activation of neutrophils. The decreasing isoprene elimination

found in our patients with ARDS and pneumonia is in contrast to the work cited above. Since we did not measure at the very onset of ARDS and pneumonia in our study, decreasing isoprene elimination was probably due to an impaired cholesterol metabolism occurring at a later stage of ARDS and pulmonary infection.

Pentane arises from peroxidation of n-6 polyunsaturated fatty acids [27]. It is considered to be a marker of in vivo lipid peroxidation [10, 28]. The increased pentane elimination in patients with a pulmonary infection may reflect an increase in radical generation during the oxidative burst of inflammatory cells in the lungs. Increased elimination of acetone in patients with ARDS may be the result of an enhanced metabolism [3].

The sensitivity of acetone and n-pentane elimination rates as markers of in vivo lipid peroxidation is limited. Acetone elimination was increased in ARDS, but unchanged after pulmonary infection. Moreover, acetone is produced by spontaneous decarboxylation of acetoacetate and it is impossible to quantitate the fraction that arises from lipid peroxidation. Pentane elimination increased during pulmonary infection, but there was no relation to ARDS, where radical mediated reactions are supposed to play an important role. Only isoprene elimination was shown to be associated with ARDS and pulmonary infection. The interpretation of isoprene excretion, however, is difficult because changes in isoprene elimination seem to depend on the type and stage of the pathological process underlying the disease. Isoprene elimination may decrease following impaired cholesterol synthesis and may increase either secondary to neutrophil activation or at the onset of repair processes [17]. Furthermore, isoprene elimination varies during the daytime [29] and with the state of alertness [30]. There is evidence that isoprene elimination is reduced during general anaesthesia [31] but little is known about the kinetics of isoprene excretion in SICU patients. In addition, the considerable variation in the elimination rates within each patient group renders any clinical conclusion in the individual patient difficult. It is certainly a weakness of this study that the many factors influencing isoprene elimination could not be held constant during the study.

Pulmonary excretion rates of volatile substances may depend on solubility, ventilation and perfusion. Since the solubility of acetone, isoprene and pentane in blood is similar in all patients, its effect on differences in pulmonary substance elimination should be negligible. In those patients in whom the effects of pulmonary infection were studied, gas exchange and haemodynamic variables were comparable between the two measurements. Since isoprene and pentane are both poorly soluble in water, their pulmonary elimination rate should be affected by ventilation and perfusion in the same way. In the ARDS/non-ARDS group, however, the isoprene elimination rate decreased only in the ARDS patients. Therefore, it seems unlikely that this change is due to differences in ventilation/perfusion ratios between the patient groups.

In conclusion, our results suggest that there is a relationship between the chemical composition of patient's exhaled gas and lung injury. In this context, the isoprene elimination rate warrants further investigation. Little is known about the origin and metabolic pathways of that substance under physiological and pathophysiological conditions. The ratio of pentane to isoprene elimination seems to be an interesting parameter for the diagnosis of pulmonary infection.

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