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BREATH BIOMARKERS OF INFLAMMATION, INFECTION AND METABOLIC DERANGEMENT IN THE INTENSIVE CARE UNIT

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Abstract

The analysis of volatile organic compounds (VOCs) in breath may be a useful non-invasive tool in the Intensive Care Unit (ICU) to monitor metabolic and oxidative stress or diagnose pulmonary infection. Acetone is produced during starvation and metabolic stress, hydrogen sulphide (H_2S) may be a marker of inflammation and infection and hydrogen cyanide (HCN) may also act as a marker of infection, particularly caused by Pseudomonas aeruginosa.

Firstly, the effects on measured VOC concentrations of the breath collection equipment and storage were assessed. Sample humidity declined faster than any analyte. Sample losses of 21%, 25% and 24% for acetone, H_2S and HCN, respectively, were seen as a result of being passed through the sampling apparatus. Over 90% of initial breath VOC concentrations were detectable after 90 min storage in Tedlar bags at 40°C.

Secondly, a breath collection method for off-line analysis was validated in 20 mechanically ventilated patients in the ICU. The effect on VOC concentrations of breath sampling from two locations after two breathing manoeuvres was explored, revealing significantly higher analyte concentrations in samples from the airways than from a T-piece in the breathing circuit, and after tidal breathing compared to a recruitment-style breath. Practical difficulties were encountered using direct airway sampling and delivering recruitment style breaths; end-tidal breath sampling from the T-piece was simplest to perform and results equally reproducible.

Breath samples from 26 healthy anaesthetised controls were used to validate a breath collection method in the operating theatre. The effects of altering anaesthesia machine settings on inspiratory and exhaled acetone concentrations were explored. A difference in median inspiratory, but not exhaled, acetone concentrations was observed between the anaesthesia machines (ADU Carestation 276 ppb, Aysis Carestation 131 ppb, p=0.0005). Closing the adjustable pressure limiting (APL) valve resulted in a reduction in exhaled acetone concentration, as did breath sampling distal to the circuit filter, due to dilution by dead space air. Median (range) breath concentrations for samples

collected on the patient side of the circuit filter with the APL valve open (n=22): acetone 738 ppb (257–6594 ppb), H_2S 1.00 ppb (0.71-2.49 ppb), HCN 0.82 ppb (0.60-1.51 ppb). Breath acetone concentration was related to plasma acetone (r_s=0.80, p<0.0001) and beta-hydroxybutyrate concentrations (r_s=0.55, p=0.0075).

Finally, breath and blood samples were collected daily from 32 mechanically ventilated patients in the ICU with stress hyperglycaemia (n=11) and/or new pulmonary infiltrates on chest radiograph (n=28). Samples were collected over a median 3 days (1-8 days). Median (range) breath VOC concentrations of all samples collected: acetone 853 ppb (162–11,375 ppb), H_2S 0.96 ppb (0.22-5.12 ppb), HCN 0.76 ppb (0.31-11.5 ppb). Median initial breath acetone concentration was higher than in anaesthetised controls (1451 ppb versus 812 ppb; p=0.038). There was a trend towards a reduction in breath acetone concentration over time. Relationships were seen between breath acetone and arterial acetone (r_s=0.64, p<0.0001) and beta-hydroxybutyrate (r_s=0.52, p<0.0001) concentrations. Several patients remained ketotic despite insulin therapy and normal, or near normal, arterial glucose concentrations. Inspired and exhaled H_2S and HCN concentrations were not significantly different. Breath H_2S and HCN concentrations could not be used to differentiate between patients with pneumonia and those with pulmonary infiltrates due to other conditions.

In conclusion, losses due to the sampling apparatus were determined and linear over the range of concentrations tested. End-tidal breath sampling via the T-piece was the simplest technique, with reproducibility comparable to other methods. It was possible to replicate the breath sampling method in the operating theatre; pre-filter samples with inspiratory gas flow rate 6 L/min and APL valve open provided repeatable results avoiding rebreathing. There was no role for the use of breath H_2S or HCN in the diagnosis or monitoring of pneumonia in critical illness. There was no relationship between breath acetone concentration and illness severity, however the utility of breath acetone in the modulation of insulin and feeding in critical illness merits further study.

Publications Arising from this Thesis

Original articles

1. Sturney S, Storer M, Shaw G, Shaw D, Epton M. Off-line breath acetone analysis in critical illness. J Breath Res 2013;7:037102

Review articles

1. Storer M, Dummer J, Sturney S, Epton M. Validating SIFT-MS analysis of volatiles in breath. Curr Anal Chem 2013;9(4):576-583.

Conference abstracts

1. Sturney S, Storer M, Shaw G, Shaw D, Epton M. Breath acetone measurement in intubated patients in Intensive Care – End-tidal breath sampling via a T-piece is better than direct sampling from the airways. Am J Respir Crit Care Med 2012;185:A3948.
2. Sturney S, Storer M, Shaw G, Shaw D, Epton M. Can exhaled hydrogen sulphide and hydrogen cyanide be used to diagnose pneumonia in the Intensive Care Unit? Thorax 2013;68(Suppl 3):A141.
3. Sturney S, Storer M, Shaw G, Kennedy R, Shaw D, Epton M. An off-line end-tidal breath sampling method in anaesthetised patients with analysis by selected ion flow tube mass spectrometry. Thorax 2013;68(Suppl 3):A158.

Conference presentations

1. Sturney S, Storer M, Shaw G, Shaw D, Epton M. Off-line breath acetone analysis in critical illness. 2012 International Breath Analysis Meeting, Sonoma, California.

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Declaration

This is to certify that the work submitted in this thesis is the result of original research carried out by me at Christchurch Hospital, New Zealand, under the supervision of Dr Michael Epton (Christchurch) and Dr Dominick Shaw (Nottingham, UK). It has not already been submitted or accepted for any other degree.

Study design, ethics application, data collection, analysis and writing were conducted primarily by me with support from my supervisors and Dr Malina Storer. Dr Storer helped with breath sample collection and processing. Dr Geoff Shaw and Dr Ross Kennedy supported me with study development in the ICU and operating theatre. I was involved in the initial centrifuging of blood samples for H_2S analysis, pipetting the plasma for processing at the University of Canterbury, Christchurch campus.

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List of Abbreviations and Symbols

A-a gradient	Alveolar-arterial gradient
ABG	Arterial blood gas
AKI	Acute kidney injury
ALI	Acute lung injury
APACHE score	Acute Physiology and Chronic Health Evaluation score
APL valve	Adjustable pressure limiting valve
ARDS	Acute respiratory distress syndrome
ATP	Adenosine triphosphate
BAL	Bronchoalveolar lavage
BMI	Body mass index
BP	Blood pressure
°C	Degrees Celsius
CAP	Community-acquired pneumonia
CBS	Cystathionine-β-synthase
CF	Cystic fibrosis
CFCs	Chlorofluorocarbons
cm	Centimetre
cmH_2O	Centimetres of water
CO	Carbon monoxide
CO_2	Carbon dioxide
COPD	Chronic obstructive pulmonary disease
CPAP	Continuous positive airways pressure
CRH	Corticotropin-releasing hormone
CRP	C-reactive protein
CSE	Cystathionine-γ-lyase
CV	Coefficient of variation
CVVHDF	Continuous veno-venous haemodiafiltration
CXR	Chest x-ray
Da	Daltons
DKA	Diabetic ketoacidosis
DVT	Deep vein thrombosis
DNA	Deoxyribonucleic acid

eNOS	Epithelial nitric oxide synthase
EPAP	Expiratory positive airway pressure
ESI-MS	Electrospray ionisation mass spectrometry
ETT	Endotracheal tube
F_{ENO}	Fraction of exhaled nitric oxide
FEV_1	Forced expiratory volume in one second
FID	Flame ionisation detection
FiO_2	Fractional concentration of inspired oxygen
FVC	Forced vital capacity
GC	Gas chromatography
GC-MS	Gas chromatography mass spectrometry
H_2O_2	Hydrogen peroxide
H_2S	Hydrogen sulphide
HAP	Hospital-acquired pneumonia
HCN	Hydrogen cyanide
HFAs	Hydrofluoroalkanes
HPA	hypothalamo-pituitary-adrenal
ICS	Inhaled corticosteroid
ICU	Intensive Care Unit
IIT	Intensive insulin therapy
IL-(10)	Interleukin-(10)
IMR-MS	Ion-molecule reaction mass spcetrometry
IMS	Ion mobility mass spectrometry
iNOS	Inducible nitric oxide synthase
IPAP	Inspiratory positive airway pressure
IQR	Interquartile range
k	Rate coefficient
kg	kilogram
kPa	Kilopascal
L	Litre
µm	Micrometre
µmol	Micromole
m	Metre
MALDI	Matrix-assisted laser desorption/ionization

MDI	Metered dose inhaled
metHb	Methaemoglobin
mg	Milligram
MI	Myocardial infarction
min	Minute
ml	Millilitre
mm	Millimetre
mmHg	Millimetres of mercury
mmol	Millimole
MODS	Multiple organ dysfunction syndrome
MPM	Mortality Probability Model
MPO	Myeloperodidase
MS	Mass spectrometry
ms	Millisecond
m/z	Mass-to-charge ratio
Na_2S	Sodium sulphide
NaHS	Sodium hydrosulphide
NF-κB	Nuclear factor-kappa B
NO	Nitric oxide
NOS	Nitric oxide synthase
NSAID	Non-steroidal anti-inflammatory drug
OA	Osteoarthritis
OGTT	Oral glucose tolerance test
OVA	Ovalbumin
$PaCO_2$	Arterial partial pressure of carbon dioxide
PaO_2	Arterial partial pressure of oxygen
$PetCO_2$	End-tidal partial pressure of carbon dioxide
PCR	Polymerase chain reaction
PEEP	Positive end-expiratory pressure
PiCCO	Pulse contour continuous cardiac output
POTS	Postural orthostatic tachycardia syndrome
ppb	Parts per billion
ppm	Parts per million
ppt	Parts per trillion

PTR-MS	Proton transfer reaction mass spectrometry
RA	Rheumatoid arthritis
ROS	Reactive oxygen species
RR	Respiratory rate
RRT	Renal replacement therapy
r_s	Spearman's rank correlation
SAPS	Simplified Acute Physiology Score
SBP	Systolic blood pressure
SD	Standard deviation
sec	Second
SEM	Standard error of the mean
SESI-MS	Secondary electrospray ionisation mass spectrometry
SIFT-MS	Selected ion flow tube mass spectrometry
SIM mode	Selected ion monitoring mode
SIMV	Synchronised Intermittent Mandatory Ventilation
SIRS	Systemic Inflammatory Response Syndrome
SOFA score	Sequential Organ Failure Assessment score
SPME	Solid phase microextraction
SPRINT	Specialised Relative Insulin Nutrition Tables
t	Time
TD	Thermal desorption
TIVA	Total intravenous anaesthetic
TNFα	Tissue necrosis factor alpha
TOF	Time of flight
V_A/Q	Alveolar ventilation-perfusion ratio
V_T	Tidal volume
VAP	Ventilator-associated pneumonia
VOC	Volatile organic compound
WCC	White cell count

1. Introduction

Exhaled breath contains many trace compounds, some of which have been associated with illness states[1-3]. If those produced by metabolic processes or pathological organisms can be identified and investigated, using techniques such as mass spectrometry, the analysis of volatile organic compounds (VOCs) in breath may provide useful information about a patient's clinical state. The non-invasive nature of breath analysis and its potential for providing rapid results in real time[4] make it an attractive tool, however there are numerous confounding variables that must be controlled or taken into account when designing studies and interpreting results.

Few investigations of breath analysis have been conducted in intubated patients in the Intensive Care Unit (ICU)[5-9], possibly due technical difficulties surrounding sample collection. Making a diagnosis of pneumonia in intubated patients can be difficult, not least because there is no single diagnostic test available and many clinical features are not specific to pneumonia. If a breath test were developed that could differentiate between pulmonary infection and other causes of inflammation, it might improve diagnostic accuracy and, therefore, the timely administration of the correct targeted treatment. Two potential breath markers of pulmonary infection and inflammation are investigated in the experimental chapters of this thesis.

Mechanically ventilated patients undergo continuous monitoring of their heart rate, blood pressure and oxygen saturation, but there are currently no techniques for monitoring metabolic status. This may be particularly important in patients with stress hyperglycaemia who have deranged glucose handling. Acetone is investigated as a marker of metabolic stress and monitored in patients with stress hyperglycaemia receiving treatment with insulin.

1.1. Thesis outline

In the introductory chapter, the features of critical illness, including associated hyperglycaemia, and the difficulty in diagnosing respiratory infection in intubated and ventilated patients are discussed. The potential applications of breath analysis for the detection of inflammation, infection and metabolic

disturbance are outlined, along with potential confounding variables, such as breathing pattern. Methodological issues concerning breath sampling, particularly in intubated and ventilated patients with critical illness, are considered. Selected ion flow tube mass spectrometry (SIFT-MS) is explained in detail, along with its utility in the analysis of VOCs in breath. Potential biomarkers of metabolic disease (acetone), inflammation and infection (hydrogen sulphide (H_2S) and hydrogen cyanide (HCN)) are highlighted and described. Finally, the hypothesis and aims of the thesis are outlined.

The first experimental part of this thesis involves the optimisation of the breath collection technique in intubated and mechanically ventilated patients in the ICU, with validation against previous study methods. Technical and patient factors which might affect breath measurement are discussed.

Secondly, normal levels of exhaled acetone, H_2S and HCN in intubated and mechanically ventilated patients undergoing elective surgical procedures in the operating theatre are studied. The effects of changing several of the anaesthesia machine settings during breath sampling were assessed to minimise any differences when compared to ICU ventilators.

Finally, potential breath biomarkers of metabolic dysfunction, oxidative stress, inflammation and infection are investigated in patients in the ICU. Patients requiring insulin treatment for stress hyperglycaemia and those with new pulmonary infiltrates on chest radiograph (CXR) were studied over the course of their illness, with repeated breath analysis and blood, urine and sputum sampling. Any relationships between VOC concentrations in breath and blood are investigated. VOC concentrations are also compared to conventional markers of disease severity. Comparisons are made between measured breath analyte concentrations in the critical illness and control groups and the results discussed.

The thesis concludes with a discussion of the studies performed, including their limitations, as well as the potential for further research in this area.

1.2. Critical Illness

1.2.1. Sepsis and multi-organ failure

Septic shock is a major cause of death in patients in the ICU[10,11], either as a result of early haemodynamic compromise or subsequently due to the development of multi-organ failure[10,12,13]. Despite many improvements in ICU care, including advances in diagnostic and therapeutic technologies and pharmacological therapies, the mortality rate from sepsis has remained above 20% for patients requiring several days' admission[10,12,14-16].

Systemic Inflammatory Response Syndrome (SIRS) is the term used to describe the clinical features of multi-organ dysfunction associated with infection or other inflammatory processes, such as pancreatitis, burns, multi-trauma or haemorrhagic shock. If two or more of the following criteria are satisfied, a diagnosis of SIRS can be made: (1) temperature >38°C or <36°C, (2) heart rate >90 beats per min, (3) respiratory rate >20 per min or $PaCO_2$ <32 mmHg, (4) white cell count (WCC) >12 x 10^9/L or <4 x 10^9/L or >10% immature neutrophils[14]. Sepsis is the term used to describe SIRS triggered by infection, severe sepsis is defined by sepsis associated with hypotension, hypoperfusion and end-organ dysfunction (for example lactic acidosis, oliguria and altered mental state), and septic shock is severe sepsis where hypotension persists despite adequate fluid resuscitation[14].

Around 27% of patients admitted to ICU departments across England, Wales and Northern Ireland between 1995 and 2000, met criteria for severe sepsis in the first 24 hours, an estimated 51 per 100,000 population per year. Patients remained in the ICU for, on average, 3.56 days and in the hospital for 18 days, representing a significant burden on health care resources[16]. In a European study of 3,147 ICU patients, sepsis, predominantly due to pulmonary infection, was associated with more severe organ dysfunction and increased length of ICU and hospital stay compared to alternative conditions[10]. In the United States, a study of the cost of ICU care in 2002 illustrated that not only was it more expensive to support a mechanically ventilated patient on a daily basis than a patient who did not require ventilation ($31,574 ± $42,570 versus $12,931 ± $20,569, respectively), but that mechanically ventilated patients

tended to require longer ICU admissions, further escalating the cost[17]. This led the authors to conclude that interventions to reduce the duration of mechanical ventilation and/or ICU length of stay could significantly reduce health care spending.

Pathophysiology

Microbial endotoxins are responsible for the initiation of a number of complex pro- and anti-inflammatory pathways that usually result in elimination of the pathogen. Imbalance between these pathways, however, can lead to excess systemic inflammation and host organ damage[15]. The exact mechanism by which sepsis-related multi-organ failure develops is unknown, however the combination of poor blood supply at the macro- and microvascular level causing hypoperfusion and impaired mitochondrial activity leading to inability to utilise oxygen have been implicated[13,18]. Distant organs may be affected by leakage of inflammatory mediators into the systemic circulation[18]. It is not clear why certain infective organisms cause damage to some organs and not others, and why the same infection in different individuals has different consequences[18].

Activation of macrophages, neutrophils, endo- and epithelial cells and the transcription factor nuclear factor-κB (NF-κB) help to regulate the immune response to infection[18]. Cytokines, chemokines, platelet activating factor, interferon-γ, leukotrienes and proteases are released, some of which cause further activation of NF-κB[15]. Immune cell stimulation is important in order to clear the offending pathogen; macrophage activation caused by microbes, pro-inflammatory mediators, and also hypoxia and acidaemia, intensifies the pro-inflammatory cascade. Sepsis can cause delayed neutrophil apoptosis, resulting in increased inflammation and further tissue injury[18].

Reactive oxygen species (ROS) are released by activated neutrophils and are produced by oxidative phosphorylation in mitochondria. Under normal conditions, ROS produced in mitochondria act as signalling molecules and are scavenged by antioxidants such as vitamins C and E and glutathione. When overproduced during episodes of tissue hypoxia or in the presence of pro-inflammatory cytokines, for example tumour necrosis factor-α (TNF-α), the

anti-inflammatory pathway is overwhelmed and ROS cause systemic inflammation and organ damage[13,15,18]. The superoxide anion can react with increased levels of pro-inflammatory nitric oxide (NO), leading to the formation of peroxynitrite[12,13]. Like ROS, peroxynitrite causes damage to cells by reacting with components of the mitochondrial electron transport chain, reducing oxygen extraction and utilisation by tissues, and also induces DNA damage, both of which lead to cell death[12,13]. Reduced antioxidant levels have been seen in sepsis and Acute Respiratory Distress Syndrome (ARDS), but interestingly the administration of antioxidants as a treatment does not appear to have consistent effects in sepsis[15].

The vascular epithelium is involved in a number of processes occurring in association with sepsis, including leukocyte trafficking, the expression of adhesion molecules and involvement in clotting pathways[18]. Activation of the epithelium results in elevated levels of NO release due to its production from L-arginine, catalysed by nitric oxide synthase (NOS). Endothelial NOS (eNOS) produces small amounts of NO with anti-adhesive and protective properties, whereas inducible NOS (iNOS) causes the synthesis of large quantities of NO in sepsis, producing pro-inflammatory effects[19]. The resulting smooth muscle relaxation causes vasodilatation and hypotension, but the administration of NO blocking drugs causes significant adverse side effects[18]. This highlights the complexity of the inflammatory pathways; vasodilatation is also mediated by activation of potassium channels and changes in hormone levels, for example cortisol and vasopressin[18].

The lungs appear to be affected early in the course of severe sepsis[20]. ROS exhibit direct and indirect effects on the lung, with injury to the epithelium leading to leakage of fluid into the airways. Shear stresses generated by recruitment and derecruitment of alveoli during mechanical ventilation can augment pulmonary damage[18].

Features of illness severity and severity scoring systems in critical illness

The severity of lung injury associated with hypoxaemia in ARDS is classified according to the ratio of the partial pressure of arterial oxygen divided by the fraction of inspired oxygen (PaO_2/FiO_2); mild 200 mmHg (26.7 kPa) <

$PaO_2/FiO_2 \leq 300$ mmHg (40 kPa), moderate 100 mmHg (13.3 kPa) < $PaO_2/FiO_2 \leq 200$ mmHg, severe $PaO_2/FiO_2 \leq 100$ mmHg. The syndrome also requires the following criteria to be fulfilled: (1) onset within one week of clinical insult or new or worsening respiratory symptoms, (2) bilateral infiltrates on CXR not fully explained by effusions, lung collapse or nodules, (3) respiratory failure not fully explained by pulmonary oedema or fluid overload, (4) use of positive end-expiratory pressure (PEEP) or continuous positive airway pressure (CPAP) >5 cmH_2O[21].

Interestingly, the severity of pulmonary dysfunction related to sepsis does not appear to be associated with 30 day mortality, which is more closely linked to neurological and renal dysfunction as well as the degree of coagulopathy[20]. Increasing age[10,11] and infection with Pseudomonas species are also risk factors for poorer outcomes[10]. Several, more specific independent variables appear to be associated with the progression from sepsis to severe sepsis and septic shock[22]; temperature >38°C, heart rate >120 beats per min, systolic blood pressure (BP) <100 mmHg, platelet count <150 $x10^9$/L, serum sodium >145 mmol/L, bilirubin >30 μmol/L, mechanical ventilation and five other features characterising the infection (pneumonia, peritonitis, primary bacteraemia, infection with Gram positive cocci or aerobic Gram negative bacilli). Most of these form part of the critical illness severity scoring systems described below.

Several validated scoring systems are used in the ICU to predict mortality based on a variety of clinical parameters and blood results in the first 24 hours of an admission. The most commonly used is the Acute Physiology and Chronic Health Evaluation (APACHE) II score[23], which is slightly simpler to use than the APACHE III score[24]. Both of these scoring systems, along with four others (the Simplified Acute Physiology Score (SAPS) versions I and II, and the Mortality Probability Model (MPM) I and II)) were compared using a multicentre, multinational patient database, concluding that reliable results could be achieved by using any of the systems and that none was superior[25]. Another commonly used scoring system in the ICU is the Sequential Organ Failure Assessment (SOFA) score[26], which can be calculated repeatedly throughout a patient's ICU admission to give an indication of extent of organ failure, prognosis and mortality[27].

Treatment of severe sepsis and septic shock

Due to the high morbidity and mortality associated with severe sepsis and septic shock, and the evidence that early appropriate treatment can influence outcomes, guidelines for the management of these conditions were formulated in 2004[28] and updated in 2008[29]. Key recommendations include early goal directed resuscitation in the first six hours, investigation of the causative organism prior to antibiotic therapy, but to administer the first dose of broad spectrum antibiotics within an hour of diagnosing the condition, and subsequent evaluation of microbiological results to narrow the antibiotic spectrum. Advice is given regarding the choice of fluid for resuscitation, the use of inotropic and vasopressor drugs, oxygen and steroid administration, ventilation in ARDS, treatment of severe renal impairment, patient positioning, correction of anaemia, coagulopathy and hyperglycaemia, protocols for sedation and weaning from the ventilator, and deep vein thrombosis (DVT) and stress ulcer prophylaxis.

1.2.2. Pneumonia in intubated and ventilated patients

Ventilator-associated pneumonia

Ventilator-associated pneumonia (VAP) affects 8-20% of ICU patients[30-32] and up to 27% of mechanically ventilated patients[32], and is associated with a high mortality rate of 20-50%[30,32]. This is increased further if VAP is caused by a multi-drug resistant pathogen[32].

Standardised criteria for the diagnosis of VAP are lacking[33]; most clinicians base their diagnosis on the presence of fever, elevated or low WCC, purulent secretions and new or persistent pulmonary infiltrates on CXR[34], none of which are specific for VAP. Bacteriological data does not improve the accuracy of diagnosis[32] and patients in whom there is a strong clinical suspicion of VAP have a high mortality rate irrespective of positive microbiological evidence of disease[35]. The difficulty surrounding diagnosis means that only half of patients with suspected VAP actually have it[31,36] and in up to a third of patients who have VAP the diagnosis is missed. Alternative diagnoses revealed at autopsy include atelectasis, thromboembolic disease, pulmonary haemorrhage, pulmonary fibrosis, carcinoma and lymphoma[32].

VAP is probably caused by microaspiration of secretions from above the endotracheal tube cuff[32,33,36], which is why the incidence rises as the duration of mechanical ventilation increases[33] and the common causative pathogens are different from those seen in community acquired pneumonia (CAP)[37,38]. It frequently complicates ARDS[30,33,36] and lengthens the duration of ventilation and ICU stay[30,33], increasing the cost of hospitalisation[31].

Rationale for accurate diagnosis of pulmonary infection

The diagnosis of pneumonia is important so that empirical antibiotic treatment can be initiated early; delayed antibiotic treatment of community-acquired pneumonia (CAP)[39,40] or VAP[31,35], or using overly narrow spectrum antibiotics in severe infection[41], are associated with increased mortality. Antibiotics are, therefore, often prescribed when infection is suspected, rather than proven, in order not to fail to treat an infective cause of a patient's symptoms[32,42]. Conversely, if a patient is suspected of having infection but the diagnosis is non-infective, delays in identifying the true cause will prevent the delivery of appropriate treatment, which could lead to increased morbidity and mortality.

There is no difference in outcome between empirically treated and pathogen directed treatment of CAP[43], however broad spectrum antibiotic use in non-severe CAP can increase the incidence of side effects[41,43] and the likelihood of development of antibiotic resistance[41]. Respiratory infections account for over 50% of antibiotic prescriptions, thus early increased certainty of the presence of infection, including identification of the organism responsible, could reduce the number of inappropriate antibiotic courses, enable narrowing of the antibiotic spectrum, allow appropriately shorter courses of antibiotics, improve compliance, and reduce costs, side-effects and antibiotic resistance[40]. Unfortunately, due to the difficulties obtaining a microbiological diagnosis, around 70% of patients with CAP never have a pathogen identified[40,43-45].

Problems with diagnosis

The diagnosis of pneumonia in ventilated patients is challenging, not least because the patient cannot articulate symptoms, and physical examination is difficult due to the patient's position and the presence of numerous lines and tubes for monitoring and delivering therapy. Many clinical features and

commonly used investigations are not helpful as they provide results that are not specific for the diagnosis of VAP[33,36] or infectious, as opposed to non-infectious, SIRS in critically ill patients[46].

Fever and elevated white blood cell count and C-reactive protein (CRP) are sensitive but not specific markers of sepsis and can be affected by other inflammatory conditions[42]. The sensitivity of these markers is lower for the diagnosis of VAP compared to other infections[34]. Temperature testing is easy, cheap, non-invasive and readily repeatable. The specificity for the diagnosis of infection increases when temperatures rise above 38°C[42], however up to 40% of patients with CAP do not present with fever[40]. White cell count is a poor marker of sepsis[42], does not correlate with response to therapy and can be affected by drugs commonly used in critical illness, for example corticosteroids, adrenaline and noradrenaline[47]. CRP, on the other hand, appears to increase with increasing severity of infection, including in VAP, and is higher in patients with SIRS due to infection than non-infective causes[42]. A CRP greater than 87 mg/L has been seen to exhibit a sensitivity of 93.4% and specificity of 86.1% for infection in critically ill patients, the specificity increasing when combined with a temperature over 38.2°C[42].

Yield for all culture-based tests is lower after antibiotic therapy[48-50], and this can be more of a problem in patients with suspected VAP as they have commonly received prior antibiotics[32]. With increasing antibiotic resistance in organisms such as Streptococcus pneumoniae and Haemophilus influenzae[45] it may become more important to culture the pathogenic organism to aid antibiotic choice. It should be noted, however, that in vitro antibiotic susceptibility testing requires more time than culture testing alone[45] and does not always correlate with clinical efficacy in vivo[51].

Any test that could improve on current methods to diagnose infection or identify the organism responsible would be of great value in the ICU setting. Currently used diagnostic tools are outlined below.

CXR A study of radiological abnormalities and autopsy findings in ventilated patients who died in the ICU highlighted the difficulty of obtaining good quality imaging, particularly due to difficulties positioning ventilated patients,

and in interpretation of portable anteroposterior CXRs[52], including the presence of tubes and lines causing the image to be less clear. The most useful radiological feature for the diagnosis of pneumonia was the air bronchogram, but this only predicted pneumonia in 64% of cases. There was a high probability that other conditions mimicked pneumonia, for example atelectasis, atypical pulmonary oedema, lung contusion and ARDS[52].

Blood culture Blood stream infections carry a high mortality rate in patients with critical illness, which increases if inappropriate antibiotics are administered[53]. The positive yield of blood cultures in CAP, hospital-acquired pneumonia (HAP) and VAP is low[47,51], with a higher chance of bacteraemia if bronchoalveolar lavage (BAL) is also positive[54]. Unfortunately, positive results do not always confirm the organism responsible for VAP, instead being associated with infection at another site[47,54]. In a prospective observational study of patients with CAP, bacteraemia was observed in only 5.7% of cases, however in over 60% of these cases the findings did not result in a narrowing of the antibiotic spectrum[51].

Other limitations of blood cultures are that results are not available immediately, some bacteria are difficult to culture from blood (including Chlamydophila, Mycoplasma and Legionella species), and it is not possible to culture viruses[51].

Sputum, tracheal aspirates and bronchoalveolar lavage Purulent secretions alone cannot be used to aid the diagnosis of pulmonary infection in the ICU as they are almost always present in the context of prolonged mechanical ventilation[36] and underlying lung diseases such as chronic obstructive pulmonary disease (COPD).

Sputum culture takes at least 48 hours, however sputum Gram stain is inexpensive, can be performed rapidly and does not require sophisticated equipment[55]. The sensitivity of a sputum Gram stain for the diagnosis of pneumonia is modest but specificity is high[44,49,55]; the better the specimen the higher the sensitivity and specificity[41]. It is therefore a useful test for the diagnosis of pneumonia if a good sample is obtained[49], which unfortunately can be significantly problematic[43,44,49,55]. Sputum culture from patients with

pneumonia is more likely to be positive in those with bacteraemia[48] and will not be of use in pneumonia related to Chlamydophila pneumoniae, Mycoplasma pneumoniae and Legionella species, as they cannot be cultured from sputum[55] and another diagnostic test must be performed.

Intubated patients cannot provide spontaneous sputum samples, so tracheal aspirates or bronchoscopy with BAL must be performed. A study of patients with suspected VAP compared a group with quantitative BAL culture and a group with non-quantitative tracheal aspirate culture, revealing no difference in number of patients receiving targeted antibiotics, days alive off antibiotics, maximal organ dysfunction scores and length of ICU or hospital stay[56]. Positive BAL culture occurs in about 50% of clinically diagnosed VAP cases, however there does not appear to be a difference in mortality between those with positive BAL culture and those who are culture negative, or a benefit in mortality if antibiotics are changed based on the BAL result[35]. Considering that bronchoscopy is invasive, not a therapeutic technique in this setting, does not seem to give superior results to tracheal aspirate samples[56] and positive BAL culture findings do not necessarily alter outcomes[35], it is not used routinely and should probably only be considered if there is a failure of therapy or a causative pathogen has not been identified using other less invasive tests[41].

It is important to remember that there is still a chance of contamination of BAL samples with upper airways organisms[41,57] and due to the heterogeneity of VAP, false positive and false negative results can be obtained if sampling from the non-affected area[32].

The interpretation of sputum, tracheal aspirate and BAL results can be difficult because hospitalised patients become colonised with bacteria within about three days of admission, more quickly if they are ventilated, therefore positive cultures may reflect the presence of colonising rather than infective organisms. The identification of a causative organism from tracheal aspirates of patients with VAP can be problematic as samples can grow two or more potential pathogenic bacteria[47].

Finally, the method of processing respiratory samples may affect the microbiological result; if there is a delay in sample preparation, causative

fastidious bacteria, for example S. pneumoniae, may fail to grow, while colonising bacteria, such as Enterobacteriacea[47], or oral contaminants may proliferate[57]. Less fastidious microorganisms grow more readily on selective media, so contaminants such as Staphlococcus aureus and Gram negative bacteria, may be overrepresented[57].

Urinary antigen tests Urinary antigen testing for Legionnaire's disease and S. pneumoniae is simple and rapid, but despite good specificity for the diagnosis of pneumonia relating to these organisms, the sensitivity of the tests is only modest[41,50,58]. False positives associated with the pneumococcal antigen test due to recent infection can be both a problem and a benefit of the test, as it will remain positive even if antibiotics have been administered[41]. The Legionella urinary antigen test appears to be highly specific for Legionella pneumophila subgroup 1 but not the other subgroups[41,58] or subspecies. Legionella longbeachae is an important cause of pneumonia in New Zealand[59], caused by its presence in potting compost[60], and a polymerase chain reaction (PCR) test is required for diagnosis[61]. Obviously, urinary antigen tests cannot be performed if the patient is anuric, which makes the test less useful in the ICU where some patients with severe pneumonia also have severe renal dysfunction.

PCR methods PCR tests have been standardised for the testing of Legionella species and Mycobacterial species. They can be used to provide a probable diagnosis of M. pneumoniae and C. pneumoniae[41] and there is likely to be an increase in the number of organisms that can be detected accurately in this way over time[57]. Advantages of PCR tests are that they provide rapid results[41,57], remain positive after antibiotic therapy has been commenced[41], and can be used to detect slow growing or difficult to culture bacteria[62]. Conversely, they are more expensive than conventional Gram stain and culture, occasionally lack sensitivity data and are not quantitative, so it may not be possible to determine whether a microorganism is colonising the patient or causing infection[57].

1.2.3. Hyperglycaemia and its effects in critical illness

Patients with critical illness commonly develop "stress hyperglycaemia", particularly in association with sepsis[63,64]. Hyperglycaemia, in the absence of previous evidence of diabetes mellitus, occurs with hyperinsulinaemia as a

consequence of peripheral and hepatic insulin resistance and subsequent increased gluconeogenesis and glycogenolysis[63,65]. Insulin resistance impairs glycogen synthesis and storage[63,66], resulting in a failure to remove glucose from the circulation. Elevated insulin levels should suppress glucose production, however in critical illness the secretion of counter-regulatory hormones (for example cortisol, growth hormone and glucagon), catecholamines and pro-inflammatory cytokines promotes further hepatic insulin resistance and increased gluconeogenesis[63,66]; particularly implicated are TNFα, interleukin (IL)-1 and IL-6[63]. Catecholamine infusions (adrenaline and noradrenaline) can cause further imbalance of glucose homeostasis through mechanisms that include the stimulation of gluconeogenesis[63]. Stress hyperglycaemia is associated with increased hospital mortality[67] and the degree of hyperglycaemia appears to correlate with the severity of critical illness[12,63,68].

Excess glucose can be toxic to cells. Increased oxidative phosphorylation within mitochondria results in the generation of more ROS than can be scavenged by antioxidants, which are already reduced due to critical illness itself[12]. The production of peroxynitrite causes further cellular damage, as discussed previously (Chapter 1.2.1.)[12,13]. Endothelial dysfunction as a consequence of hyperglycaemia may exacerbate multi-organ failure, and may be why improved outcomes have been seen with insulin treatment[66]. Other potential complications of hyperglycaemia include lipid profile derangement[12] and the development of a catabolic state, platelet activation, acid/base disturbances and electrolyte and fluid shifts[66]. Neuronal cell uptake of glucose is independent of insulin; consequently critical illness polyneuropathy seems to correlate with glucose level[12]. Reduced neutrophil activity[12], including impaired respiratory burst, may explain the increased risk of infection associated with hyperglycaemia[63,66].

1.2.4. The effect of insulin treatment in critical illness

Initial trials of aggressive insulin therapy to maintain normoglycaemia in the ICU setting revealed improvements in mortality, particularly in those with sepsis[65,69]. This was in agreement with earlier studies showing a reduction in

mortality in hyperglycaemic patients receiving insulin following myocardial infarction (MI)[70-72]. Administration of insulin to maintain normoglycaemia was associated with improved insulin sensitivity[68]. One detrimental effect of insulin administration was the development of hypoglycaemia, which was more common in patients with sepsis and conveyed an independent increase in mortality rate[64,69].

The inaugural study of insulin therapy in critical illness involved just over 1,500 patients in a surgical ICU by Van den Berghe et al.[65]. Participants were randomised to receive intensive insulin therapy (IIT) aiming for blood glucose of 4.4-6.1 mmol/L or conventional treatment with insulin only if blood glucose rose to over 11.8 mmol/L, aiming for 9.9-11.0 mmol/L. Overall mortality was lower in the IIT group (4.6% versus 8.0%), with more marked improvements in mortality for ICU stays over four days. Reductions were seen in the incidence of bacteraemia, critical illness polyneuropathy, acute kidney injury (AKI) requiring renal replacement therapy (RRT) and liver dysfunction. Length of ICU stay was reduced, possibly as a result of a reduction in duration of mechanical ventilation.

Further analysis of this study[65] suggested an anti-inflammatory effect of IIT, indicated by an increased rate of reduction of CRP and attenuated increase in manose-binding lectin levels[73]. Significantly lower serum NO levels were seen in survivors than non-survivors, with elevated NO correlating with the risk of developing AKI. Suppression of iNOS in liver biopsy samples of those in the IIT group was believed to be partially due to a reduction in NF-κB activity[19]. Structural and functional mitochondrial abnormalities seen in liver biopsies appeared to be prevented or reversed by IIT[74].

The follow-up study in a medical ICU[69] involving 1,200 patients failed to report a significant reduction in mortality with IIT overall, in fact the mortality rate for those patients admitted to ICU for less than three days was elevated in the IIT arm. In contrast, for stays in ICU of three days or more mortality decreased from 52.5% to 43.0%. Similar morbidity benefits to those seen in the surgical ICU trial were described, with the exception of a reduction in bacteraemia rate. This discrepancy was argued to be as a result of medical

patients being admitted to the ICU due to sepsis, irrespective of their diagnosis at hospital admission.

The difficulty of achieving normoglycaemia in patients with stress hyperglycaemia[75,76], discrepancy in the results of subsequent trials with similar aims[77,78] and anecdotal evidence from ICU clinicians that tight glycaemic control did not seem to result in the type of improvements seen in the trials by Van den Berghe et al.[65,69], led to a large, international, parallel-group, randomised, controlled trial of 6,104 medical and surgical ICU patients[76]. Hyperglycaemic patients were randomised to receive IIT (glucose target range 4.5-6 mmol/L) or conventional glucose control (8-10 mmol/L). There was a 2.6% higher mortality rate in the ITT arm, with no difference between the two groups in terms of median duration of ventilation, RRT, ICU stay or hospital stay. Severe hypoglycaemia affected 6.8% of patients in the ITT group compared to only 0.5% in the conventional glucose control group. The authors concluded that the number needed to harm with tight glycaemic control was 38, recommending maintenance of glucose concentration below 10 mmol/L to reduce mortality whilst avoiding hypoglycaemia.

An Australian study of 168,337 blood glucose measurements in 7,049 critically ill patients[79] demonstrated that intra-subject mean glucose concentration and fluctuations in glucose concentration (determined by the standard deviation and coefficient of variability of glucose concentrations), were independently predictive of ICU and hospital mortality. The authors hypothesised that controlling the variability, not just total concentration, of blood glucose may be beneficial.

As a consequence of the NICE-SUGAR trial[76] and two meta-analyses performed to assess the outcomes of IIT[75,80], international guidelines now recommend the control of glucose to below 10 mmol/L in critical illness, the prevention of large fluctuations in glucose level, and the avoidance of tight glycaemic control due to potential detrimental effects. It is suggested that intravenous glucose infusions are limited to reduce the risk of hyperglycaemia[81].

1.2.5. The SPRINT protocol

In Christchurch Hospital ICU, New Zealand, a unique system for glycaemic control was implemented as a change of practice in August 2005. The Specialised Relative Insulin Nutrition Tables (SPRINT) protocol is a simple wheel-based system that modulates not only insulin dose but also volume of feed (enteral or parenteral) administered hourly[82,83]. The system was developed from validated computer models that predict insulin sensitivity by looking at the handling of previous insulin and nutritional inputs[83]. Low carbohydrate feeding regimens aim to prevent further elevation of blood glucose level[82,83]. Unlike other protocols in the literature, insulin is administered as an intravenous bolus to avoid an inappropriate infusion dose running until the next blood glucose measurement. The protocol is commenced when a patient has two consecutive blood glucose measurements greater than 8 mmol/L during standard monitoring, or can be started by a specialist doctor if a patient with severe critical illness has a glucose level persistently over 7 mmol/L. If blood glucose levels remain high despite a maximum insulin dose of 6 units/hour, a background infusion of 0.5-2 units/hour can be initiated. This is most commonly required for patients with underlying diabetes mellitus. Unlike some insulin regimes where blood glucose is monitored every four hours, measurements are taken at one to two hourly intervals, with the aim of maintaining tight glucose control whilst preventing hypoglycaemia[82]. Hypoglycaemia is also avoided by withholding insulin when blood glucose drops by more than 1.5 units/hour if the glucose level is below 7 mmol/L[83]. The protocol is stopped when stable blood glucose measurements of 4-6 mmol/L have been achieved over six hours or more, at an insulin dose of 2 units/hour or less, with feed running at a minimum of 80% of the goal feed rate[82].

When compared to a retrospective cohort (413 patients), the SPRINT protocol (371 patients) led to a tightening of glucose control without an increase in hypoglycaemia seen in other studies[65,69]. A reduction in hospital mortality from 34.1% to 25.4% was seen in patients with an ICU stay of three days or more, and 31.9% to 20.6% in patients staying over four days. There was no

significant difference in illness severity between the two groups, as determined by the APACHE II score[82].

A significant reduction in organ failure, as determined on a daily basis by the SOFA score, was seen when the SPRINT group was compared to the historical cohort. Organ failure also appeared to resolve more quickly in the SPRINT group[83].

1.2.6. Feeding in the ICU

The assessment of a patient's nutritional status is problematic in the ICU as it is difficult to weigh the patient accurately, especially on a repeated basis, and even when it is possible the presence of oedema and fluid shifts make weight and body mass index (BMI) results unreliable. Energy expenditure is also not easy to calculate, but it is known to be a time of increased metabolism with subsequent development of a catabolic state[84,85]. Controversy surrounds the timing of initiation of feeding, the amount of feed administered and route of administration.

European guidelines recommend early enteral feeding in critically ill patients who are not expected to be eating normally within three days, avoiding excessive caloric intake in the initial phase of critical illness[86]. Clear evidence for recommending early enteral feeding is lacking, however meta-analyses suggest a reduction in infection rate and length of stay[86].

Feeding via the enteral route is advised by both European and American guidelines[84,86] to preserve gut integrity, and its barrier and immune functions[84]. Due to problems with gut motility associated with severe illness, adequate enteral intake is often not achieved[85,87-89]. In these cases supplementary parenteral feeding is recommended[86], but not as the only route of feeding due to higher cost and increased rates of infection, particularly line sepsis, when compared to enteral feeding[84,86].

The appropriate amount of feeding required has also been of interest to ICU clinicians. Overfeeding, especially via the parenteral route, increases the likelihood of developing stress hyperglycaemia[63] and can lead to increased carbon dioxide (CO_2) production, which may be more concerning in patients

with respiratory compromise[84]. Some, mainly observational studies, have shown that significant underfeeding is related to increased mortality[90], infection rate[85,91], duration of mechanical ventilation[85,90] and length of ICU stay[85]. In contrast, two studies showed that moderate underfeeding corresponded to improvements in mortality[88,89] and return of spontaneous ventilation[88] when compared to feeding at estimated maximum feed rate. The authors suggest that lower calorie intake might lead to improvements in outcome due to a reduction in metabolic rate and oxidative stress, thus a reduction in ROS generated in mitochondria, improvement in insulin sensitivity, and altered neuroendocrine and sympathetic nervous system function[89].

1.3. The analysis of volatile organic compounds in breath

1.3.1. The components of exhaled breath

Exhaled breath is composed of a mixture of nitrogen, oxygen, CO_2, water vapour, inert gases and numerous VOCs at the low parts per billion (ppb) level[1,2]. For centuries, physicians have recognised characteristic odours on a patient's breath or skin, caused by these trace gases, and related them to disease states; the sweet smell of diabetic ketoacidosis (DKA), the fishy smell of liver failure and the urine smell of renal failure[1-3]. Initially about 250 substances were separated from human breath, without specific identification[92], however over 1,000 substances have now been identified of both endogenous and exogenous origin[1,93]. VOCs produced by both normal and pathological metabolic processes are transported in the blood stream and can be exhaled via the lungs.

1.3.2. The potential applications of breath analysis

Breath analysis is an attractive method of obtaining information about a patient's metabolic or inflammatory status as it is non-invasive and, using certain techniques such as SIFT-MS, can be performed rapidly and in real time[4]. Much research has been undertaken into the clinical applications of breath analysis, however there are currently very few examples of breath

testing in clinical practice; capnography is an important tool for monitoring CO_2 level, used in operating theatres and the ICU, "breathalysers" are used by the police to detect breath alcohol levels, the ^{13}C-urea breath test is an important tool in the detection of Helicobacter pylori in the stomach, which can promote the development of ulcers, and eosinophilic inflammation in asthma can be monitored using the fraction of exhaled nitric oxide (F_{ENO}), for which a protocol for obtaining breath samples exists[94].

One of the important issues that needs to be addressed is the choice of VOC(s) for analysis, because to be used as a biomarker it needs to fulfil certain criteria. A biomarker is defined as "a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathologic processes, or pharmacological responses to a therapeutic intervention"[95]. Ideally a biomarker would be simple to measure, repeatable and reproducible, reflect changes associated with treatment and predict outcome or disease progression[96]. It is important that the relationships between the marker and underlying condition[96], and for systemic processes between breath and blood concentrations, are clear[8,97]. The monitoring of F_{ENO} in asthma is a good example; F_{ENO} rises with increased eosinophilic inflammation and is reduced by steroid treatment[98]. F_{ENO} can be used to predict response to inhaled corticosteroids (ICS)[99], to modify ICS dose without unwarranted side effects or loss of asthma control[100], and elevated levels after the cessation of steroid treatment can predict relapse[98]. Elevated F_{ENO} can also discriminate between individuals with asthma (as determined by symptoms suggestive of the diagnosis and a positive bronchial provocation test or positive response to inhaled bronchodilator) and those without[101,102], with a higher sensitivity than peak flow measurement and other spirometric parameters[102].

A number of potential breath biomarkers have been identified. Ethane and pentane appear to be linked to lipid peroxidation, acetone to glucose metabolism, isoprene to cholesterol biosynthesis and ammonia to protein metabolism[1,2]. Ethanol and hydrogen appear in human breath due to the action of gut bacteria[2]. Possible clinical applications have been investigated, including elevated ammonia as a biomarker in end-stage renal failure[103] and the

assessment of dialysis efficacy[104], the effects of surgery on metabolic stress[105] and the monitoring of intravenous anaesthetic drug levels[106].

Markers of infective pathogens have also been identified; 2-pentylfuran as a marker of Aspergillus fumigatus[107], methyl nicotinate as a marker of Mycobacterium tuberculosis[108,109], and hydrogen cyanide (HCN)[110] and 2-aminoacetophenone[111] as markers of Pseudomonas aeruginosa in the cystic fibrosis (CF) lung. By using a combination of 22 VOCs, children with CF could be separated from those without CF with 100% reliability and using 14 VOCs those with P. aeruginosa infection could be separated from those without[112].

1.3.3. The potential of breath analysis for the detection of pneumonia and metabolic disturbance in critically ill patients

For breath analysis to be clinically useful, results need to provide the clinician with information not readily available via conventional analysis of blood and urine samples[97], and also be of diagnostic or prognostic benefit, or be useful in following response to treatment. Breath samples may be simpler to process as they might not need to undergo preparation as blood and urine samples do[113] and results may, therefore, be more rapidly available, particularly when compared to samples for microbiological culture. Blood and urine samples are generally used to measure levels of large molecular weight proteins and ions rather than very small quantities of volatile substances[114]. There is a suggestion that due to the variability of individual measurements of VOCs, such as acetone, in blood that breath analysis may be more reliable[115]. When evaluating airway inflammation, sputum analysis for inflammatory cells is not practical on a daily basis due to the difficulty of obtaining adequate samples, and identification and interpretation of inflammatory cells requires highly skilled laboratory staff[116]. Other methods of sample collection, such as bronchoscopy, can be more invasive and impractical on a regular basis. In addition, breath sampling can provide details that other investigations cannot, such as information about pulmonary function[113] and may be able to provide information about several VOCs at the same time.

As described in Chapter 1.2.2., it is difficult to differentiate between inflammation due to infection and that due to other causes, especially in the lung and in patients with critical illness, on the basis of currently available diagnostic tests[97]. The differentiation between pneumonia, aspiration lung injury, pulmonary oedema and ARDS due to a cause other than pneumonia by a bedside test is currently not possible. Any test that could do this would be extremely useful in the ICU setting, where patients are unstable, their condition is rapidly changing, and management of the conditions above is different. Pneumonia requires antibiotic treatment and investigation of the organism responsible; aspiration lung injury usually requires supportive care without antibiotic therapy; pulmonary oedema requires pharmacological maintenance of cardiac function, and possibly mechanical support via a balloon pump; low volume ventilation or other ventilation strategies may be necessary in ARDS, as well as treatment of the underlying cause. It is important, therefore, to instigate appropriate management plans early and avoid unnecessary antibiotics, for example, with potential side effects of renal dysfunction or the promotion of bacterial resistance.

The ventilatory and haemodynamic status of a ventilated patient is closely and continuously monitored in the ICU or operating theatre, however there are currently no tests available to quickly and accurately monitor rapidly changing levels of metabolic stress, inflammation and infection. Currently available tests, for example Swann-Ganz catheters, pulse contour continuous cardiac output (PiCCO) monitors and blood tests are often invasive and results are not always available immediately[105].

Two studies of exhaled NO in intubated and ventilated patients in the ICU have shown promise in the diagnosis of pneumonia, with higher concentrations in the breath of patients with pneumonia than those without[117,118]. Neither study found a difference in exhaled NO concentration between patients with sepsis and those without, or a correlation between breath NO and plasma nitrate concentration[117,118]. There was no relationship between exhaled NO concentration and degree of consolidation on CXR[117] or markers of systemic inflammation, haemodynamic instability or disease severity[118]. Elevated NO

appeared to be associated with pulmonary infection and was not related to systemic production[117,118].

If the correct breath markers are identified, non-invasive breath sampling could be used to provide rapid, accurate, repeatable or continuous information to diagnose and monitor changes in breath marker concentrations as a consequence of infection, metabolic derangement or treatment in ventilated patients.

1.3.4. Breath analysis techniques

There has been a great deal of progress in the identification and quantification of trace gases in breath since the first studies of Pauling et al.[92] in the 1970s using gas-liquid partition chromatography. Several techniques are now in use, each with their own advantages and disadvantages, although only a few can accurately quantify VOCs at the low ppb level, on-line and in real time[3].

Gas Chromatography

Gas chromatography (GC) is the most commonly used method of breath analysis, whereby different compounds in a mixture are separated by their reactions with the substance of the GC column[119]. It can be combined with various methods of ion detection and analysis, for example flame ionisation detection (FID), time-of-flight (TOF) and MS. Tandem mass spectrometry (MS-MS) can be used to increase the sensitivity and confidence in identification of compounds detected. Conventional ionisation, for example electron impact ionisation, is used. This results in many ion fragments to be analysed and enables substances to be confidently identified by their characteristic fragmentation pattern[2]. The instrument must be calibrated for each VOC with known gas standards.

It is not possible to identify very low molecular weight compounds with accuracy using GC[3,4,120], thus pre-concentration, requiring off-line sampling, is important. Breath is humid and condensation of water vapour interferes with this method of analysis, affecting results. Water vapour must, therefore, be removed from samples prior to analysis[121], hampering the development of a method for directly breathing into a GC-MS instrument. Real-time breath

analysis is also precluded by the response time of the analyser to detect a change in concentration, which is too slow to accurately determine the concentration of a VOC over the duration of a single exhalation[3,4]. Pre-processing of raw GC-MS data involves de-noising, alignment and normalisation, prior to analysis. Due to the preparation steps of the sample and raw results data, this technique is time-consuming and requires a skilled operator. Instrumental variation can be improved by analysing samples in batches[119].

Ion mobility mass spectrometry

Ion mobility mass spectrometry (IMS) can be used to detect and quantify trace gases at the low ppb level. The device is smaller than other mass spectrometers as samples do not require heating prior to analysis[122]. A carrier gas of purified air or nitrogen, transports precursor ions, generated by a radioactive source, into a drift tube. A mass spectrum is produced when product ions with various molecular masses arrive at the detector at different times due to their different drift velocities. By changing the drift length, drift gas, electrical field strength, temperature or pressure, product ion separation can be improved[119]. This method is usually coupled with GC in order to separate complex gas mixtures[3,4,122]. Calibration of ion mobility for each ionic species at different humidity levels is necessary[4], which can be time-consuming.

Proton transfer reaction mass spectrometry and selected ion flow tube mass spectrometry

Proton transfer reaction mass spectrometry (PTR-MS) and SIFT-MS are both able to identify and quantify multiple VOCs at the sub ppb level, simultaneously, from a mixed sample. Both techniques allow rapid, on-line, real time measurement of single breath samples without pre-concentration[3,4,120]. The response time of PTR-MS instruments is around 100 ms[119] and 20 ms for SIFT-MS[123]. Instruments have reduced in size over the last 10 years, but are still bulky and expensive. Analysis of breath samples, however, is cheap once a machine is available, as consumables are inexpensive and significant preparation of samples is unnecessary[124].

"Soft" ionisation is used, preventing complicated analysis of multiple fragments[125], with the H_3O^+ reagent ion being used in PTR-MS and either H_3O^+, NO^+, or $O2^{+\bullet}$ in SIFT-MS. Reagent ions in the buffer gas react with trace gases to form product ions, which are detected downstream. Identification of product ions relies on their mass-to-charge ratios (m/z), the data for which is held in an inbuilt computer library, negating the need for calibration with known standards[2-4,120]. Isobaric product ions cannot be further separated by PTR-MS, thus a different method of analysis may be necessary. The choice of three precursor ions when using SIFT-MS resolves this problem and allows conclusive identification of compounds[3]. A detailed explanation of the SIFT-MS technique can be found in Chapter 1.4. of this thesis.

Secondary electrospray ionisation mass spectrometry

Secondary electrospray ionisation mass spectrometry (SESI-MS) involves passing a gaseous sample in a stream of air or CO_2 through a cloud of electrospray solution with which the volatile analytes react to become protonated. Product ions are driven by an electrical field and are sucked into the inlet of the triple quadrupole mass spectrometer for detection and analysis[126,127]. Analytes in the gaseous phase and those condensed in water droplets can be ionised by this technique[128] with no need for sample preparation[127]. Excellent sensitivity, down to the low parts per trillion (ppt), is possible over a wide range of masses (over 600 Da)[126], with results of a full mass scan available in less than 2 min[127]. Unlike PTR-MS and SIFT-MS, the fragmentation of compounds of interest by SESI enables exact compound identification by MS[128].

Optical spectroscopy

Optical spectroscopy can be used to detect and quantify a single substance in breath, on-line and in real time. One example of this type of instrument is the chemiluminescent analyser for measuring $F_{E_{NO}}$, which measures light produced by the reaction of NO in the airway with ozone[98,116]. A smaller, more portable tool has been developed that works on the electrochemical cell technique[116]. Standardised guidelines for on- and off-line breath collection have been produced by the American Thoracic and European Respiratory Societies[94,129].

Electronic noses

Sensor array devices, or electronic noses, utilise chemical vapour sensors to detect a pattern of trace gases in breath in order to diagnose a disease state[130,131]. The "smellprint" or "breathprint" is recognised from stored patterns in a simple to use hand-held device[130,131]. Electronic noses derived their name from the comparison with the human nose, having the ability to recognise a unique smell without knowing the exact constituent compounds[132]. Results are generated immediately; however complex statistical methods are often required for data analysis[133]. The electronic nose has acceptable within day and between day repeatability; slightly lower than the repeatability of F_{ENO} and spirometry[133].

1.3.5. Off-line breath collection and storage

As discussed above, some techniques, such as GC, require off-line breath collection and others can be used off-line if it is not possible to move the instrument to the subject or vice versa. Off-line collection is used for sampling atmospheric gases[134] and the headspace above blood samples and urine or cell cultures[114]. Sample loss and contamination are potential problems associated with off-line collection, pre-concentration, storage and subsequent desorption[1]. A comparative study of on-line versus off-line breath HCN quantification in polymer bags reported higher analyte concentrations in on-line samples; however, the difference may have been confounded by comparing mixed breath (off-line) and alveolar (on-line) samples[135].

Storage containers

Gaseous samples can be collected into various containers, but the properties of stainless steel canisters[134,136] and polymer bags[120,134,135,137-140], importantly their inertness, have led to their common use. Despite being durable and long-lasting, stainless steel canisters are complicated to use and expensive to purchase[134]. Polymer bags, on the other hand, are more popular as they are simple to use, cheap, inert and relatively durable[134,140]. Some bags can be cleaned and reused, whereas others are disposable, for example Nalophan® (polyethylene terephthalate)[135,140]. Damage from reuse can affect the inertness

of the bag and also lead to increased substance adsorption onto the inner wall[140].

Sample integrity within a polymer bag depends on the rate of diffusion out of the bag and adsorption of VOCs onto the inner wall, as well as contamination of the sample by diffusion of substances into the bag from the environment[134]. Bag thickness and surface area to volume ratio are important in these processes, with water vapour more likely to diffuse out of the bag than other gases[134,138,139]. Previously collected trace gases adsorbed onto the inner bag wall may also contaminate subsequent samples if the bag is not cleaned effectively[134,141], thus it is advisable to clean bags as soon as possible and store them in a collapsed state if their re-use is intended[141]. Contamination from the bag material itself is a problem with Tedlar® (polyvinyl fluoride) bags; N,N-dimethylacetamide at m/z 88, a solvent used in the bag production, and phenol at m/z 95 are released[134,138], particularly if the bag is heated[138].

Different bags have different storage properties. Tedlar is the most commonly used polymer bag[134] and, along with Mylar® bags, is recommended for the off-line analysis of F_{ENO}[94]. It is suitable for the storage of sulphur compounds[140] and acetone[134,135] for up to six hours with minimal loss and HCN for six hours with 35% loss[135]. Nalophan and FlexFoil® bags have been reported to show no significant loss of contents in the first six hours, unlike Teflon® (polytetrafluoroethylene) which demonstrated losses of between 10% and 20%. All bags showed significant losses at 24 hours[134,135,137,140]. The rate of reduction in volatile concentration is higher when bags are stored at room temperature compared to body temperature[135], possibly as a result of sample condensation and adsorption onto the bag's inner surface.

Due to differences in storage properties of different bags for a variety VOCs, it is important to assess the storage capability of a chosen container for the particular VOCs of interest, so that any losses can be accounted for. It is desirable to store samples for the shortest time possible to minimise loss and contamination[141]. It has been recommend that the contents of all polymer bags are analysed within ten hours of collection, when recovery of alcohols, nitriles, aldehydes, ketones and aromatic compounds is over 80%[134].

Sample pre-concentration techniques

Discontinuous sampling techniques are attractive because the small size of some of the newer pre-concentration materials makes sample collection convenient[142,143]. As well as being a useful technique if the patient is remote from the device[144], the ability to store samples for several days or more with little sample loss[144,145] may allow a large number of samples collected at the same time to be processed over several days. This can improve the cost-effectiveness of an analyser and aid collaboration of research work where only one site has direct access to an MS instrument[145].

As with any pre-concentration technique, despite improvements in sensitivity, some of the sample may be lost or contaminated in the collection and desorption processes[3]. Subsequent GC-MS analysis can be lengthy, so the results are not available in real time, which may limit the usefulness of this technique in clinical practice. Analytical techniques with instantaneous generation of results, such as SIFT-MS, may overcome this problem and allow easy and frequent measurement of breath volatiles, when combined with off-line sampling[146].

A popular method of sample pre-concentration is solid phase microextraction (SPME), which is often used with GC-MS as substances can be automatically desorbed onto the GC column at the time of analysis. Other methods include direct cryogenic trapping[136] and sorbent traps, for example activated carbon[93,121], where desorption is achieved by heating or microwave energy.

Thermal desorption (TD), onto a sorbent such as Tenax®, has been successfully used with SIFT-MS for the collection and storage of environmental gases[146] and breath[144]. On-line breath sample and TD recovery rates were almost the same[144]. It was possible to collect samples from a single exhalation and store them for several days before rapid analysis[144]. The small cartridges loaded with analyte can be transported easily, including by air freight, without significant loss of sample over a period longer than two months, including time spent at altitude[145].

1.3.6. Technical and methodological considerations

There is little standardisation for the analysis of trace gases in exhaled breath. In order to effectively compare results from different studies and use any data for clinical purposes the following factors must be taken into account and, if possible, controlled for[2,147].

Location of VOC production and gas exchange and the effect on breath concentrations

VOCs in breath may originate in the blood and cross the alveolar membrane, or may be produced in the airways, sinuses, mouth, nose or gastrointestinal tract[2]. Sampling from the mouth or nose can result in differences in observed VOC concentration, for example volatiles produced by oral bacteria can contaminate orally exhaled breath samples[148]. H_2S[149], HCN[150,151] and ammonia[150] concentrations are higher in oral than nasal breath samples, therefore uncontrolled oral exhalations of these trace gases are inappropriate, bringing into question the relevance of some previous study results[110]. In contrast, F_{ENO} is higher in nasal than oral exhalations, although lower concentrations have been noted in children and in patients with CF and Kartagener's syndrome[152].

The composition of expired gases changes with the phases of expiration. The concentration of exhaled CO_2 and other endogenous low blood-soluble trace gases is very low on initiation of exhalation (Phase 1 or anatomical dead space air) and climbs during expiration to a plateau (Phase 3), which represents alveolar air reaching the mouth (Figure 1-1 parallel lines). The concentration of low blood-soluble gases in alveolar air is in equilibrium with the concentration in the pulmonary circulation. The rate of diffusion across the alveolar-capillary membrane is dependent upon the polarity of the molecule, its solubility, volatility and blood:gas partition coefficient[124]. Pathological conditions that affect pulmonary blood flow and capillary or alveolar surface area, membrane thickness and permeability will also have an effect on gas exchange of low blood-soluble gases[153].

In contrast, highly blood-soluble VOCs, such as ethanol and acetone, exchange wholly or predominantly in the airways[154,155]. There is very little or no dead space air as the trace gas is present in breath as soon as exhalation is

initiated[156,157]. There is a "wash-in/wash-out" effect, whereby high blood-soluble gases present in airway tissue and the mucus layer transfer into luminal air during inspiration, saturating the air so that by the time it reaches the alveoli very little or no more gas exchange can occur. There is insufficient time for the tissues and mucus to be completely replenished with soluble gas from the bronchial circulation at the end of inspiration, so the saturated air deposits soluble gas down the concentration gradient back into the mucus during expiration[154,155,157]. As a consequence, the concentration of a highly soluble gas at end-exhalation is always lower than the alveolar concentration[154,155,157] and, therefore, may not reflect its concentration in the pulmonary[158] or systemic circulation[156] or correlate with end-tidal CO_2[147] (Figure 1-1 solid line). A rebreathing technique may result in equilibrium between airway and mucus layer concentrations, therefore improving the accuracy of breath concentration measurements[154,156,159,160], however subjects may not be able to rebreathe for long enough before hypoxia[154,160] or hypercapnia become a problem[160].

The process of airways gas exchange is limited by airway tissue thickness, bronchial blood flow, airway temperature and blood:gas partition coefficient[154-156]. Variation in breath VOC concentrations has been seen in different temperature environments, some VOCs showing an increase and others a reduction in concentration[161], although the changes may be related to differences in humidity rather than temperature alone. Airway gas exchange is also affected by changes in ventilation[156,162,163], with increased bronchial exchange during exercise resulting from a combination of increased alveolar ventilation[162], increased tidal volume[163] and/or increased bronchial blood flow[162].

Bronchial blood flow can be affected by drugs[157] and pathological respiratory states, for example increased bronchial blood flow is associated with airway inflammation in asthma; higher bronchial blood flow was seen in ICS naïve asthmatics than treated asthmatics, with the lowest flow in healthy controls[164]. As CF-related lung disease progresses the bronchial circulation becomes increasingly hypertrophied. This appears to augment gas exchange in the airways, with a greater percentage of cardiac output going to the lung[165].

Airway remodelling in pulmonary disease may lead to thickened airway walls and impaired diffusion from the bronchial circulation to the airway lumen[157].

For gases with a blood:gas partition coefficient greater than 1,000 gas exchange occurs entirely in the airways and if the coefficient is less than 10 gas exchange occurs entirely in the alveoli. For gases with a blood:gas partition coefficient between those two values, gas exchange occurs in both the alveoli and airways, predominantly in the airways for coefficients greater than 100[155,157].

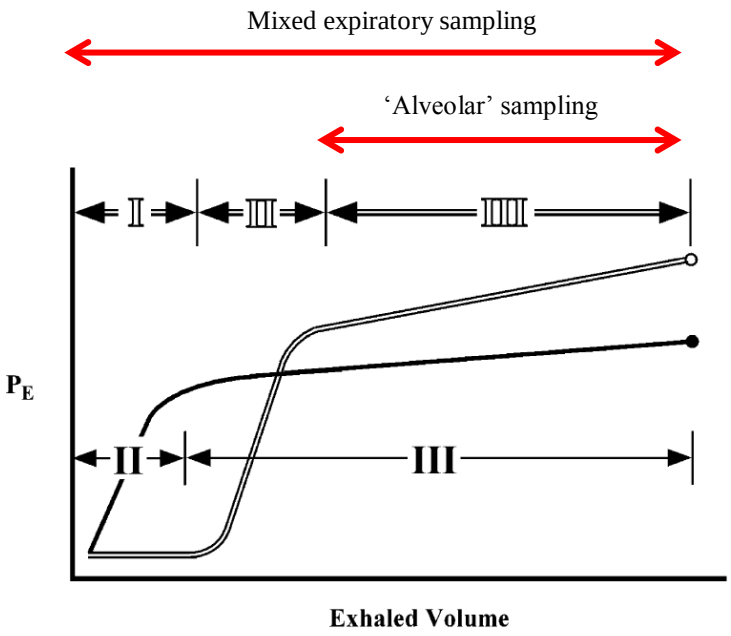


Figure 1-1. Schematic showing the phases of an expirogram of a low blood-soluble gas which exchanges in the alveoli (parallel lines) and a high blood-soluble gas that exchanges in the airways (solid line). The partial pressure of exhaled gas (P_E) is plotted against the exhaled volume. Red arrows show the phases of breath collected in mixed expiratory and 'alveolar' sampling (Modified from the original[157]).

Different breath sampling techniques and the effect on measured VOC concentrations

Much importance has been given to the collection and/or analysis of alveolar gas samples because, as well as containing fewer exogenous contaminants[166], they contain higher levels of some VOCs[7,166], whereas whole breath or mixed

expiratory samples are diluted by dead space gas[147] (Figure 1-1). Multiple mixed breath samples generally contain lower VOC concentrations than a single large exhalation, probably as a result of a greater volume of dead space gas[161].

Alveolar samples can be identified using breath humidity[167], via CO_2-controlled sampling, whereby the portion of exhaled breath containing ≥3.5% CO_2 is collected for analysis[7], or by using computer software to select only the last 20% of a breath by volume for analysis[151,168]. Discarding a fixed volume of breath at the beginning of an exhalation[121] involves an incorrect assumption that all subjects have the same dead space volume[169], and time-controlled sampling starting breath collection 1 sec after the initiation of expiration, for example, when compared to CO_2-controlled sampling, leads to highly variable results[166].

Effect of breathing pattern on measured VOC concentrations

The rate of alveolar ventilation is affected by aerobic cellular metabolism and acid-base balance, aiming to maintain CO_2 at a steady state[147]. Spontaneously breathing subjects tend to hyperventilate when asked to perform breathing tests, reducing end-tidal CO_2 concentration and possibly that of other low blood-soluble trace gases such as isoprene[169,170], via a combination of increased tidal volume and minute ventilation[169,171]. Hyperventilation also causes a reduction in the end-tidal concentration of high blood-soluble gases like ethanol, where 20 sec hyperventilation can reduce the exhaled concentration by 11% and only three deep breaths can result in a concentration reduction of 4%[154]. This is due to an increased volume of air passing over the airway mucosa per unit time[154]. The control of respiratory rate and tidal volume, calculated according to body mass, reduces breath VOC concentration variability and improves inter-individual comparison, however paced breathing does not always result in improved correlation of VOC concentration with end-tidal CO_2[169], presumably as a consequence of differences in blood:gas partition coefficient.

Breath holding before exhaled breath sampling causes an increase in almost all VOC[161], including F_{ENO}[152] and ethanol; a 30 sec breath hold can increase exhaled ethanol concentration by 16%[154].

Expiratory flow rate affects F_{ENO}; during a slower exhalation there is more time for NO to be released into the airway lumen causing an increase in measured breath concentration[152,172]. To allow comparison, F_{ENO} is reported as a concentration, however the exhaled flow rate must be kept constant and noted to allow the calculation of NO output per unit time[94,152]. Due to the wash-in/wash-out effect, exhaled ethanol concentration increases over an exhalation at a constant flow rate[154].

Many investigations of exhaled flow rate, volume and duration do not control for the other variables, and have been performed in mixed and alveolar samples; therefore the behaviour of individual volatiles is not completely clear. The concentration of exhaled isoprene measured on-line increases over the duration of a single breath without reaching a plateau, with the concentration dependent on the duration of exhalation, making it impossible to accurately quantify during an uncontrolled on-line single breath exhalation[159]. In contrast, when whole breath samples are collected and analysed off-line, isoprene concentration does not increase when greater than 400 ml breath is exhaled[144]. Acetone concentration increases with greater breath volume in on-line exhalations[159,168] and off-line mixed breath samples[144], although the gradient of the Phase 3 slope for acetone is much shallower than that for isoprene[159]. This is, however, only the case if tidal breath samples are compared with each other or exhalations from vital capacity to residual volume are compared with each other, as a greater volume of air passing over the airway mucosa during a vital capacity breath has a diluting effect on breath acetone concentration[163].

For high blood-soluble gases like acetone, increasing the volume of an inspiratory breath, and the subsequent exhaled breath, moves the location of gas exchange deeper into the lungs; a larger volume of air will become less saturated with the same quantity of soluble gas, thus more gas exchange is possible in the alveoli than with shallower breaths[157]. Exhaled acetone has been reported to be dependent on expiratory flow rate[156], however this was not the

case in subsequent studies[159,168]. Exhaled breaths were only analysed to the volume of the smallest breath, therefore it is possible for results to be explained by differing percentages of total lung volume analysed[156].

Due to possible effects of the breathing pattern on VOC concentration, if breath samples are not collected over a given time period but instead over a single exhalation, that breath must be representative of the whole breathing pattern otherwise averaged breath results are recommended[1,97].

Effect of V_A/Q changes on breath VOC concentrations

Changes in alveolar ventilation-perfusion ratio (V_A/Q) affect the concentration gradient of VOCs and therefore their concentration in exhaled breath, which is also dependent on the blood:gas partition coefficient[173,174]. When the blood:gas partition coefficient equals V_A/Q, 50% of the gas will remain in the pulmonary circulation and 50% will move into the alveolus. A greater proportion of the gas will be transferred to the alveolus as V_A/Q increases and more will be retained in the circulation as V_A/Q is reduced[173]. This means that highly soluble gases can still exchange in areas of high V_A/Q, whereas in areas of low V_A/Q even the smallest amount of perfusion allows exchange of low blood-soluble gases[173]. This explanation does not, however, completely take into account airway gas exchange[157].

V_A/Q is not homogeneous throughout the lung, with areas of V_A/Q mismatch and shunt more marked in critical illness[1,174]. Physiological dead space is greater during anaesthesia than spontaneous breathing; alveolar dead space fraction (alveolar dead space/tidal volume) but not anatomical dead space can be reduced by increasing tidal volume and reducing respiratory rate[175]. Chronic lung diseases tend to increase V_A/Q mismatch without significant shunt, however in acute lung diseases shunt is more common with less V_A/Q mismatch (fluid in alveoli prevent gas exchange)[176]. Pronounced haemodynamic compromise can cause alterations in breath VOC concentrations, however small changes in cardiac output may not have any effect[1,2,176].

V_A/Q alters as a subject moves from a semi-supine to supine posture due to increased cardiac output, mainly as a consequence of increased stroke volume, causing increased pulmonary blood flow[177], with less of an effect on ventilatory parameters. Breath isoprene concentration increases as a result of greater cardiac output in the supine position, whereas posture has no effect on acetone concentration[171] or F_{ENO}[152].

Effect of exercise on breath VOC concentrations

Exercise causes changes in cardiac output, thus pulmonary and bronchial perfusion, minute ventilation as a consequence of increases in tidal volume and respiratory rate, and alveolar ventilation, which all have an effect on measured breath VOC concentrations[162,177,178]. Breath isoprene increases at the onset of exercise then decreases back to baseline within 15 min[171,177-179], due to an increase in cardiac output overwhelming the reduction in breath concentration that would be expected with an increase in respiratory rate[171,178]. An increase in airway gas exchange of acetone, as a consequence of increased cardiac output and subsequent bronchial blood flow[162], is balanced by increased ventilation, however other metabolic factors influence breath acetone concentration. Breath acetone concentration climbs during exercise due to the production of acetone from carbohydrate and fat sources[177,178] to anaerobic threshold, after which it is seen to fall as a consequence of increased lactate and decreased acetoacetate production during anaerobic metabolism[178]. The relationship between F_{ENO} and exercise is unclear[116], however because one study found that NO output increased with exercise and F_{ENO} and nasal F_{ENO} fell, it is recommended that subjects refrain from exercise for one hour prior to breath testing (reviewed[94]).

Effect of inspired VOCs on exhaled concentrations

The rationale for investigating breath biomarkers involves a belief that endogenous breath VOCs in disease states will differ from healthy controls, however this does not take into account current or previously inspired VOCs. Most VOCs in breath are exogenous[180] and are either inspired from the surrounding environment or absorbed through the skin, affecting exhaled VOC concentrations[181]. Rates of uptake, distribution in body tissues and excretion vary depending on the compound[180], as can be seen in a recent study of

subjects breathing air polluted with seven VOCs of interest[182]. Breath volatile concentrations were examined as inspired air concentrations were reduced, showing some retention of all inspired VOCs under investigation and a close linear relationship between inspired and exhaled concentrations. The authors calculated VOC retention coefficients, concluding that it may be possible to ignore inspiratory concentrations of substances with low coefficients, such as acetone, methanol, formaldehyde and deuterated water[182]. Breath acetone concentrations have also been examined in workers exposed to industrial acetone, revealing higher concentrations than in non-exposed workers. Correlations existed between breath, blood and urine concentrations and environmental concentrations; a stronger relationship was seen between breath and blood concentrations in exposed workers than controls[183].

Even within the hospital, environmental levels of some common VOCs show significant variation in different locations at different times of the day[184]. Interestingly, there seemed to be less variability in VOC concentrations in the piped medical air supply than ambient room air when measured over three months[185]. Both indoor and outdoor pollutants could be responsible for the differences, including building and furnishing materials, disinfectants and air conditioning systems recycling air around the building[180]. This may have implications for the clinical application of breath testing in certain settings, particularly if environmental concentrations of VOCs of interest are high and endogenous concentrations are low.

There is no standard way of dealing with different inspired trace gas concentrations. It is possible to calculate a VOC gradient by subtracting the atmospheric concentration from that found in breath samples[93,121], although due to the physiology of some trace gases the relationship between inhaled and exhaled concentrations is not linear and the subtraction method may not give accurate results[8,181]. Another possible solution is to "scrub" the inspired air to remove trace gases, as is recommended for the determination of F_{ENO}[94], but for some analytes this method can be difficult, time consuming, impractical for most clinical use and not always reliable[93,147,182]. The amount of time an individual must breathe scrubbed air before equilibrium is reached between the

inhaled gas and circulating blood is also unclear[147]; it may take days in the case of very lipid soluble gases such as isofluorane[6].

If inspired VOC concentrations are the same or higher than corresponding exhaled concentrations it can be difficult to interpret the results[182]. This has led some authors to recommend that results should be treated with caution if the inspired concentration is greater than 5%[8], or more practically 25%[2], of the expired concentration.

Effect of cigarette smoking on breath VOC concentrations

Cigarette smoke contains many VOCs[123,186-189], some of which can be detected in human breath and could potentially confound breath analysis; pentane, isoprene[190], HCN[191] and methyl-nicotinate[189] have all been investigated as potential biomarkers with higher concentrations in the breath of smokers than non-smokers. In one study, a combination of seven VOCs could be used to discriminate between smokers and non-smokers, the most important appearing to be HCN, acetonitrile and benzene[192]. F_{ENO} is lower in smokers than non-smokers, increasing over time from the last cigarette smoked, but decreasing with increasing total number of cigarettes smoked over a lifetime[193]. The mechanism by which smoking causes a reduction in F_{ENO} is unclear. It is possible that NO in cigarette smoke causes a reduction in endogenous production in the airways due to a negative feedback loop, supported by lower eNOS and iNOS in smokers, however, due to its volatile nature, endogenous NO may simply be more readily oxidised in the lungs of smokers (reviewed[193]).

Effect of food and drink on breath VOC concentrations

Different foods and the timing of eating relative to breath sample collection can affect the results of breath testing. As a ketone body, acetone production is induced by fasting and reduced by eating[167,194] (discussed in more detail in Chapter 1.5.). Breath acetone concentration is increased by eating a diet high in protein and fat and low in carbohydrate[195,196]. Following particular diets, for example a gluten-free diet[197], can affect the concentration of a range of VOCs in breath. An initial reduction and then increase in breath ammonia

concentration has been seen after eating, the cause of which is unclear[194], however it may be related to changes in salivary pH[198].

The consumption of alcohol will obviously affect the concentration of ethanol in the circulation, which will be reflected in breath concentrations[154,199]. Breath ethanol concentration also rises slightly after meals as a result of small quantities of alcohol in food and fermentation in the gut due to bacterial activity, especially in the presence of slow gastric emptying[169,200,201].

As with alcohol, it is possible that consumption of foods containing VOCs of interest will contaminate breath samples, particularly if oral exhalations are used. Examples include 2-aminoacetophenone for the detection of P. aeruginosa, which can be found in corn chips, canned tuna and some beer[202], and three potential breath markers of M. tuberculosis; methyl p-anisate is found in some throat sweets, toothpaste and face cream, methyl nicotinate is found in cigarettes and chilli powder and methyl salicylate is the main component of "Oil of Wintergreen", which enhances the mint flavour of products like chewing gum and toothpaste[189]. Increased breath concentrations of 2-aminoacetophenone were seen after ingestion of corn chips, falling back to baseline within two hours[202]. Controlling oral intake, breath sampling more than two hours after the last meal or snack, or enquiring about recent food and drink is therefore important in breath analysis.

Effect of age and gender on breath VOC concentrations

Ammonia in exhaled breath seems to rise with increasing age[167,203], however this is not the case with acetone[167,203], propanol[167] or HCN[203]. Different VOC concentrations have been seen in children compared to adults[203,204] and there is also a suggestion that breath acetone is affected by gender[167,205].

Effect of time of day on breath VOC concentrations

Diurnal variation in breath isoprene concentration, being highest around 6 a.m. and lowest at about 6 p.m., has been linked in some way to the circadian rhythm[1]. Significant day-to-day variation[167,168], as well as diurnal variation[168], exists in the measurement of acetone, which is likely to be a consequence of eating or fasting.

Standardisation of results and normal values

There are currently no agreed normal ranges available for exhaled trace gases and there is an ongoing need to standardised breath sampling methods and analysis[161,166] and/or to normalise data[1,2] so that results from different studies can be accurately compared.

It has been suggested that the exhalation manoeuvre performed for F_{ENO} assessment[94], an oral exhalation at a fixed flow against a fixed resistance, is used for all breath VOCs[2]. Cope et al.[169] suggest normalising VOC concentrations in exhaled breath to CO_2 concentration, therefore allowing comparison of mixed and alveolar samples, however this may be affected by breath pattern and sampling method[7,169], or normalising results to body mass or surface area, however this may over-normalise results of obese patients[169].

It can be seen, however, from the discussion in this thesis so far that different trace gases have different properties and a single standardised manoeuvre may not be appropriate for all. The issue is so important to the breath research community that a "Task Force" was recently set up to look into a standardised approach, which may involve different standards for individual VOCs[206].

1.3.7. Specific considerations when breath sampling from mechanically ventilated patients

Technical difficulties exist in obtaining breath samples from critically ill patients as they are often intubated and mechanically ventilated. On-line sampling is challenging due to the nature of the ventilator circuit, the size and portability of the instrument used for breath analysis, health and safety and infection control issues. Off-line sampling is often required, with its associated benefits and drawbacks (see Chapter 1.3.5.). Breath sampling has been performed during anaesthesia, to assess breath concentrations of endogenous VOCs and the intravenous anaesthetic agent propofol. Real-time techniques such as SIFT-MS[106], PTR-MS[207,208] and ion-molecule reaction MS (IMR-MS)[209,210] have been used for continuous monitoring, and discontinuous techniques involving SPME[8,143] and TD[142] with GC-MS analysis have also been explored.

The physical dimensions of the devices used for continuous monitoring are still not suitable for positioning adjacent to a patient[106,207,209]. To overcome the physical limitation of the device, previous studies have used long sampling lines, which can affect sample integrity (compounds can be lost by adsorption onto the tubing) and have been found to change the humidity of the sample[106,207]. As with any technique these effects can be quantified and controlled for, however this method may be unsuitable for sampling some VOC.

Mixed breath rather than alveolar sampling is sometimes performed in ventilated patients due to ease of collection[97], however end-tidal breath has been determined by various methods; breath sample temperature[208], breath CO_2 levels using valves in the respiratory circuit to identify and collect breath containing CO_2 of at least 3.5% by volume[7], and dual MS systems to trace CO_2 concentrations for software controlled collection[210]. An experimental circuit for use with SIFT-MS was developed to exclude the first 30% of each exhaled breath as dead space and collect the remaining 70% into Tedlar bags[211,212]. This method is likely to be less accurate than a CO_2-controlled sampling technique, as a consistent expired breath volume is required for the mechanism to work and the alveolar portion of gas may not be the same percentage of the total volume when the breathing pattern changes.

The downside of these techniques is the presence of additional equipment both in the operating theatre or ICU and in the ventilator circuit, which may introduce difficulties with the synchronisation of two devices' flow or sampling rates and data collection[210], may not be appropriate or feasible in these environments, and would introduce additional factors that must be controlled for.

Breath samples can be contaminated by inspired gases and compounds emitted by the ventilator and tubing, whilst dilution can occur due to the presence of dead space gas in the tubing[7,97]. Exhaled breath samples from anywhere along the expiratory limb of the ventilator tubing have been shown to contain similar trace gas levels, however it is advisable to collect inspiratory gas samples close to the ventilator to reduce contamination from expired gas[6]. Humidified

breathing circuits used in the ICU can result in higher water vapour concentration in exhaled samples, which may affect analysis by some techniques[97].

Ventilator settings vary from patient to patient and cannot be altered if a patient is breathing spontaneously. The differences in respiratory pattern can affect exhaled VOC concentrations[169], potentially making interpretation and comparison of results difficult. Unlike awake volunteers, sedated patients will not be able to perform specific manoeuvres for breath collection.

VOC concentrations in the breath of patients with critical illness may differ from healthy subjects due to gases not passing through the upper airways and greater shunt and dead space ventilation[8], which is likely to affect the way VOCs are excreted in breath[176] and the relationship between their concentrations in breath and blood[8].

1.4. SIFT-MS

1.4.1. The development of SIFT-MS

The SIFT-MS technique was originally developed in 1976 as an alternative to GC. It was first used to study ionic reactions in the atmosphere[213] and to quantitatively assess the production of molecules exiting interstellar clouds[214]. It is possible to analyse samples more quickly using this method than GC techniques, which usually require adsorption and concentration of samples onto traps, with subsequent release of samples to be analysed off-line. It can rapidly sense small changes in trace gas concentration allowing for analysis over a single exhalation. The SIFT-MS technique enables samples to be collected into storage containers and then analysed off-line or breath samples to be directly exhaled into the instrument for on-line analysis. Traditional MS produces a large number of mass fragments for any compound analysed, however SIFT-MS has overcome this by using chemical ionisation via reagent ions (H_3O^+, NO^+ and $O_2^{+\bullet}$), resulting in a reduction in the degree of collision fragmentation. These reagent ions do not react with the major components of breath. Higher flow rates of air or breath through the chemical reactor than with other techniques have improved the sensitivity of this method[4,114,123].

1.4.2. The SIFT-MS technique

A mixture of ions is produced when water vapour or air is passed through a microwave discharge ion source. A reagent ion species (H_3O^+, NO^+ or $O_2^{+\bullet}$) is selected by a quadrupole mass filter and injected into a fast-flowing stream of inert carrier gas, usually helium at a pressure of around 1 Torr, passing through a Venturi-type opening into a flow tube around 1 m long. The sample gas, for example breath, is injected into the analyser at a set flow rate via a heated, calibrated capillary. The trace gases in the sample react with the precursor ions to produce one or more product ions, their characteristic patterns allowing identification. The reagent and product ions then pass through another quadrupole mass spectrometer and ion-counting system for detection and analysis (Figure 1-2). The decay rate of the reagent ions and production rate of the product ions are analysed. The SIFT-MS technique has been described in more detail previously[4,123].

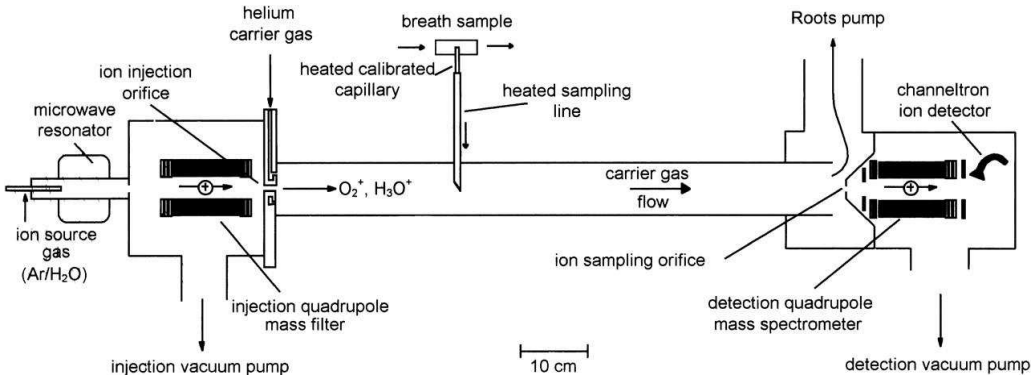


Figure 1-2. Schematic of the SIFT-MS instrument[194].

The device can be set up in two modes: Mass Scan mode (screening of a number of VOCs by their m/z ratios over a selected mass range) and Selected Ion Monitoring (SIM) mode, which targets only VOCs of interest, giving slightly more accurate quantitation[4,114,123].

An example of the reaction of trace gas, M, and the H_3O^+ reagent ion resulting in the product ion, MH^+, is shown in equation (1):

$$M + H_3O^+ \rightarrow MH^+ + H_2O \qquad (1)$$

The relationship between count rates of reagent and product ions, detected downstream by the ion-counting system, are shown in equation (2):

$$[MH^+]_t = [H_3O^+]\ k\ [M]\ t \quad (2)$$

Quantification of product ions is possible because the integration time (t) and rate co-efficients (k) of reactions between analytes and reagent ions are known, and this information is stored within an on-board database[4,114,123]. Flow rate, temperature and pressure are important in the calculation of reaction rates[215].

The water vapour content of on-line breath samples can be used to determine the end-tidal portion of a breath[119], however it can affect the analysis of gases by SIFT-MS[216]. The sample capillary tube and connecting tubing are heated to 100°C to prevent condensation in the instrument[215] as the error caused by water vapour is around 2%[216].

When using the H_3O^+ reagent ion to analyse a humid gas sample, hydronium ions are produced and form clusters: $H_3O^+.(H_2O)_{1,2,3}$. These ions subsequently react with the trace gas molecules to produce product ions, for example $MH^+.(H_2O)_{1,2,3}$. Accurate quantitation of a trace gas requires the identification and addition of all product ions[4]. The results of these reactions are available for several trace gases[216-218].

Various product ions are produced by the reactions of the NO^+ precursor ion with different trace gases: $(M-H)^+$, M^+ or $NO^+.M$[3].

The $O_2^{+\bullet}$ precursor ion is mainly used to detect small molecules that do not react with the other precursor ions and do not fragment during the reaction with $O_2^{+\bullet}$. Such molecules include NO, NO_2 and CS_2. The molecules are protonated with concurrent formation of oxygen[4].

Accuracy and precision

Initial studies of the accuracy of the SIFT-MS technique used dry gases including ethanol, benzene, toluene, xylene, acetone, 2-butanone, 1-methoxy-2-propanol and trichloroethylene, at concentrations of 10 ppb to 20 parts per million (ppm) over a range of partial pressures, producing results to within 10% of the true concentration using the syringe injection[219] and permeation tube methods[220]. Further studies have examined the accuracy of analysis of humid samples[216-218]. Dummer et al.[168] observed an 8% instrument measurement bias when using known concentrations of acetone at 100%

relative humidity. The inter-day and intra-day coefficients of variation (CVs) were 5.6% and 0.0%, respectively.

The precision, or repeatability, of measurements depends upon the number of product ions counted; the standard error of an individual measurement is calculated as the square root of the total number of product ions. Typical standard errors for acetone and ammonia in single breath exhalations range from ±5% to ±20%[4]. The repeatability of SIFT-MS for breath analysis was investigated in an off-line study of breath samples collected from ten volunteers[221]. Samples were analysed for 60 sec and then immediately reanalysed for another 60 sec. Volatiles at higher concentrations, for example acetone and ammonia, had low instrument CVs (1-2%), whilst those at lower concentrations, such as HCN, had much higher CVs (19%). This can be explained by the differences in analyte concentration and count rate.

Specificity

The choice of precursor ion depends upon the trace gas to be analysed. Some compounds only react with a single precursor ion and others react with all three. The various product ions produced by the different reactions allow precise identification of the compound[114]. For example, the reactions between H_3O^+ precursor ions with acetone and propanal give isobaric product ions at m/z 59, however reactions of the NO^+ reagent ion form product ions for acetone at m/z 88 and for propanal at m/z 57, so they can be accurately separated and identified[3].

Few compounds cannot be identified by SIFT-MS; hydrogen and smaller alkanes have high ionisation energies and low proton affinities, making identification impossible[4].

Dynamic response time

The dynamic response time is the time taken for changes in analyte concentration to be detected by the instrument and differs depending on the VOC to be analysed. This has been reported for SIFT-MS as 20 ms[4,123]. Another study reported the 0-90% response time for the quantitation of acetone at physiological concentration as 500 ms[222]. The cause of the discrepancy is not

clear, although the instrument set-up may have differed up-stream of the sample inlet[223]. It has been suggested that for accurate measurement of an analyte the 90% response time should be ≤10% of the total exhalation time, therefore both dynamic response times mentioned above still allow the use of SIFT-MS for on-line measurement of single exhalations over 5 sec[224].

1.4.3. The use of SIFT-MS for breath analysis

Several studies have used the SIFT-MS technique for on-line[106,110,150,167,168,179,194,201,203,223,225,226] and off-line[135,144,184,221] breath analysis. Most of these studies have taken healthy volunteers to determine normal trace gas values, before comments can be made on concentrations in disease states. The distributions of exhaled trace gases appear to be log-normal and an attempt to determine the normal ranges of some common VOCs has been made (Table 1-1)[200].

Table 1-1. The concentrations of common volatile organic compounds in the breath of 30 healthy volunteers, as determined by SIFT-MS[200].

Analyte	Mean (SD) (ppb)	Range (ppb)
Ammonia	833 (1.2)*	248 - 2935
Acetone	477 (1.58)*	148 - 2744
Methanol	450 (1.62)*	32 - 1684
Ethanol	196 (244)	0 - 1663
Propanol	18[§]	0 - 135
Acetaldehyde	24 (17)	0 - 104
Isoprene	118 (68)	0 – 474

*, geometric mean (multiplicative SD); [§], median.

1.5. Acetone

1.5.1. Acetone production and metabolism

Acetone (2-propanone, dimethyl ketone, β-keto-propane, pyroacetic ether) is one of the most abundant VOCs found in breath. It is responsible for the distinctive smell of “pear drops” on the breath of patients with DKA. It is

produced as a result of ketosis, which is the consequence of increased lipolysis in adipose tissue and ketogenesis in the liver.

When more acetyl-CoA is produced than can be utilised in the Krebs cycle, ketone bodies (acetoacetate and beta-hydroxybutarate) are formed and released into the circulation[227,228] (Figure 1-3). To enter the Krebs cycle, acetyl-CoA must join with oxaloacetate, produced during glycolysis, but when glucose levels are low oxaloacetate is preferentially used in the process of gluconeogenesis, therefore driving ketogenesis[229].

Acetoacetate can be converted to acetone by enzymatic or non-enzymatic decarboxylation[227,228,230], the acetone formed being excreted in breath. The enzymatic catalyst responsible for decarboxylation, acetoacetate decarboxylase, is induced by starvation and inhibited by acetone[227,228]. This process helps to regulate pH by removing excess acetoacetic acid[227,229]. During fasting in healthy individuals about 37% of acetoacetate is converted to acetone (up to 50% in those with diabetes), however acetone is by far the least abundant of the ketone bodies[227].

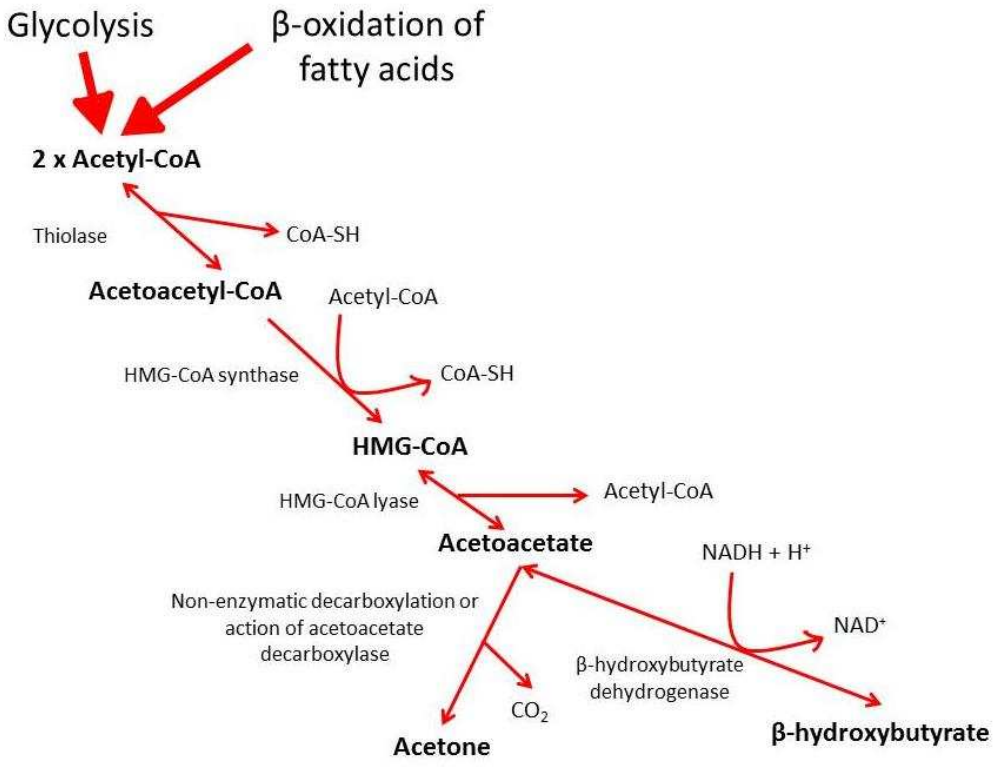


Figure 1-3. The pathway of ketone synthesis from excess acetyl-CoA.

The ratio beta-hydroxybutarate:acetoacetate depends on the redox state of the hepatic cells and is normally 1:1, rising to 6:1 after fasting and up to 10:1 in DKA[229,231]. Other pathological states that cause higher concentrations of NAD^+ and, therefore, an increased ratio of beta-hydroxybutarate:acetoacetate include alcoholic ketoacidosis, salicylate poisoning, severe hypoxia, end-stage liver disease, hepatic ischaemia, metabolic disorders and multi-organ failure[229].

Ketone bodies perform a protective function as an energy source for vital organs in times when glucose is not readily available[227,229]. Ketones provide 2-6% energy after an overnight fast and 30-40% after a three day fast. Unlike other tissues, the brain cannot use fatty acids as an energy source, so is reliant on ketones during starvation; nearly two thirds of its energy requirements are provided by ketones during prolonged fasting and starvation. Despite protective properties, ketones have also been implicated in the generation of ROS[229].

Acetone can be broken down via several pathways to pyruvate, lactate or acetate[227,228]. Very small amounts of glucose can be produced from acetone in an attempt to regulate glucose level[227,228,232]. Radiolabeled carbon within acetone molecules has subsequently been detected in glucagon, urea, cholesterol, amino acids, fatty acids and exhaled carbon dioxide in animal models[228], and in lipids and proteins in humans[232], showing that metabolised components of acetone play a role in anabolic processes.

In the fasting state in the non-diabetic, ketone production increases in the presence of decreasing glucose and then insulin levels, with a concurrent increase in glucagon and stress hormone secretion (glucagon, corticosteroids, catecholamines and growth hormone). This sequence of events is not as evident in Type 1 diabetes, whereby insulin levels remain high despite hypoglycaemia, and there is a lack of glucagon and stress hormone release[225,228]. In DKA, elevated stress hormone levels promote ketogenesis, a concomitant abnormal increase in glycogenolysis and gluconeogenesis, and a reduction in the insulin:glucagon ratio. There is an associated reduction in peripheral uptake of glucose as a consequence of insulin resistance[233,234]. Glucagon stimulates further gluconeogenesis and ketogenesis and reduces the oxidation of fatty

acids to triglycerides[234,235]. Insulin is administered to switch off ketogenesis[231], usually causing a reduction in beta-hydroxybutarate before acetoacetate[229]. Acetone levels appear to be elevated in hyperglycaemic diabetic patients without DKA[230] and in diabetic patients without hyperglycaemia, in which case acetone may act as an early marker of loss of glycaemic control[236].

1.5.2. Acetone in breath

As with other highly soluble compounds, rather than exchanging in the alveoli, acetone appears to exchange in the airways[155]; its blood:gas partition coefficient has been quoted in the literature between 146 and 400[154,174,183,237]. As a consequence of the wash-in/wash-out phenomenon, the concentration of acetone in end-exhaled breath may not be in equilibrium with that in the systemic circulation[156]. This relationship may be improved by a rebreathing technique[115], which allows at least partial equilibration of acetone in the airways and mucus lining, increasing the concentration of exhaled acetone[156,160]. Rebreathing concentrations of acetone, however, have been shown to be within measurement uncertainty of single-exhalation concentrations; therefore end-exhaled gas sampling and the rebreathing technique may be equivalent[159].

Due to its volatile nature, acetone is difficult to accurately analyse in breath and blood. Significant intra-individual variation in blood acetone was seen in a study by O'Hara et al.[115], whereas, despite significant inter-individual variation, breath acetone concentration appeared to be more consistent. Other studies have also shown low intra-individual within session CVs for breath acetone concentration (1.6% - 2.6%)[168], but significant intra-day (36%) and inter-day (15%) variation[167,168], as well as inter-individual variation (38%)[105,167,168,196,238]. Exhaled breath acetone concentration appears to correlate better with arterial than peripheral venous blood concentration[115].

As described in Chapter 1.3.6., exhaled acetone concentration appears to be dependent on breath volume but not expiratory flow rate[159,168]. A difference in breath acetone concentration between children and adults[204] was not seen in an earlier study[203], and some studies have reported higher breath concentration in men than women[167,205], independent of height, weight and diet[205]. The observed

differences may be due to differences in energy consumption[167] or may be attributed to discrepancies in exhaled lung volume[168]. No significant difference in acetone concentration was seen when nasal and oral exhalations were compared[150,222,239] and cigarette smoking does not affect breath acetone concentration[183,190].

1.5.3. Physiological and pathophysiological changes in exhaled acetone concentration

For a long time it has been known that fasting causes a rise in acetone levels in blood, urine and breath[230,232]. Crofford et al.[230] reported a linear relationship between the concentration of acetone in breath and blood during fasting. A more recent SIFT-MS study in healthy volunteers showed elevated breath acetone concentration after an overnight fast[194], with a fall to nadir within five hours of ingestion of a protein-calorie meal. Acetone seems to be formed more slowly in obese compared to non-obese individuals during fasting[230,232], yet there is no correlation between breath acetone concentration and BMI[167,204].

Exercise results in a small increase in breath acetone concentration, as has been described in Chapter 1.3.6., believed to be due to the breakdown of fat and carbohydrate as energy sources up to anaerobic threshold[171,178]. It should be noted, however, that the volunteers in the study by King et al.[171] had high baseline acetone concentrations and had been starved for at least seven hours, so changes in breath acetone concentration may have been due to ongoing fasting.

Breath acetone has been used to approximate blood glucose[240] or plasma ketone level[195,240] and has been proposed as a useful non-invasive method of monitoring response to treatment in diabetes mellitus, however a relationship between breath acetone and blood glucose concentrations has not always been seen[205,226]. Lee et al.[241] found breath acetone concentration alone did not accurately predict blood glucose level, however when used in conjunction with other breath markers of glucose metabolism (ethanol, methyl nitrate and ethylbenzene) the accuracy of prediction was increased.

Differences exist between glucose metabolism in healthy subjects and those with diabetes mellitus. In various studies, patients with diabetes mellitus have been reported to have higher[238,242,243] or similar breath acetone concentrations[203,226] when compared to healthy individuals. Unfortunately, it was not always clear whether patients had Type 1 or Type 2 diabetes[238], whether patients were fasted or not[243], and the blood glucose level at the time of breath analysis was not always measured[242], making the data difficult to evaluate. In any case, the difference in breath acetone concentration between patients with diabetes mellitus and healthy controls appeared to be smaller than the variation of breath acetone concentration seen in healthy subjects due to the time of day or change in diet[196]. Some individuals with Type 1 diabetes were mildly ketotic despite normal blood glucose concentrations[243], which, as previously mentioned, may help to identify early loss of glucose control[236].

In patients with Type 1 diabetes, an insulin induced reduction in blood glucose has been associated with a reduction in breath acetone concentration, possibly because insulin has more of an effect on ketogenesis than glucose concentration[226]. In contrast, in healthy individuals a reduction in breath acetone concentration following ingestion of glucose as part of an oral glucose tolerance test (OGTT) has been associated with an initial increase followed by a reduction in blood glucose concentration[225,240]. Breath acetone concentration during OGTT could not distinguish between patients with diabetes, impaired glucose tolerance and healthy controls[238]. A correlation between breath acetone and grouped glucose and grouped haemoglobin $A1_C$ concentrations was not present when glucose and haemoglobin $A1_C$ were used as continuous variables[243]. These negative findings were confirmed in a later study in patients with Type 2 diabetes[205].

The detection of acetone in breath has been used to monitor the rate of dietary fat loss[244], to check for ketosis when using ketogenic diets for the treatment of epilepsy associated with intractable seizures[195], and to investigate metabolic stress during cardiac surgery[105] and congestive cardiac failure[245]. During cardiac surgery, breath acetone increased slightly after sternotomy and significantly after cardiac bypass, mirroring plasma CRP and troponin levels[105]. In individuals with congestive cardiac failure, breath acetone levels

were elevated compared to controls, and much higher in the subgroup with marked signs of right heart failure. It was suggested that elevated breath acetone was due to a combination of elevated stress hormones, increased metabolic rate and malnutrition[245].

Breath acetone has been measured in ventilated patients in the ICU[5,7,8] with no significant difference in acetone concentration seen in studies using whole breath compared to CO_2-controlled breath sampling[7]. Exhaled acetone concentration appeared to be higher in patients with ARDS than those who did not meet the criteria, although there was no significant difference in concentration when patients with and without pneumonia were compared[5]. It is important to note that the inter-individual CVs in this study were high. Acetone concentration in blood was lower in patients with sepsis than post-operative patients, although exhaled acetone concentration was not significantly different between the two groups[8]. It was suggested that the discrepancy in blood levels was due to septic patients being fed and receiving insulin to control blood glucose, whereas surgical patients had been starved prior to their operation. This does not completely explain, however, why there was no significant difference in breath acetone concentration.

The measurement of breath acetone concentration does not appear to be a surrogate for blood glucose monitoring, but has the potential to evaluate glucose metabolism and starvation, by way of ketosis, and the degree of metabolic stress in critical illness or due to surgery. Elevated levels may indicate reduced response to insulin treatment.

1.6. Hydrogen Sulphide

1.6.1. The metabolism and biological role of hydrogen sulphide

H_2S is a colourless gas with a distinctive smell of rotten eggs. The human nose can detect H_2S down to levels as low as 100 ppb[246], however at higher concentrations of around 50 ppm it causes inflammation and irritation of mucous membranes[246,247], and at 250-500 ppb causes severe irritation of the respiratory tract and the development of pulmonary oedema[246]. At atmospheric

concentrations over 700 ppm, inhalation of H_2S is fatal within minutes, due to inhibition of cytochrome c oxidase and therefore cellular respiration[246-249].

The majority of H_2S is metabolised via a series of oxidation steps, mainly occurring in mitochondria, to sulphate and thiosulphate, which are excreted via the kidney[250,251]. Activated neutrophils can also oxidise H_2S[252]. It binds strongly to and can be scavenged by methaemoglobin (metHb)[249]; haemoglobin acts as a common "sink" for H_2S, NO and carbon monoxide (CO)[247,253]. The administration of hyperbaric oxygen and nitrate in the treatment of H_2S poisoning are intended to increase the rate of oxidation and the formation of metHb, respectively, preventing binding to cytochrome c oxidase[254].

H_2S has been proposed as the third gaseous inflammatory mediator after CO and NO[253]. Its formation from amino acids L-cysteine, L-cystathionine, or L-homocysteine, is catalysed by the pyridoxal-5'-phosphate-dependent enzymes cystathionine-γ-lyase (CSE), cystathionine-β-synthase (CBS) and, to a lesser extent β-mercaptopyruvate[255] (Figure 1-4). The expression of these enzymes is tissue specific, for example CBS is mainly present in the brain[253,256,257] and CSE predominant in vascular tissue[253,258,259] and liver[252,260,261]. The rate of H_2S production differs across organs, higher in the liver compared to the brain at all substrate concentrations, and under anaerobic compared to aerobic conditions[262]. At least partly via a direct effect on K_{ATP} channels, H_2S can cause smooth muscle relaxation (resulting in vasodilatation, possibly bronchodilatation, and regulation of peristalsis) and a reduction in neuronal excitability, providing a neuromodulatory effect with involvement in learning and memory[253,263].

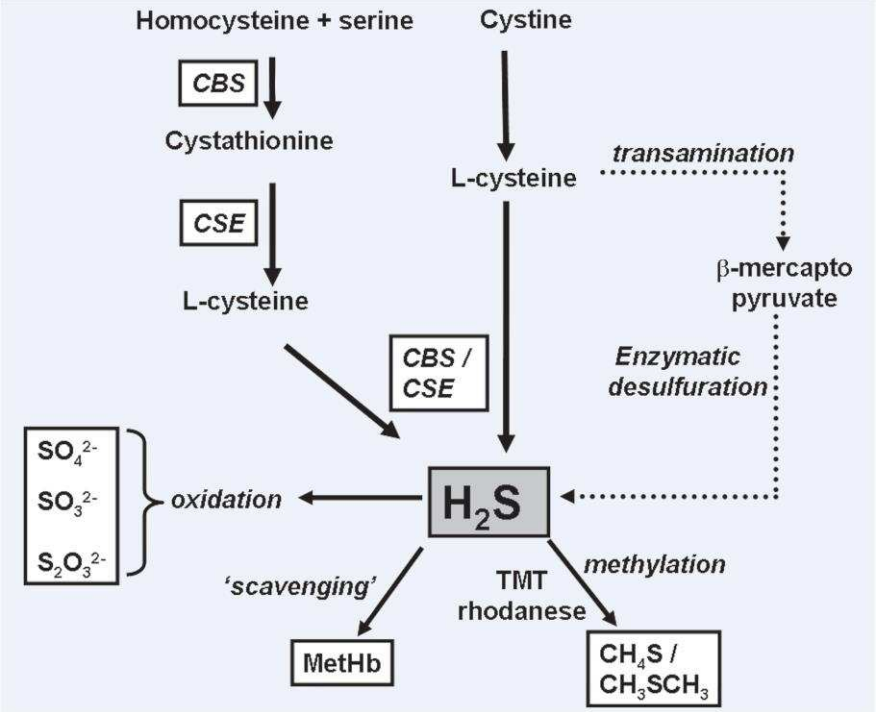


Figure 1-4. Main pathways of H_2S synthesis and removal[255].

There is conflicting evidence in the literature around whether H_2S acts as a pro- or anti-inflammatory mediator. Its role in the inflammatory process is not clear; it seems to influence leucocyte function, it may modulate the activity of NO and appears to be involved in the release of cytokines and chemokines, such as substance P, calcitonin gene-related peptide and neurokinin A[248], at least partly via the NF-κB pathway[264,265]. A protective function has been suggested because by reacting with several cytotoxic species, such as perioxynitrite, hypochlorite, H_2N_2, O_2^- and NO, H_2S can prevent oxidant-related cell death[255].

H_2S is a weak acid and at physiological pH readily dissociates (reaction equation below), with around 18.5% remaining as H_2S and 81.5% present as HS^-[255].

$$H_2S \leftrightarrows HS^- + H^+ \leftrightarrows S_2^- + 2H^+$$

It is therefore unclear whether H_2S, the hydrosulphide ion[266] or sulphite is responsible for any effects seen[252]. In in vitro and live animal experiments, variable results have been produced by differences in the choice and dose of H_2S donors and the concentration of CSE and CBS inhibitors[266]. The specificity of CSE and CBS inhibitors has been questioned, as well as the comparability of the effects of a bolus of H_2S produced by the administration

of a common donor (NaHS or Na_2S) and the endogenous release of H_2S which is likely to be slower[266]. High experimental concentrations of H_2S may inhibit cytochrome c oxidase, but at physiological concentrations could act as a substrate for the respiratory chain[251]. As a result of these problems, a newer H_2S donor, GYY4137, has been developed, causing a slower and more sustained vasodilatory effect on rat aortic tissue than NaHS[267] and may, therefore, produce a more physiological change in H_2S concentration.

Most interest has been focussed on the effects of H_2S on the vasculature. Bacterial endotoxins appear to cause vasodilatation and hypotension by activation of K_{ATP} channels, and it has been proposed that H_2S causes hypotensive shock via the same pathway[266]. H_2S causes vasodilatation of rat aortic tissue in vitro, with and without an intact endothelium, implying a direct action on smooth muscle cells via K_{ATP} channel modulation[258]. Injection of an H_2S donor causes vasodilatation and subsequent hypotension in rats in vivo[258,267], with the administration of GYY4137 attenuating the development of chemically induced hypertension[267].

Elevated plasma H_2S is seen in animal models of haemorrhagic shock[259], endotoxic shock[252,260,261] and pancreatitis[268], and a small study of sepsis in humans[261]. By blocking CSE activity it is possible to cause a partial reduction in the degree of hypotension[259] and reduce plasma H_2S elevation and multi-organ inflammation[260,261,268]. This suggests that organ damage associated with sepsis is not due to hypotension alone, and that the effects of H_2S in septic shock are independent of its potential role in blood pressure regulation[260]. Injection of NaHS in mice produced similar effects to those of endotoxic shock, with increased myeloperoxidase (MPO) activity, neutrophil accumulation identified histologically, and increased levels of TNFα[261].

In contrast, administration of GYY4137 to anaesthetised rats in a model of endotoxic shock attenuated the developing hypotension, inhibited TNFα production and reduced the rise in iNOS, NF-κB activation and other measures of inflammatory cascade activity[269]. In the same model but using conscious rats, GYY4137 administration reduced the rise in pro-inflammatory cytokines and CRP, reduced the rise in pulmonary MPO activity, increased the

production of anti-inflammatory IL-10 and reduced lung tissue damage. It is possible, therefore, that the role of H_2S at physiological concentration is anti-inflammatory.

Oxygen concentration appears to modulate the effects of H_2S on in vitro animal studies; the same H_2S concentration that mediated aortic contraction at high oxygen concentration caused relaxation at lower oxygen levels, including physiological concentration[270]. Exogenous H_2S caused the same vasoactive effects as hypoxia in isolated vascular tissue of rats[270], cows and sea lions[271]. In renal tissue, improvements in medullary blood flow have been linked to hypoxia-induced H_2S formation[272]. H_2S metabolism, therefore, appears to be important in oxygen sensing pathways[271,272]. Progression of renal disease in rats corresponded with a reduction in plasma H_2S concentration, due to a reduction in CBS and CSE expression in kidney and liver tissue[272].

At physiological concentration, H_2S appears to exert anti-inflammatory effects in the brain and gut, hypothesised to be as a consequence of its influence on the regulation of gastric[273] and cerebral[256] microcirculation. Animal models of gastric mucosal damage demonstrated a reduction in plasma H_2S and CSE expression, inflammatory changes improving on administration of NaHS, with an associated attenuation in TNFα level and leucocyte adherence in mesenteric venules[273]. An in vitro study by Whiteman et al.[257] demonstrated the inhibitory effect of H_2S on hypochlorous acid-induced oxidative stress in brain tissue. In Alzheimer's disease, human brain tissue contains significantly less H_2S than expected and showed evidence of oxidative stress[256].

Several studies have examined the effect of H_2S on insulin secretion. CBS is expressed in the whole of the mouse pancreas but CSE is mainly seen in exocrine pancreatic cells[274,275]. In in vitro animal models of insulin-secreting cell lines, increased H_2S concentration, due to NaHS or L-cysteine administration or stimulation of CBS, caused a reduction in insulin secretion[274,276], via opening of K_{ATP} channels[274,277]. These effects were reversed by CSE[277] and CBS inhibition and the administration of glibenclamide[274], a drug used to treat diabetes mellitus and known to block K_{ATP} channels, suggesting that H_2S modulated the secretion of insulin via K_{ATP} channel

activity[274]. Hyperglycaemia was associated with a reduction in H_2S production, increased insulin secretion[277], but a slight increase in CSE expression[275]. Hyperglycaemia-related islet cell apoptosis was suppressed by the addition of NaHS, therefore it was suggested that H_2S may be involved in the mechanism by which pancreatic β-cells are protected from glucotoxicity in diabetes[275].

In diabetic rats, increased pancreatic and hepatic H_2S production was associated with greater CBS expression in the pancreas and CSE and CBS expression in liver, when compared to non-diabetic animals[278]. Insulin administration restored these changes, but resulted in higher plasma H_2S concentration compared to diabetic rats and controls, the cause of which is unclear[278]. H_2S also appears to modulate glucose uptake in rat adipose tissue[279]. Endogenous formation of H_2S due to CSE activity in rat adipose tissue caused a reduction in basal and insulin-mediated glucose uptake in adipocytes, which was reversed by CSE inhibition. As previously described in pancreatic tissue[277], high glucose concentration was associated with lower H_2S concentration in adipose tissue[279]. Inhibition of glucose uptake by H_2S appeared to involve the PI3K rather than the K_{ATP} channel pathway[279].

In humans, plasma samples from male subjects with non-insulin dependent Type 2 diabetes mellitus (median concentration 10.5 µmol/L) were lower than those from BMI-matched overweight subjects (22.0 µmol/L) and lean men (38.9 µmol/L)[280]. There was a negative correlation between H_2S concentration and blood pressure, microvascular function, glycaemic control and insulin sensitivity. Waist measurement and other measures of adiposity were independent predictors of H_2S concentration, which may be confounded by peripheral insulin sensitivity[280].

1.6.2. Hydrogen sulphide and airways disease

H_2S, via the addition of NaHS, has been demonstrated to cause tracheal smooth muscle cell relaxation, but had no effect on muscle tension when added to cells that had not been pre-contracted[281]. In isolated airway smooth muscle cells from resected or donor lungs, the administration of both NaHS and GYY4137 resulted in inhibition of both cell proliferation and pro-inflammatory IL-8 release[282]. By blocking CBS but not CSE activity, the effects of H_2S were

reversed and further inflammation was seen, hence the effects of H_2S seem to be anti-inflammatory and dependent on CBS activity[282].

Acute lung injury (ALI) in the oleic acid-induced rat was associated with reduced plasma and pulmonary H_2S concentration[283]. Injection of NaHS reduced the degree of pulmonary injury and inflammatory cell infiltrate, and caused an elevation in the level of the anti-inflammatory cytokine IL-10[283]. Similar effects were also observed in a murine model of ALI associated with burns and smoke inhalation, where there was an associated reduction in mortality and improved survival rate[284]. These results differ from those of Bhatia et al.[268], who saw elevated plasma H_2S in a rat model of pancreatitis and ALI. In their experiment, the addition of a CSE-inhibitor reduced the degree of lung inflammation and MPO activity.

The effects of administering NaHS and a CSE blocker were similar in animal models of asthma-related[285] and cigarette-exposure-related lung inflammation[281]. Administration of NaHS led to a reduction in the previously induced lung inflammation, whereas injection of a CSE blocker further aggravated airway hyperresponsiveness. Injection of NaHS in ovalbumin (OVA)-treated rats caused a reduction in iNOS activity, indicating that H_2S may modulate airway inflammation via interactions with the NO pathway[285]. The administration of NaHS in control rats had no effect on pulmonary function, lung pathology or cell counts in BAL fluid[285]. There were some important baseline differences between the two studies; lung tissue and serum from OVA-treated rats contained lower H_2S concentration, in association with reduced CSE expression, when compared with controls[285], however in cigarette-exposed rats lung CSE expression and plasma H_2S were higher than controls[281]. In the asthma model, serum and lung tissue H_2S concentrations negatively correlated with inflammatory cells (neutrophils and eosinophils) in BAL fluid[285]. The difference in CSE expression and production of H_2S between the two models of lung inflammation may be in the chronicity of the exposure; OVA-treated rats may show an acute inflammatory response, whereas rats exposed to cigarette smoke daily for four months may display a pattern of chronic inflammation.

Stable patients with asthma have been observed to have lower serum H_2S concentrations than controls, the lowest concentrations seen in patients with severe acute exacerbations (reviewed[254,286]). There was a positive correlation between H_2S concentration and severity of airways obstruction, and a negative correlation with sputum cell count and neutrophilia. Serum H_2S was lower in smokers than non-smokers in all groups. The authors hypothesised that airway H_2S production in asthma causes inflammation and bronchoconstriction, however it is difficult to determine whether lower serum H_2S in asthma patients is the cause or consequence of the condition.

In contrast, studies of H_2S in patients with COPD revealed higher serum concentrations when compared to controls, however worsening lung disease was associated with a reduction in H_2S concentration, including during acute exacerbations. An inverse relationship was seen between H_2S and sputum neutrophil count, but positive correlation between H_2S and sputum lymphocyte and macrophage levels and lung function, as measured by forced expiratory volume in 1 sec (FEV_1). The effect of smoking on serum H_2S was not consistent in the two studies, with significantly lower H_2S in smokers compared with non-smokers in the first study and no significant difference in the second[287,288]. Treatment with theophylline for one month did not affect serum H_2S concentration, however it was associated with a reduction in sputum neutrophil count[288]. It was concluded that higher H_2S levels were protective, with reduced inflammatory cells in sputum and increased smooth muscle-related bronchodilation[287,288].

A further study of serum H_2S and airways disease focussed on the relationship with pulmonary infection[289]. No significant difference in H_2S concentration was seen between patients with acute exacerbations of COPD and controls. Acute infection was associated with significantly lower H_2S concentration in those with pneumonia versus controls and those with infective exacerbations of COPD compared to non-infective exacerbations. There was a weak inverse relationship between serum H_2S concentration and CRP.

1.6.3. Hydrogen sulphide in breath

The administration of intravenous Na_2S resulted in a dose dependent increase in H_2S concentration, measured by a sulphide gas detector, in the exhaled breath of mice[290] and humans[291]. The concentration of exhaled H_2S increased rapidly after commencement of the infusion with an equally rapid reduction when the infusion was stopped[291]. Although less than 1% of the total amount of sulphide injected was removed via the lungs[291], it still seems possible that increased concentrations of H_2S in exhaled breath could be used as a marker of elevated plasma H_2S, as long as changes in breath could be measured at the ppb level. The quantification of H_2S in humid air by SIFT-MS has enabled the use of this technique for the detection of H_2S in human breath[217].

Oral bacteria produce H_2S, along with other sulphur compounds, which is detectable in human breath[292]. Several methods have been employed in an effort to reduce the concentrations of these sulphur compounds that cause halitosis, the most effective being rinsing the mouth with hydrogen peroxide solution[292]. When measuring H_2S in exhaled breath, levels were found to be up to ten times higher in oral than nasal samples, although this was a small study of only two subjects and it was unclear whether breath samples were taken following mouth or nose breathing[149]. By first rinsing the mouth with hydrogen peroxide solution and then performing a nasal exhalation, contamination by oral H_2S can be avoided[223]. H_2S is present in small quantities in cigarette smoke[187] but there is no evidence that it is detectable in the breath of smokers.

End-expiratory breath samples were collected orally from healthy volunteers, after a 15 sec breath hold with the mouth wide open, and analysed by a gas chromatograph equipped with a chemiluminescence sulphur detector. Results for exhaled H_2S were only slightly higher than concentrations in ambient air[262]. These results are mirrored by those from nasal exhalations in experiments by Dummer[223] using a SIFT-MS device. The concentration of H_2S in exhaled breath correlated with the ambient air, thus the possibility of contamination by inspired air could not be ruled out. It was, therefore, not possible to identify whether exhaled flow or volume had any effect on H_2S concentration[223].

When examining H_2S in breath as a potential marker of inflammation in airways disease, Dummer[223] found no significant difference between mean-exhaled or end-exhaled breath concentrations in subjects with COPD or asthma versus controls. There was a significant negative correlation between H_2S and sputum neutrophil count in COPD patients and a positive correlation between H_2S and markers of eosinophilic inflammation in asthmatic patients, although neither relationship reached statistical significance.

Two studies of breath H_2S have been performed in other inflammatory states, revealing higher breath concentrations in chronic pancreatitis compared to healthy controls[293] and lower concentrations in lung transplant recipients with evidence of acute rejection compared to those with stable lung function, although the difference did not reach statistical significance[294]. Unfortunately, it was not possible to distinguish patients with disease from their respective controls using breath H_2S results due to concentration overlap between the groups[293,294].

If H_2S behaves in the same way as NO, it is possible that pulmonary infection will result in higher breath concentrations as a result of local production in the airways, as seen in two previous studies of exhaled NO in ventilated patients[117,118].

These findings suggest further studies are required to investigate whether H_2S could act as a biomarker of airway inflammation. The relationship between breath and serum H_2S requires further exploration as it is possible that breath H_2S may act as a marker of systemic inflammation as well.

1.7. Hydrogen Cyanide

1.7.1. Hydrogen cyanide exposure and its biological role

At physiological pH and temperature, cyanide is largely found as volatile HCN gas[295] which has a bitter almond smell. Its toxicity is well known, being lethal if inhaled at concentrations over 300 ppm[296] and causing hypoxia and collapse at lower concentrations[297]. Its toxic effects are caused by the inhibition of aerobic respiration in mitochondria by the competitive inhibition of

cytochrome c oxidase[249,295,298]. Despite its toxicity, HCN has been found in small quantities in blood[299] and breath of healthy individuals[300].

HCN is commonly detected at concentrations above 1,000 ppm in domestic fires, produced by the combustion of nitrogen-containing materials in solid and upholstered furniture and bedding[297]. Death probably results from rapid collapse (only requiring 2 min exposure at 200 ppm) preventing escape and allowing further exposure to poisonous levels of HCN and CO[297]. Blood cyanide levels in fire victims are usually low[297], possibly because over 98% is bound to metHb[298,299,301] and that remaining in the plasma disperses into tissues[297] where it is detoxified by conversion to thiocyanate[191,301]. It is unbound HCN in plasma that causes toxic effects[301]. Antidotes for cyanide poisoning induce methaemoglobinaemia in the belief that the strong affinity of metHb for HCN[249,298,299,301] will prevent it binding to cytochrome c oxidase[249,297,298].

HCN is found in cigarette smoke[123,186-188] and in higher concentrations in the breath[123,192,301], saliva[191] and blood of smokers than non-smokers[191,301]. The detoxification of HCN to thiocyanate results in higher plasma[191,301] and salivary thiocyanate[191] concentrations in smokers than non-smokers, allowing distinction between the two groups[191]. Plasma thiocyanate concentrations decrease slowly, taking up to 14 days to return to normal after smoking cessation[301], however a study of volunteers breathing HCN at 10 ppm for 1 min (equivalent to smoking one cigarette), showed that the half-life in exhaled breath was very short, only 10-22 sec[302]. This led the authors to conclude that even at fatal inspired concentrations the exhaled concentration of HCN should be low, therefore high breath concentration may indicate systemic intoxication.

Oral exposure to HCN in food is not uncommon as many plants, particularly food crops, are cyanide producing, such as maize, rice, wheat, barley, rye, oats, apples, sugar cane and cassava. This property is believed to protect the plants against pests and ward off herbivores. Humans have overcome the problem by pre-processing food and detoxifying any HCN consumed by the activity of sulphurtransferases in the gut. These enzymes require sulphur-containing amino acids, which is why low protein diets in countries where large quantities

of high HCN-containing crops, particularly cassava, are eaten can lead to chronic HCN poisoning[303].

Leucocytes contain thiocyanate, MPO and hydrogen peroxidase, mixtures of which in other settings have been seen to contain HCN[304], therefore the observed production of HCN within these cells is plausible. Under physiological conditions, thiocyanate has been found to be a major substrate for MPO[305] and eosinophil peroxidase[306], although the main product of these reactions was hypothyocyanite rather than HCN, which is believed to play an antimicrobial role in the oral cavity[307]. Further studies have confirmed the production of HCN by phagocytosing polymorphonuclear lymphocytes in the presence of Staphlococcus epidermidis and Escherichia coli[308]. Via the $MPO/H_2O_2/Cl^-$ system, more HCN was produced by the chlorination of S. epidermidis than E. coli and this was explained by a larger amount of the substrate glycine in S. epidermidis cell walls. HCN production was augmented following damage to S. epidermidis cells by a sublethal dose of penicillin G[308]. In subsequent experiments, Stelmanszynska showed that, in the presence of S. epidermidis, leucocytes can produce cyanide from thiocyanate, again via the $MPO/H_2O_2/Cl^-$ system. Only minute quantities of HCN were formed, however, by leucocytes without bacterial stimulation[309]. The biological role of HCN itself is still unclear, although it appears to stimulate the "respiratory burst" during phagocytosis[310] and may therefore have a role in the inflammatory process.

1.7.2. Hydrogen cyanide production by Pseudomonas aeruginosa

P. aeruginosa is a cyanogenic bacterium producing HCN from glycine, catalysed by the enzyme HCN synthase associated with its cell membrane. The function of this phenomenon is not fully understood[295], however by excluding other microorganisms it may improve its chances of colonisation[311]. P. aeruginosa is not affected by high HCN concentrations itself, having developed a respiratory chain that is not inhibited by HCN and employing several mechanisms to detoxify its environment[311]. P. aeruginosa produces many other VOCs[218,312] however the production of HCN[311] and 2-

aminoacetophenone[111] appear to be almost unique to this organism, potentially allowing its identification.

P. aeruginosa is an opportunistic pathogen, colonising and causing infection in patients with chronic lung diseases, particularly those with CF, and immune system compromise. It is one of the most common nosocomial pathogens and commonly causes VAP (reviewed[313]). The presence of P. aeruginosa in the CF lung is associated with poorer lung function and increased mortality[314], therefore its detection is crucial in order to delay CF lung disease progression[315] by modifying treatment and attempting eradication.

Four different methods of detection of HCN or the cyanide ion in microbiological samples have been employed in previous studies; detection of HCN in the headspace above solid or liquid culture media by SIFT-MS[218,312,316] or GC-MS[312], detection of the cyanide ion in sputum[314] and BAL samples[315] using a cyanide ion selective electrode, and by liberating cyanide from sputum by acidification followed by cyanide assay test[317].

HCN is more consistently detected in the sputum of P. aeruginosa positive patients than in non-infected individuals[314,315,317]. It is not always detected in infected samples[314], however, and can be detected in those that do not culture P. aeruginosa[315,317]; non-infected samples contained HCN at lower concentrations than infected samples[315]. Two studies show conflicting results in terms of correlation of cyanide concentration in sputum and lung function[314,317]. In the study by Ryall et al.[314], the cyanide positive group showed significantly lower FEV_1 and forced vital capacity (FVC) which could not be explained by age, sex or P. aeruginosa sputum load. Stutz et al.[315] proposed that HCN production was related to pulmonary inflammation rather than infection with P. aeruginosa because their study of 226 BAL samples from 96 children with CF reported an association between cyanide concentration and neutrophil count. The relationship between HCN and poorer lung function[314] may also be explained by greater levels of neutrophil inflammation. In the CF lung, both neutrophils and Pseudomonas species may be producing HCN, therefore the relative effects of HCN from both of these sources is unknown[307].

The use of antibiotics may affect the quantity of HCN produced by P. aeruginosa in the CF lung. In the study by Sanderson et al.[317], the administration of intravenous antibiotics to treat P. aeruginosa chest infections was followed by a reduction the concentration of HCN to undetectable levels in the sputum of four of seven cases and the highest concentration was seen in a sputum sample from a patient with chronic P. aeruginosa infection who was not taking antibiotics. It was not clear whether decreased HCN production was related to a reduction in bacterial load, the level of pulmonary inflammation, or a direct effect of antibiotics on HCN production.

A study by Gilchrist et al.[311] revealed higher concentrations of HCN in sputum samples containing non-mucoid compared to mucoid strains, a study contradicted by Stutz et al.[315] who found higher HCN concentrations in association with mucoid strains. Another study of several P. aeruginosa strains showed a difference of up to two orders of magnitude in the concentration of some VOCs produced, and that culturing P. aeruginosa on solid media produced higher concentrations of HCN than when the same sample was cultured in liquid media[312]. This suggests the difference in HCN concentration between mucoid and non- mucoid strains between the two studies could be the result of differences in culture media, or duration of incubation. It is, however, clear that the production of HCN by different strains of P. aeruginosa is variable, which probably explains why the production of HCN does not appear be related to the concentration of P. aeruginosa[314].

1.7.3. Hydrogen cyanide in breath

HCN is soluble with a blood:gas partition coefficient greater than 100, thus it is likely to behave like acetone and exchange predominantly in the airways[302,318].

HCN can be detected in breath after the administration of sodium nitroprusside as an anaesthetic agent. Breath concentrations follow changes in plasma concentration, with a linear relationship between $\log_{10}$ breath and plasma concentrations[301].

HCN can be identified in orally exhaled breath of healthy subjects[203,319] at levels around 50 times higher than would be expected from plasma levels[319].

Higher concentrations of HCN are found in orally compared to nasally exhaled air[150,223,239,318], the highest concentrations being found in the oral cavity[150]. In order to investigate the source of HCN in the oral cavity, Lundquist et al.[319] performed in vitro experiments on saliva, demonstrating the formation of cyanide in aerated saliva at 37°C, enhanced by the presence of glucose. Further heating prevented cyanide production, implying enzymatic involvement, in agreement with previous experiments showing catalysation by salivary peroxidase[304]. A similar process is responsible for HCN production in the lung, catalysed by lactoperoxidase in the healthy lung and MPO in the presence of pulmonary inflammation[307].

HCN produced in the mouth can contaminate nasally exhaled breath, so for accurate HCN quantitation it has been suggested that nasal breathing followed by a nasal exhalation should be performed[151]. Oral, but not nasal, breath HCN concentration is increased after eating and drinking, implying HCN generation in the mouth[318].

Variation in HCN concentration at low levels in ambient air does not appear to affect exhaled concentrations[151], however higher inspired concentrations result in higher exhaled concentrations[302]. Exhaled breath volume has no effect on HCN concentration[151], however, due to low concentrations of HCN in the exhaled breath of healthy subjects and a correlation with ambient air, it was not possible for Dummer[223] to fully examine the effects of exhaled flow rate on physiological concentrations of breath HCN. Due to differences in breath sample collection (oral versus nasal, mixed versus end-exhaled breath) and analysis, there is discrepancy in the literature around the effects of gender, age and oral intake on breath HCN concentration[200,318,320,321].

In studies by Dummer[223], the concentration of exhaled HCN in patients with COPD and asthma was not statistically different when compared to healthy individuals. A negative correlation existed between HCN in breath and sputum neutrophil count in the COPD group and a positive correlation between HCN in breath and sputum eosinophil count in asthma, neither of which reached statistical significance. Larger studies are needed to further examine whether there is a true relationship between these markers. The role of HCN in the

inflammatory process is not fully understood, both in localised airway inflammation or systemic inflammation, and this requires further studies in the clinical field.

Three SIFT-MS studies have been performed to assess the utility of breath HCN as a marker of P. aeruginosa colonisation or infection. Significantly higher breath HCN concentrations were seen in children with CF and known P. aeruginosa colonisation when compared to children with asthma[110], but oral exhalations were performed in this study, which must be borne in mind when interpreting the results. In a recent study of 20 adults with CF, oral breath HCN concentrations were similar in P. aeruginosa colonised and non-colonised subjects, but higher median concentrations were observed in nasally exhaled breath of those with P. aeruginosa (11 ppb versus 0 ppb)[239]. The two groups could not be separated by breath HCN concentration, however, due to considerable overlap between the two groups; four subjects with chronic infection had nasal breath concentrations lower than 5 ppb and two without infection had nasal breath concentrations greater than 10 ppb[239]. In another recent study of 30 adults with bronchiectasis, including three with CF, there was a small but significant difference in nasal end-exhaled HCN concentration between those with bronchiectasis and healthy controls (median 3.7 ppb versus 2.0 ppb), but no difference in breath concentration between patients colonised with P. aeruginosa and those who were not[151]. These results reveal that, at present, despite the known production of HCN by P. aeruginosa it cannot be used as a reliable breath marker for the presence of this bacterium in the lung.

1.8. Hypotheses and aims

There are two main hypotheses to be explored in this thesis. Firstly, that increased metabolic and oxidative stress in critical illness, as well as the development of stress hyperglycaemia, leads to increased breath acetone concentration. It is believed that elevated breath acetone concentration, despite adequate insulin therapy for hyperglycaemia, indicates an increased risk of developing further organ failure. Secondly, that exhaled H_2S and HCN are elevated in the presence of infection and inflammation in this pro-inflammatory state. If these compounds are detectable in breath, there is uncertainty around

whether they originate in the airway or are due to systemic production. The utility of these VOCs for the diagnosis of pneumonia in intubated and ventilated patients is explored.

The aims of the thesis are:

- To evaluate the efficacy of the breath sampling apparatus and Tedlar bags for the collection and storage of acetone, H_2S and HCN in breath. This is necessary to confirm the suitability of these materials for subsequent parts of the study, enabling causes of sample loss and contamination to be identified and quantified, if possible.
- To develop an off-line end-exhaled breath sampling technique for use in intubated and ventilated patients in the ICU, ensuring that it is repeatable and will provide reproducible results.
- To develop a breath sampling technique for use in anaesthetised intubated patients, comparable to that used in the ICU. The rationale for this aim was to develop a technique that allowed samples to be collected from a healthy control group and to explore how altering anaesthesia machine settings might alter the concentration of VOCs collected.
- To explore changes in breath acetone concentration over time in two groups of patients in the ICU; patients with stress hyperglycaemia and those with new pulmonary infiltrates on CXR. Also, to assess the utility of breath acetone as a marker of illness severity and clinical outcomes. The rationale for this aim is that if acetone is a marker of oxidative and metabolic stress, seen as an alteration in glucose metabolism[105,245], then it should correlate with conventional measures of illness severity.
- To explore specifically the variation in breath acetone concentration with insulin administration and feeding, and to assess whether it could be used to guide insulin or feeding regimes.
- To investigate any change in concentrations of H_2S and HCN over time in ICU patients with new pulmonary infiltrates on CXR and to correlate these changes with clinical parameters, as well as assessing the utility of these VOCs for the diagnosis of pneumonia.

- To examine the effects of factors known to influence exhaled VOC concentration, for example breathing pattern[152,157,163,168] and inspired VOC concentration[181,182].
- To investigate any relationship between breath and serum VOC concentrations.

2. The effect of breath collection apparatus and sample storage on breath analyte concentration

2.1. Introduction and aims

It is not always possible for an individual to travel to a SIFT-MS instrument and, due to its size, weight and infection control issues, the instrument cannot always be moved to an individual, therefore breath analysis may have to be performed off-line. Appropriate breath collection equipment and containers are thus required for the collection, storage and transport of breath samples in order to preserve their integrity.

Volatile organic compounds (VOCs) behave differently in different containers, thus it is important to understand how the compounds of interest will behave in a chosen container. When stored in polymer bags, all breath samples are affected by a degree of volatile adsorption onto the bag's inner surface, diffusion out of the bag and contamination from pollutant diffusion into the bag and from compounds emitted by the bags themselves[134,135,137-140]. Effective bag cleaning is necessary to prevent analytes from one sample causing contamination of subsequent samples[134,138,140].

It is important to consider whether other components of the breath collection equipment might release or absorb volatile gases and, therefore, affect sample composition prior to analysis. In order to compare breath VOC concentrations across different studies, the effect of different equipment and sampling processes on sample concentration must be investigated.

The aim of this part of the study was to evaluate any change in analyte concentration and humidity of breath samples due to the breath collection apparatus and storage in incubated Tedlar bags. It was important to use breath in the experiments wherever possible, rather than humid gas mixtures, to enable changes in physiological concentrations of VOCs and humidity to be explored. The process of bag cleaning will be described and evaluated.

2.2. Methods

2.2.1. Bag cleaning

A standard cleaning procedure was established at the start of the study. Transparent 1 L Tedlar bags with single polypropylene hose/valve fittings (film thickness: 50 μm) (SKC Inc, USA) were employed in all sections of this thesis. Bags were filled with medical air from the wall supply, left for over an hour then evacuated. This process was repeated three times, with the bags being left full of air overnight at least once. Bags were refilled a fourth time for analysis of the contents by SIFT-MS. Bags were not heated. Bags containing VOCs at concentrations less than 110% of medical air supply concentrations were accepted as clean. 'Dirty' bags, containing higher VOC concentrations, were flushed and analysed again until concentrations were no more than 110% of medical air concentrations. For all other parts of this thesis, clean Tedlar bags were used unless otherwise stated.

2.2.2. The effect of sample storage on analyte concentration and humidity

Six Tedlar bags were filled with breath by healthy members of laboratory staff exhaling directly into them and each was analysed immediately by SIFT-MS. Bags were then incubated at 40°C (MIR-162, Sanyo Electric Co. Ltd, Japan) to prevent condensation of the humid samples. Higher temperatures were not used in order to avoid potential bag wall damage and impairment of storage integrity[242]. The contents of each bag were analysed by SIFT-MS at 30 min intervals for two hours, then hourly for another two hours. The concentrations of acetone, H_2S and HCN were calculated along with a measure of the H_3O^+ precursor and hydrated ion counts. The concentration of Tedlar specific contaminant compounds was assessed. Each bag was removed from the incubator for the duration of analysis only, then immediately returned. Bags were stored in the incubator for a total of four hours, because breath samples were unlikely to need longer storage periods before analysis in subsequent parts of this thesis.

2.2.3. The effect of the breath collection apparatus on sample concentration

Firstly, the proposed breath sampling equipment was tested for the emission of volatile gases at the mass-to-charge ratios (m/z) of the three volatiles of interest. Air in a sealed inert container was analysed by SIFT-MS, then an item of proposed breath sampling equipment was placed inside. The container and its contents were incubated at 40°C for 30 min. The headspace above the piece of equipment was then analysed by SIFT-MS. This process was repeated with each piece of equipment that could be incubated.

The breath collection apparatus was set up as if to collect a patient's breath sample. Two pieces of suction catheter tubing (4.7 mm x 53 cm, Ch14, Pennine Healthcare, UK) had their tips cut off up to the side holes and the tapered ends trimmed. The two suction catheters were connected by a sampling filter (DISMIC®-25, Advantec®, Toyo Roshi Kaisha Ltd, Japan) (total 8 ml dead space in tubing and filter). One of the suction catheters was joined to the 'in' port of a handheld pump (Gilian® Personal Air Sampler, Sensidyne, USA) via the tapered end. The pump flow rate was set to 1.8 L/min (checked by a pneumotachometer (RSS 100, Hans Rudolph Inc, USA)).

A Tedlar bag was filled with breath by a member of laboratory staff exhaling directly into it and was analysed immediately by SIFT-MS. It was then attached to the free end of the suction catheter. An empty Tedlar bag was attached to the pump output tube and the pump switched on for 4 sec (the duration over which a breath sample would be taken) (Figure 2-1). A breath sample of approximately 150 ml was transferred from the full to the empty bag. The pump was switched off and the new sample placed to one side whilst another empty Tedlar bag was attached to the pump and the process repeated to give a total of three new samples. Breath samples were analysed in turn by SIFT-MS. The remainder of the initial sample was re-analysed to look for any changes in volatile concentration and humidity. The process was performed with a total of seven original breath samples containing VOCs at different concentrations. The pump was turned on to suck room air through the

equipment for at least 2 min between different initial breath samples in order to flush out the previous sample and prevent contamination of further samples.

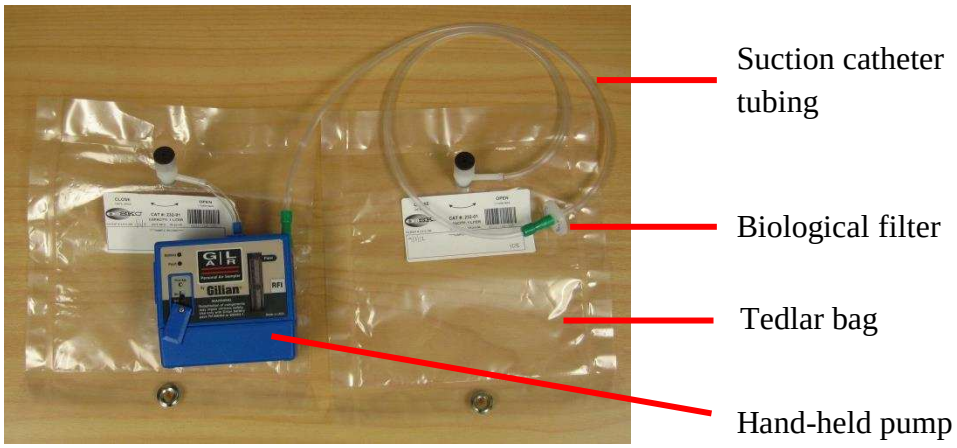


Figure 2-1. Photograph displaying initial breath sample in Tedlar bag (right) and bag for collection of sample after it had passed through the suction catheters, biological filter and handheld pump (left).

To eliminate any effect of dead space gas in the pump and tubing on the first sample drawn through the apparatus, the experiment was performed again, as above, with three further Tedlar bags of breath. This time, the first sample drawn through the pump was discarded. A fourth sample was drawn through the apparatus to give a total of three samples for analysis. The effect of discarding the first new sample on mean VOC concentrations and percentage CVs of VOC concentration for the three new samples from each initial sample bag were investigated.

2.2.4. The effect of the pump on sample concentration

A Tedlar bag was filled with breath by a member of laboratory staff exhaling directly into it and was analysed immediately by SIFT-MS. It was then attached directly to the ‘in’ port of the pump and an empty Tedlar bag attached to the output tube (Figure 2-2). The pump was switched on for 4 sec and approximately 150 ml of breath was transferred from the full to the empty bag. The pump was switched off, the new sample placed to one side and another empty Tedlar bag was attached to the pump. The process was repeated to give a total of three new samples. Samples were analysed in turn by SIFT-MS and

the rest of the initial sample was re-analysed. This process was repeated with five different breath samples containing VOCs at different concentrations.

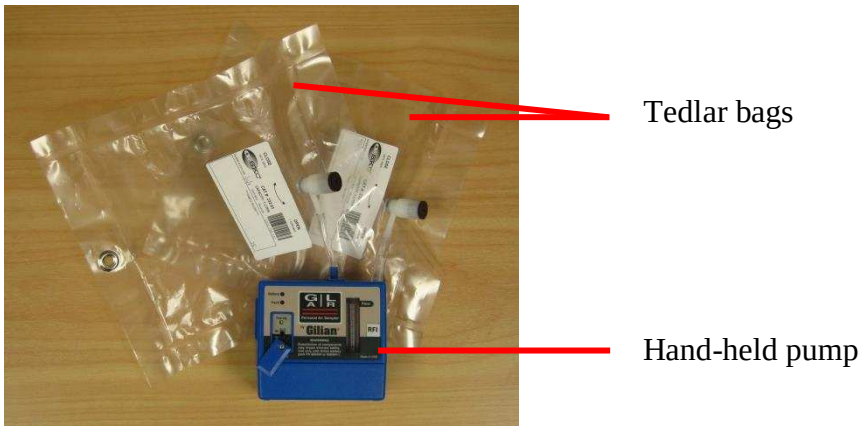


Figure 2-2. Photograph of the hand-held pump, original breath sample in a Tedlar bag (right) and the sample after it had passed through the pump (left).

2.2.5. The effect of the sampling filter on sample concentration

A test gas of 100% relative humidity was produced using dilution apparatus (Syft Technologies Ltd, NZ), a permeation chamber (Dynacalibrator Model 150, VICI Metronics, USA) and permeation tubes of acetone (Kin-tek, USA) and H_2S (Metronics, USA). The permeation tubes had known emission rates of 1269 and 489 ng/min at 40°C, respectively. Test gas flowed directly into the instrument and the concentration of acetone or H_2S was analysed. The sampling filter was then attached to one end of the flow tube and to the SIFT-MS instrument (Figure 2-3). The test gas was then analysed at least twice after passing through the filter. The concentration of each test gas was altered using the dilution apparatus to enable the experimental process to be repeated with six different acetone and six different H_2S concentrations.

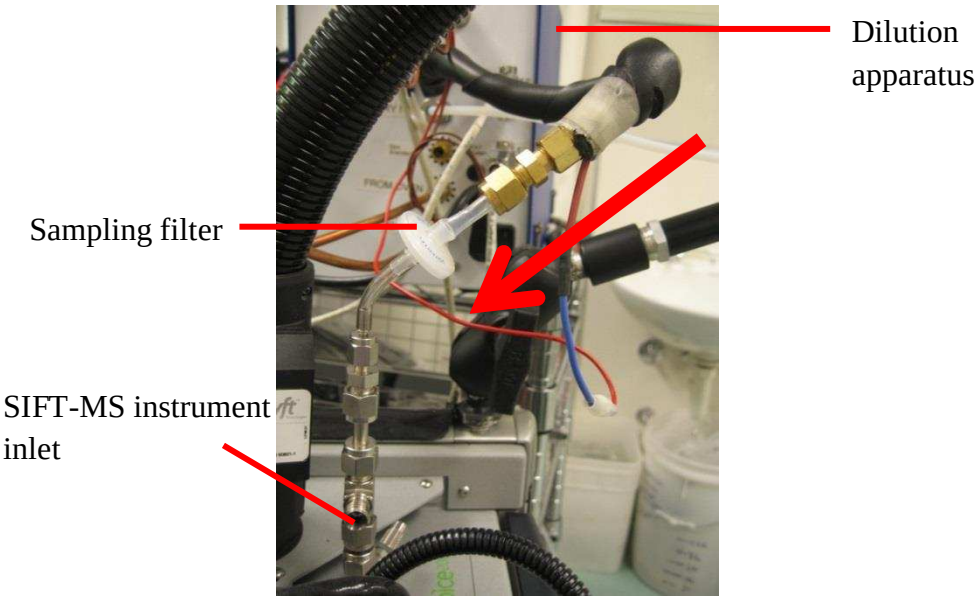


Figure 2-3. Photograph displaying the biological filter in-line with the test gas flow and SIFT-MS input port. Arrow shows direction of gas flow.

2.2.6. Selected ion flow tube mass spectrometry

The concentration of volatiles in exhaled breath and humid gas samples was measured by SIFT-MS, Voice200® (Syft Technologies Ltd, NZ), described in detail in Chapter 1.4. of this thesis. Bag samples were attached to the sampling capillary end cap of the heated inlet extension of the SIFT-MS and analysed for 30 sec (Figure 2-4).

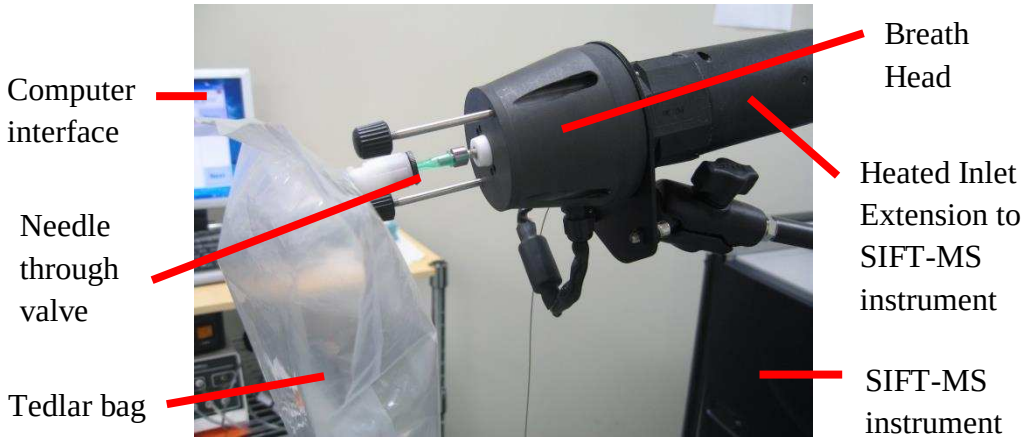


Figure 2-4. Photograph showing a Tedlar bag attached to the Breath Head and Heated Inlet Extension of the SIFT-MS instrument via a needle through the bag's valve.

Scans were performed in mass scan (MS) and selected ion monitoring (SIM) modes. Using the touch screen to control the instrument, results were displayed on a computer interface using LabSyft software. In SIM mode, the average concentration of an analyte over a desired time interval was calculated by dragging the cursor over the selected area of the trace, causing the concentration to appear in a pop-up box.

Ion reactions

The NO^+ reagent ion was used to analyze breath acetone. The reaction is an ion-molecule collisional association with He atom stabilization[322]:

$$NO^+ + CH_3COCH_3 (+ He) \rightarrow NO^+.CH_3COCH_3 (+ He)$$

The NO^+ reagent ion was monitored at m/z 30, and the $NO^+.H_2O$ hydrated reagent ion at m/z 48 as this ion is formed in humid air mixtures. The $NO^+.CH_3COCH_3$ product ion was monitored at m/z 88.

The H_3O^+ reagent ion was used to analyse breath H_2S and HCN. The reactions are proton transfer reactions[217,218]:

$$H_3O^+ + H_2S \leftrightarrows H_3S^+ + H_2O$$

$$H_3O^+ + HCN \leftrightarrows H_2CN^+ + H_2O$$

The H_3S^+ and H_2CN^+ product ions were monitored at m/z 35 and 28, respectively. The H_3O^+ reagent ion was monitored at m/z 19 and the hydrated ions $H_3O^+.H_2O$, $H_3O^+(H_2O)_2$ and $H_3O^+(H_2O)_3$, formed in humid air mixtures, at m/z 37, 55 and 73, respectively.

The H_3O^+ ion is the only reagent ion that can be used to measure H_2S. It does not react with NO^+ and the product of the reaction with O_2^+, H_2S^+, reacts quickly with H_2O[217]. A proton donation reaction can only occur if the proton affinity of the acceptor is greater than that of the donor. The difference in proton affinities of H_2S (705 kJ/mol) and H_2O (691 kJ/mol) is 14 kJ/mol, which is less than the suggested 20 kJ/mol for proton donation to occur, therefore it is possible for the reverse reaction to occur if extra energy is generated by the protonated product ions[323]. The rate coefficient of the reaction

of H_2S with H_3O^+ has been calculated in humid air[217] allowing accurate calculation of concentration.

The difference in proton affinity between HCN (713 kJ/mol) and H_2O (691 kJ/mol) is 22 kJ/mol, thus driving the reaction forward. Again, the rate coefficient of this reaction in humid air has been calculated[218].

Instrument accuracy and precision

Before the start of the experiment the SIFT-MS instrument was calibrated with known concentrations of acetone, H_2S and HCN in humid air from a commercial gas cylinder supply. The accuracy and precision of the instrument for the measurement of acetone, H_2S and HCN in humid air has previously been investigated[151,168,223] and found to be appropriate for the quantification of these compounds at physiological concentrations in breath.

Indicators of sample humidity

Sample humidity was investigated by analysing changes in relative concentrations of the H_3O^+ precursor ion and its hydrated ions.

2.2.7. Statistical analysis

The percentage change in analyte concentration over storage time was calculated. Means, standard deviations and CVs of analyte concentrations in bag samples after being drawn through the sampling apparatus were calculated. Linear regression was performed to look at the difference between initial breath sample analyte concentrations and concentrations after breath was passed through the apparatus. Statistical analyses were performed using GraphPad Prism version 5.01 for Windows (GraphPad Software, USA).

2.3. Results

2.3.1. The effect of sample storage on analyte concentration and humidity

Acetone, H_2S and HCN concentrations all decreased over time, as did the humidity of the breath samples. Figure 2-5 shows the absolute change in

analyte concentrations during storage and Figure 2-6 shows the percentage change in analyte concentration when compared to baseline concentration.

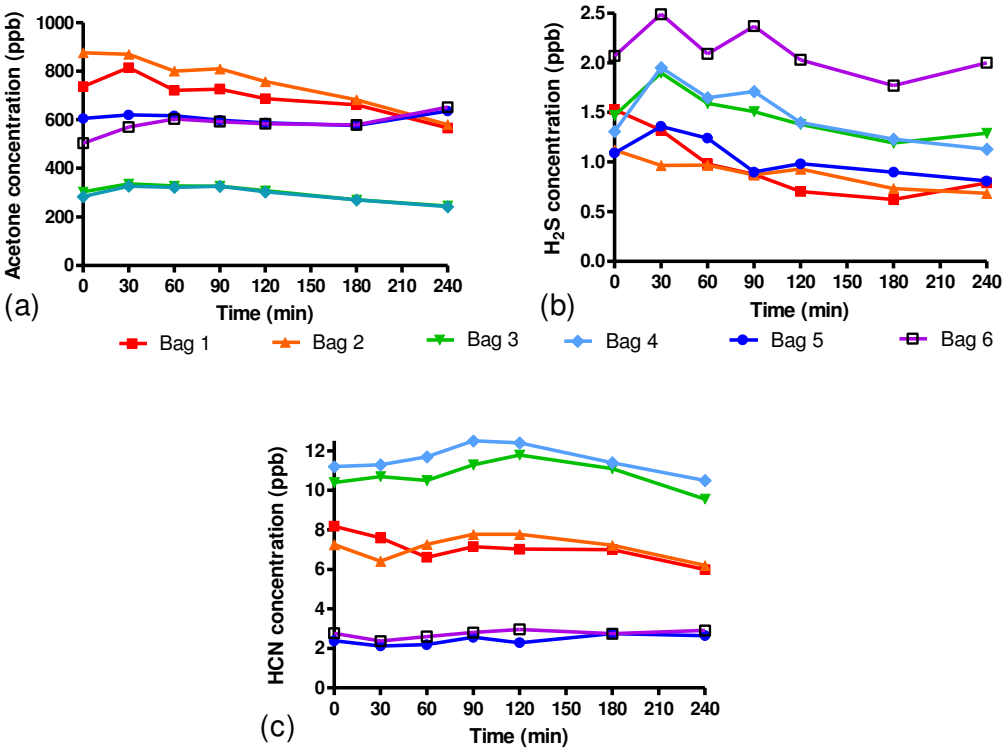


Figure 2-5. Change in analyte concentration over time in each of the six Tedlar bags; (a) acetone, (b) H_2S, (c) HCN.

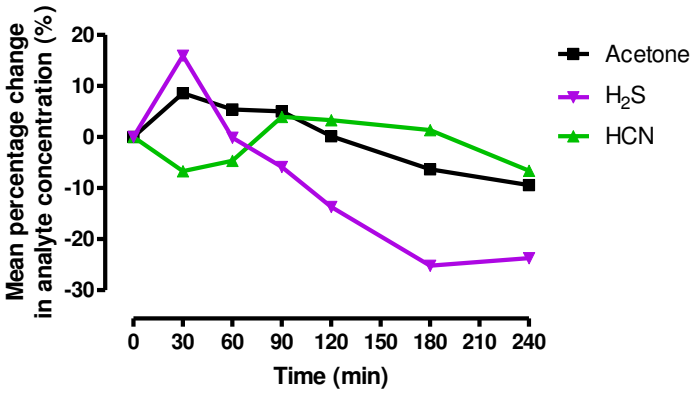


Figure 2-6. Mean change in analyte concentration over time as a percentage of the starting concentration.

Acetone

There was a greater reduction in acetone concentration in the two bags containing the highest starting concentration (Figure 2-5). Losses from the

other bags were minimal over four hours. Over 92% of the initial sample concentration was detected in each bag after 90 min of storage, and the concentration had dropped by more than 10% in only one bag after three hours. A small increase in acetone concentration was seen in most of the samples in the first 30 min of incubation.

Hydrogen sulphide

The concentration of H_2S was stable for 2 hours in five out of six of the bags and then tended to slowly decline (Figure 2-5). When looking at the mean data of all of the bags, the concentration of H_2S appears to be stable for 90 min and satisfactory for two hours (Figure 2-6). A small initial increase in H_2S concentration was noted in 4 of the bag samples (Figure 2-5).

Hydrogen cyanide

The concentration of HCN was stable over the full four hours of sample storage (Figures 2-5 and 2-6), with 93% of the initial concentration detected at that time. There was a fluctuation in HCN concentration over time, with a tendency for initial reduction and subsequent increase in concentration.

Humidity

The reduction in humidity was assessed by looking at changes in the percentage of H_3O^+ precursor ion counts (Hz) compared to hydrated ion counts. The reduction in hydrated ions as a percentage of the total counts can be seen in Figure 2-7. The percentage of H_3O^+ hydrated ions fell from 64% to 32% of the total H_3O^+ ion count with a corresponding increase in H_3O^+ 19 ions. NO^+ does not form hydrated ions as readily as H_3O^+, however there was still a reduction in the percentage of hydrated ions over the experimental time. There was a slight increase in the percentage of hydrated precursor ions in all six bags when analysed at 30 min, however humidity fell more rapidly over the four-hour storage period than any of the analyte concentrations.

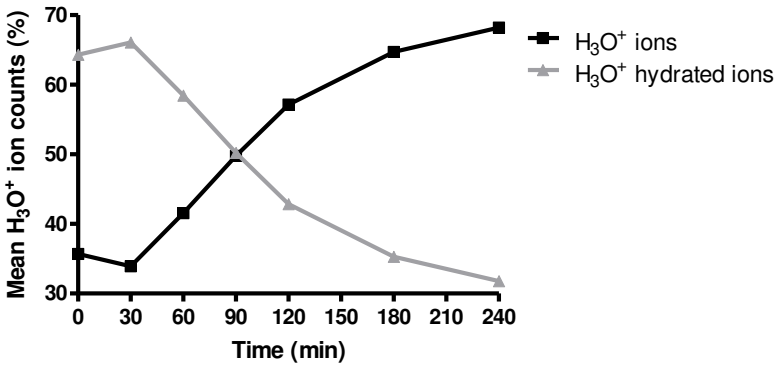


Figure 2-7. Change in H_3O^+ m/z 19 precursor and hydrated precursor ion counts (m/z 37, 55 and 73) as a percentage of the total H_3O^+ counts.

Tedlar contaminants

There was an increase in the mean concentration of contaminants specific to Tedlar bags during incubation at 40°C (Figure 2-8). The concentration of phenol (m/z 95) appeared to plateau after a peak in concentration at 120 min, whereas the concentration of N,N-dimethylacetamide (m/z 88) continued to rise throughout the incubation period.

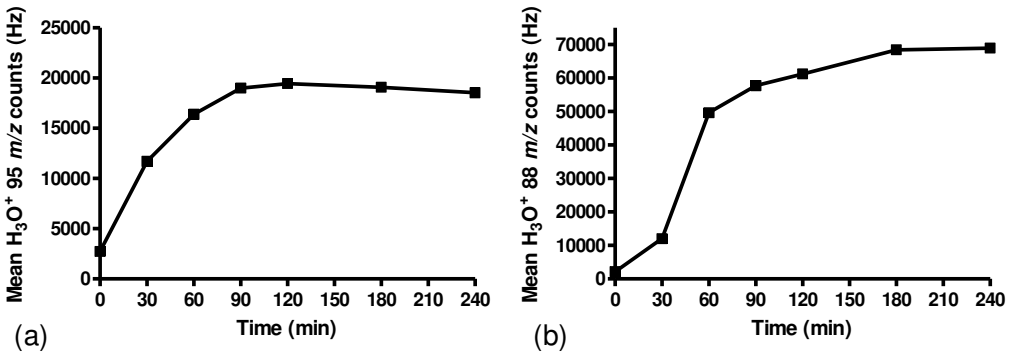


Figure 2-8. Change in mean contaminant counts over storage time; (a) phenol (H_3O^+ m/z 95), (b) N,N-dimethylacetamide (H_3O^+ m/z 88).

2.3.2. The effect of the breath collection apparatus on sample concentration

There was no change in acetone, H_2S or HCN concentrations of headspace air in the storage container before and after incubation of the breath sampling equipment. The apparatus tested was as follows: suction catheter tubing (Pennine Healthcare, UK), sampling filter (DISMIC®-25, Toyo Roshi Kaisha

Ltd, Japan), oxygen enrichment attachment (Respironics Inc, USA), tubing adaptor (Care Medical Ltd, NZ), cut-off tip of a 1 ml syringe (Becton, Dickinson and Company, Singapore).

There was a reduction in the concentrations of acetone, H_2S and HCN after the breath sample was passed through the two suction catheters, sampling filter and hand-held pump. Data for acetone can be seen in Table 2-1. When the first post-apparatus breath sample was discarded, the CV of the three post-apparatus samples improved. This did not alter the mean percentage reduction in acetone concentration (Table 2-2). Similar improvements in the CVs of H_2S and HCN samples were seen (median CV H_2S 12.9 improved to 4.2, HCN 11.8 improved to 5.3).

Table 2-1. Initial and post-apparatus breath acetone concentration and the percentage change in acetone concentration. The percentage coefficient of variation (CV) of the three post-apparatus samples is shown.

Initial acetone concentration (ppb)	Post-apparatus acetone concentration (ppb)				CV (%)	% change
	Sample 1	Sample 2	Sample 3	Mean		
1360	786	1030	1070	962	16.0	29.3
1020	593	682	670	648	7.4	36.4
992	566		805	532	54.9	46.4
803	571	643	669	628	8.1	21.8
719	491	545	638	558	13.3	22.4
644	273	328	333	311	10.7	51.7
439	280	325	410	338	19.5	22.9
				median	13.3	29.3

CV, coefficient of variation; % change, percentage change between initial sample and mean acetone concentrations after sample was passed through the apparatus. Missing value was due to sample leakage via a hole in the Tedlar bag.

Table 2-2. Initial and post-apparatus breath sample acetone concentration and the percentage change in acetone concentration after the first post-apparatus sample is discarded. The percentage coefficient of variation (CV) of the three post-apparatus samples is shown.

Initial acetone concentration (ppb)	Post-apparatus acetone concentration (ppb)				CV (%)	% change
	Sample 2	Sample 3	Sample 4	Mean		
843	578	619	647	615	5.6	27.1
665	468	470	506	481	4.4	27.6
484	301	330	315	315	4.6	34.8
				median	4.6	27.6

CV, coefficient of variation; % change, percentage change between initial sample and mean acetone concentrations after sample was passed through the apparatus.

According to the linear regression equation comparing initial and subsequent acetone concentrations ($y = 0.79x – 46.14$), there was a fixed loss of 46 ppb and a variable loss of 21%. The equation of the linear regression line for H_2S concentration ($y = 0.75x – 1.43$) explains a fixed reduction of 1.4 ppb followed by a loss of 25% from the rest of the sample. The linear regression equation for the reduction in HCN concentration ($y = 0.76x + 0.68$) reveals a decrease of 24% associated with the sampling equipment. Losses were reproducible across the analyte concentrations measured (Figure 2-9).

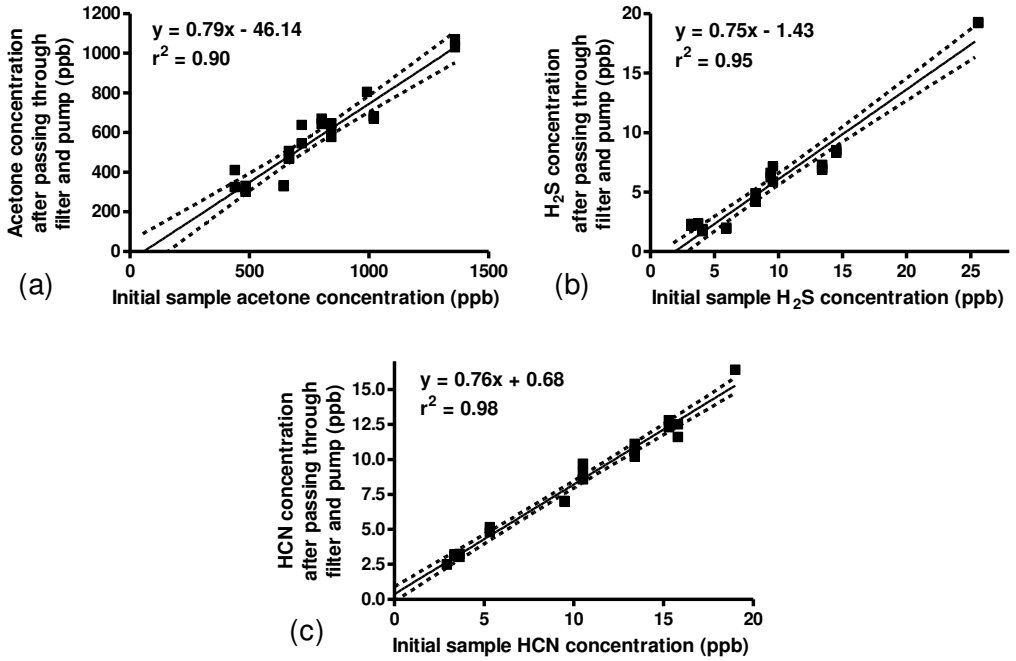


Figure 2-9. The relationship between the concentrations of (a) acetone, (b) H_2S and (c) HCN before and after the gas sample was passed through the tubing, filter and pump (linear regression lines with 95% confidence intervals marked). Individual data points for analyte concentrations, first post-apparatus samples not included.

2.3.3. The effect of the pump on sample concentration

There was a reduction in the concentrations of acetone, H_2S and HCN in the breath samples caused by being passed through the pump alone (Table 2-3). In a similar way to the results presented in section 2.3.2., there appeared to be an effect of dead space dilution on the first sample passed through the pump, therefore the first post-pump samples were excluded from linear regression analysis (Figure 2-10). There was a higher degree of variability in H_2S concentration in post-pump samples than was seen for acetone and HCN concentrations within the same breath samples.

Table 2-3. Initial and post-pump breath sample analyte concentration and the percentage change in analyte concentration. The percentage coefficient of variation (CV) of the three post-apparatus samples is shown.

Analyte	Initial analyte concentration (ppb)	Post-pump analyte concentration (ppb)			CV (%)	% change
		Sample 1	Sample 2	Sample 3		
Acetone	1160	862	907	890	2.5	23.6
Acetone	684	544	536	542	0.8	20.1
Acetone	502	338	398	393	8.8	25.0
Acetone	378	260	281	304	7.8	25.5
Acetone	361	299	301	303	0.7	16.6
H_2S	1.10	0.69	0.70	0.73	3.0	35.9
H_2S	1.01	0.99	0.95	1.06	5.6	1.0
H_2S	0.94	0.80	0.91	0.83	7.2	9.8
H_2S	0.83	0.76	0.70	0.73	4.2	12.9
H_2S	0.66	0.56	0.55	0.54	1.7	17.2
HCN	7.24	4.62	5.92	6.31	15.8	22.4
HCN	6.68	4.35	5.22	5.61	12.7	24.3
HCN	5.69	3.64	4.65	4.96	15.6	22.4
HCN	4.61	3.27	3.47	3.79	7.5	23.9
HCN	4.47	3.69	3.87	3.70	2.7	16.0

CV, coefficient of variation; % change, percentage change between initial sample and mean analyte concentrations after sample was passed through the pump.

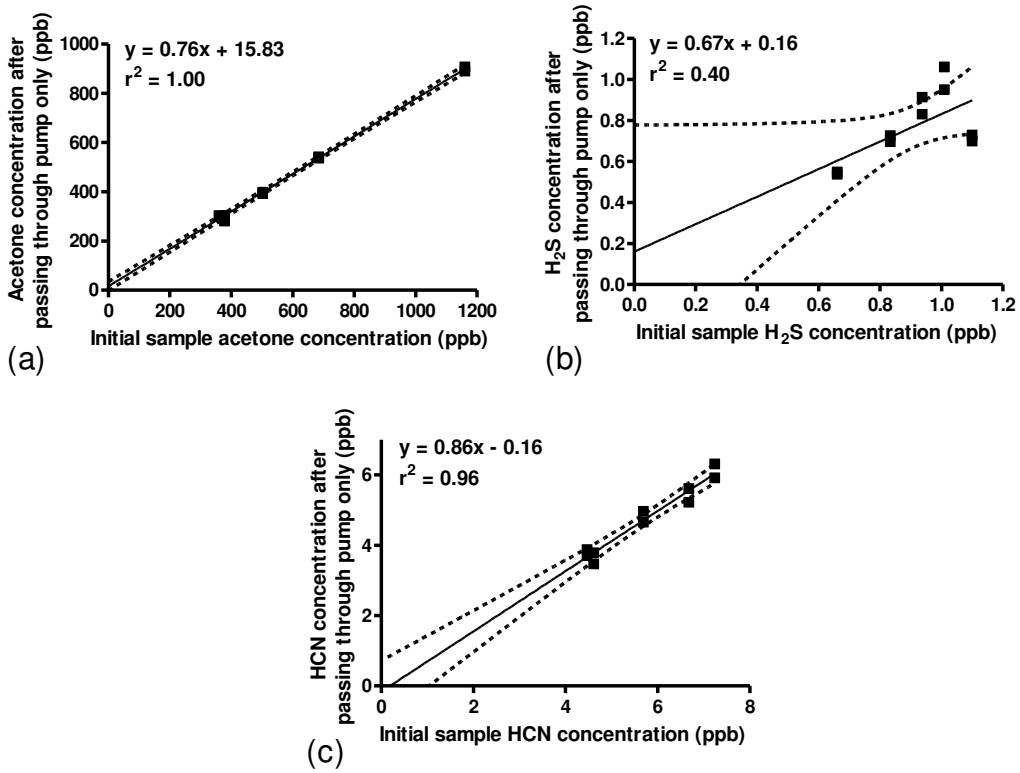


Figure 2-10. The relationship between the concentrations of (a) acetone, (b) H_2S and (c) HCN before and after the gas sample was passed through the pump alone (linear regression lines with 95% confidence intervals marked). Individual data points for analyte concentrations, first post-pump samples not included.

Linear regression equations suggest a 24% reduction in acetone concentration, a 33% reduction in H_2S concentration and a 14% drop in HCN concentration as a result of breath samples being passed through the pump.

2.3.4. The effect of the sampling filter on sample concentration

A small fixed reduction in acetone and H_2S concentration was seen due to the sampling filter alone (Figure 2-11). When the filter was placed in the line of gas flow from the dilution apparatus to the SIFT-MS instrument, it was noted that no analyte could be detected if the filter was turned through 180°. This highlights that the filter must be positioned in the correct orientation or no analyte will be detected downstream.

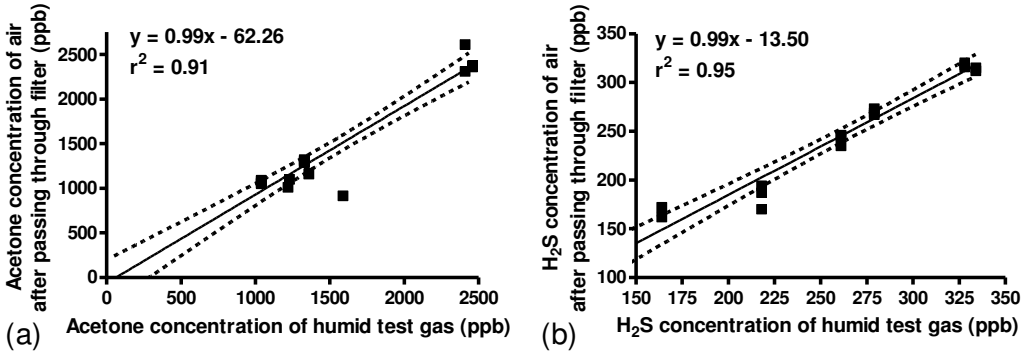


Figure 2-11. The relationship between the concentration of acetone (a) and H_2S (b) in humid air and that detected by SIFT-MS after passing through a small biological filter (linear regression lines with 95% confidence intervals marked).

2.4. Discussion

2.4.1. Storage of breath samples

This study confirms the appropriateness of Tedlar bags for the collection and short-term storage of acetone, H_2S and HCN at physiological concentrations in breath. The concentration of each of these analytes decreased over the four hours of incubation, however on average over 90% of the initial sample concentration was detected at 90 min and over 80% at 120 min. The results described here are more applicable to the rest of the thesis than previous studies using dry gases instead of breath, which is humid, with VOCs at concentrations far exceeding physiological concentrations, where the concentration gradients across the bags were steeper and would be expected to result in greater losses of analyte due to diffusion out of the bags[134,139,140]. Few studies have explored the storage of HCN[135] and sulphur compounds[140] in Tedlar bags, therefore it was important to evaluate the utility of the bags for the storage of these compounds in particular.

The concentration of acetone in Tedlar bags at physiological concentration was stable over four hours of incubation at 40°C, confirming findings of previous studies[134,135,242]. The two bags with the highest initial starting concentrations showed the greatest percentage losses over time. This may have been due to a higher acetone concentration gradient between the inside of the bag and the atmosphere (room air acetone concentration 25–50 ppb), leading to increased

diffusion out of the bag. It is also possible that these two bags were older and had more microdamages causing greater losses[140].

The concentration of H_2S dropped most rapidly, a finding similar to that seen in a previous study of sulphur compounds stored in transparent Tedlar bags[140]. In that study, H_2S recovery was at least 90% at six hours compared to 77% at four hours in this study. The difference in percentage H_2S recovery between the two studies may be due to the difference in initial H_2S concentration; less than 2.5 ppb in this study, 61.8 ppb in the study of Mochalski et al.[140]. Humid breath was analysed in this study, whereas the previous study used a dry test gas. H_2S dissolves readily in water droplets present in humid breath, which may have reduced the concentration of H_2S in the gaseous phase available for detection by SIFT-MS in this study.

A recent study of breath samples stored in Tedlar and Nalophan bags for off-line analysis of HCN showed that, when incubated at 37°C, HCN concentration was stable for up to six hours[135], in agreement with this study. They did not, however, measure volatile concentrations immediately, but at 1, 6, 24 and 48 hours, so any loss over the first hour was not quantified.

As seen in other studies, humidity declined faster than analyte concentrations[134,135]. There was an initial increase in humidity after 30 min incubation. It is possible that this increase was due to larger water droplets in the breath samples turning to water vapour as the sample was heated above body or room temperature. This may lead to an increase the concentration of hydrated ions in the mixture. This process may explain the initial increases in analyte concentration in some bags. Analyte dissolved in water droplets is unable to be analysed by SIFT-MS, however heating may have moved the substances into the gas phase, therefore enabling their detection. In contrast to this idea, Steeghs et al.[138] did not find any difference in analyte concentration when the relative humidity of bag samples was increased.

Other possible causes of an increase in analyte concentration are contamination from VOCs previously adsorbed onto the bag's inner wall being mobilised into the sample gas on heating, and substances diffusing into the bag from the environment.

Increases in the concentrations of known Tedlar contaminants, N,N-dimethylacetamide and phenol, were seen during incubation, confirming the findings of previous studies[134,138]. These compounds, found in clean Tedlar bags, can affect the analysis of other compounds with peaks at m/z 88 and m/z 95, however they do not affect the detection or calculation of acetone, H_2S and HCN necessary for the rest of this thesis. The product of the reaction of NO^+ with acetone has a peak at m/z 88, but it can be separated from the peak at m/z 88 due to the reaction of N,N-dimethylacetamide with H_3O^+.

2.4.2. The effect of breath sampling apparatus on analyte concentration

None of the separate pieces of breath sampling equipment caused a change in the m/z peaks of acetone, H_2S and HCN after incubation. It was concluded, therefore, that the sampling equipment would not contaminate breath samples. It was also necessary to explore whether the sampling apparatus would lead to a reduction in analyte concentration.

Reductions in acetone, H_2S and HCN concentrations due to the breath sampling apparatus were seen. However, because the losses were linear over the range of concentrations tested it is possible to use the apparatus, since actual analyte concentrations can be calculated from observed concentrations. Loss of analyte was due to the pump, tubing and filter. Adsorption of analyte onto the surface of the filter and tubing, and reduction in humidity as a sample travelled through the pump are the most likely causes of a reduction in observed concentrations. Unfortunately, when the filter was tested separately, breath could not be used and the concentration range of H_2S in humid gas was not within the physiological range. The concentration range of acetone used was also at the upper end of physiological concentration. It is therefore possible that the filter has a different affect at the lower concentrations that will be seen in the rest of this thesis. It was also not possible to repeat the experiment with HCN in humid gas as HCN permeation tubes were not available.

By assessing analyte loss using all of the equipment together, then removing the pump and filter and testing them separately, the identification of the

sources of analyte loss was attempted. The reduction in acetone concentration was similar using the pump alone or all of the apparatus, with narrower 95% confidence intervals when breath was passed through the pump alone. The increased variability in concentration using all of the equipment might have been due to a variable concentration of acetone sticking to the inner surface of the suction catheter tubing. HCN concentration reduction by the full set of breath sampling apparatus was greater than with the pump alone. As with acetone, it is likely that HCN in the samples was adsorbed onto the inner surface of the suction catheter tubing.

The concentration of H_2S was significantly lower and more variable when using the pump alone compared to using all of the sampling apparatus together. It is difficult to explain this increased loss; it may be related to differences in laboratory temperature or humidity on the days of testing. The concentration of H_2S in both sets of initial samples was very low, lower in the pump only experiment, close to the limit of detection of H_2S by the SIFT-MS instrument. All H_2S concentrations were determined by physiological concentrations in breath of the volunteers who provided samples. Any small changes in absolute concentration resulted in large percentage changes in concentration; therefore, it would be useful to repeat the experiments with breath or humid gas containing higher H_2S concentrations to improve accuracy.

Although it was not possible to accurately identify the relative loss of analyte due to individual parts of the apparatus, the most important of the equipment testing experiments was that using all of the equipment together with test bags of breath, as this is the proposed apparatus for the rest of the thesis and investigates changes in concentration of VOCs in a humid mixture at physiological concentrations.

Dead space gas in the tubing and pump led to dilution of the first sample collected after being passed through the pump or pump, filter and tubing. Deposition of analyte from the first sample onto the surface of the filter and tubing, possibly saturating the filter, may also have contributed to lower analyte concentrations measured in the first sample compared to subsequent samples. Discarding the first sample improved the CV of the three samples run

through the apparatus without greatly affecting the percentage reduction in analyte concentration. The first breath sample collected from each subject in other parts of this thesis will be discarded to minimise analyte concentration variability due to the sampling method. Running the pump for at least two minutes after collecting breath from each subject should flush the pump and prevent, or at least reduce, contamination from a previous sample.

2.4.3. Summary

It is recommended that breath samples are incubated to prevent sample cooling and condensation prior to analysis, and that samples are analysed as soon as possible after collection, preferably within 90 min. According to the results of this investigation, the recovery of, on average, greater than 90% of the initial sample concentration is possible if samples are stored in transparent Tedlar bags at 40°C.

The breath sampling apparatus did not emit analytes at the m/z peaks of acetone, H_2S or HCN and its use resulted in losses of up to 25% of analyte concentration. Losses were linear over the range of concentrations studied; therefore, it should be possible to calculate the absolute concentrations of VOCs in breath from measured concentrations. The apparatus studied is therefore suitable for the collection of breath in the following parts of this thesis.

3. Are breath volatile concentrations affected by sampling location and breathing manoeuvre?

3.1. Introduction and aims

In order to accurately quantify volatile compounds in the breath of intubated and ventilated patients, a robust and reproducible method of breath collection is necessary. End-exhaled breath is often analysed because the composition of VOCs better reflects that of alveolar breath, thus reducing the chance of sample contamination or dilution with dead space gas[7,147,166]. In spontaneously breathing ambulatory subjects, end-exhaled breath has been identified by the elimination of the first few seconds of an exhaled breath[240], use of humidity[150,167], and analysis of the last 15-20% of a breath sample by volume[168]. These studies used forced vital capacity manoeuvres, with or without expiratory flow rate restriction.

Several breath collection and analysis techniques have been used in intubated patients in the operating theatre and in the ICU; off-line breath collection with pre-concentration prior to analysis[5,7,105], mixed breath continuous on-line sampling[106,207], or on-line sampling using computer software to pinpoint the alveolar portion of breath[143,209,210]. A CO_2 switch has also been used to determine alveolar breath for collection[7].

The aims of this study were to compare end-exhaled single breath concentrations of acetone, H_2S and HCN collected from two different locations in the breathing circuit following tidal breathing and a larger volume breath, replicating a vital capacity breath. Attempts were made to collect samples as similar to alveolar composition as possible by sampling directly from the airways. The reproducibility of each sampling method and the ease of performing each technique were assessed.

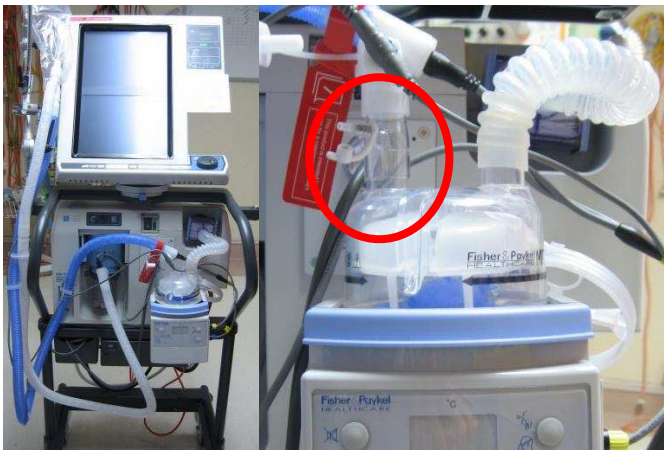
3.2. Methods

3.2.1. Subjects

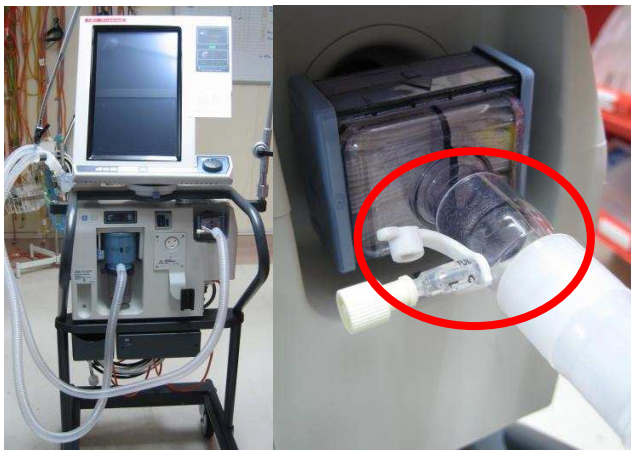
Study procedures were approved by the Upper South A Regional Ethics Committee, New Zealand. Non-diabetic adult patients admitted to the ICU, Christchurch Hospital, requiring intubation and ventilation on a controlled mode due to lack of spontaneous breathing were recruited between September and October 2011. Patients or their next of kin provided written consent prior to sample collection. In addition, if the patient had not given their own consent prior to inclusion, and regained adequate cognitive functioning, they confirmed their consent to participate in retrospect. Each patient was ventilated using a Nellcor Puritan Bennett™ ventilator system (USA) on Synchronised Intermittent Mandatory Ventilation (SIMV) mode with volume control or Bi-Level mode with pressure control. Some ventilator circuits included a humidifier (Fisher & Paykel MR 730 Respiratory Humidifier, NZ). Initial ventilator modes and settings, including fraction of inspired oxygen (FiO_2), were determined by ICU staff. Drug infusion rates were kept constant throughout breath sampling. The following data was collected for each patient: diagnosis and reason for admission, age, gender, ethnicity, smoking status, ventilator settings and end-tidal CO_2.

3.2.2. Inspiratory air sampling

All methods utilised a T-piece (oxygen enrichment attachment, Respironics Inc, USA) inserted into the breathing circuit at the ventilator gas outlet, or humidifier outlet, for inspiratory gas sampling (Figures 3-1(a) and (b)).



(a)



(b)

Figure 3-1. Photographs display the location of the T-piece in the ventilator circuit (red circle) for inspired gas sampling, with (a) and without a humidifier in situ (b).

3.2.3. Exhaled breath collection

Four different methods of breath collection were explored in each patient, using two different sampling locations within the breathing circuit and two different exhalation techniques (Table 3-1). Full experimental set-up can be seen in Figure 3-2 and a photograph of the location of the T-piece for breath sampling in Figure 3-3 (Written patient consent obtained).

Table 3-1. Exhaled breath collection methods.

Method	Sample location	Exhalation manoeuvre
A	T-piece	Tidal breath
B	T-piece	Recruitment-style breath
C	Airway	Tidal breath
D	Airway	Recruitment-style breath

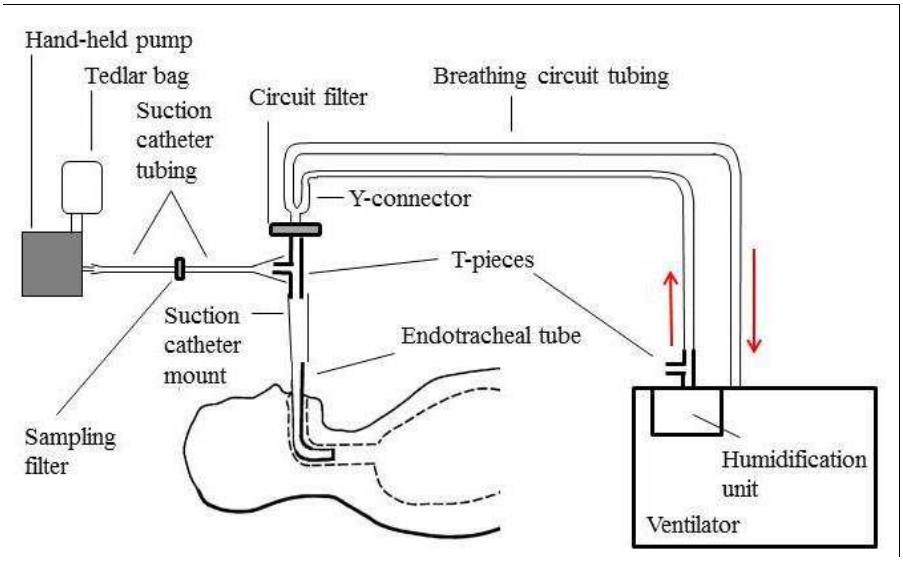


Figure 3-2. Schematic of the experimental set-up using the T-piece.

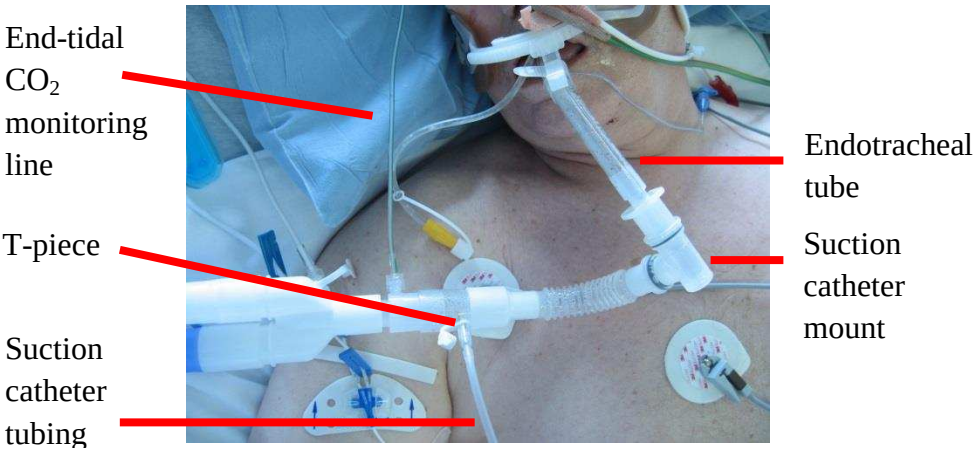


Figure 3-3. Photograph of the location of the T-piece within the breathing circuit.

Three breath samples were collected using each sampling method into separate 1L transparent Tedlar® bags (SKC Inc, USA). During breath sampling, the pressure sensitivity setting on the ventilator was temporarily altered so the collection of exhaled breath did not trigger the ventilator to deliver another inspiratory breath. All inspiratory and exhaled breath samples were incubated at 40°C and analysed within 40 min of collection by SIFT-MS. The pump was flushed by running through room air for at least 2 min between patients.

Sampling location

The first sampling location utilised a T-piece inserted into the respiratory circuit on the ventilator side of the suction catheter mount (Fisher & Paykel, NZ). Suction catheter tubing (4.7 mm x 53 cm, Ch14, Pennine Healthcare, UK) was connected to the side port of the T-piece using the cut-off tip of a 1 ml syringe (Becton, Dickinson and Company, Singapore). The other end of the tubing was connected via a sampling filter (DISMIC®-25, Advantec®, Toyo Roshi Kaisha Ltd, Japan) and another length of suction catheter tubing to a handheld pump (Gilian® Personal Air Sampler, Sensidyne, USA) (Figure 3-4(a)).

The second sampling location allowed collection of breath samples directly from the airways. Two lengths of suction catheter tubing, with a sampling filter between them, were connected to the hand-held pump. The free end of the suction catheter was inserted into the suction catheter mount and down the endotracheal tube (ETT) (Portex® Tracheal Tube, Smiths Medical Australasia Pty Ltd, Australia) until it was 1 cm from the end (Figure 3-4(b)). With the suction catheter in situ, the opening of the suction catheter mount was sealed with two Tegaderm™ film dressings (6 cm x 7 cm, 3M HealthCare, USA) (Figure 3-5).

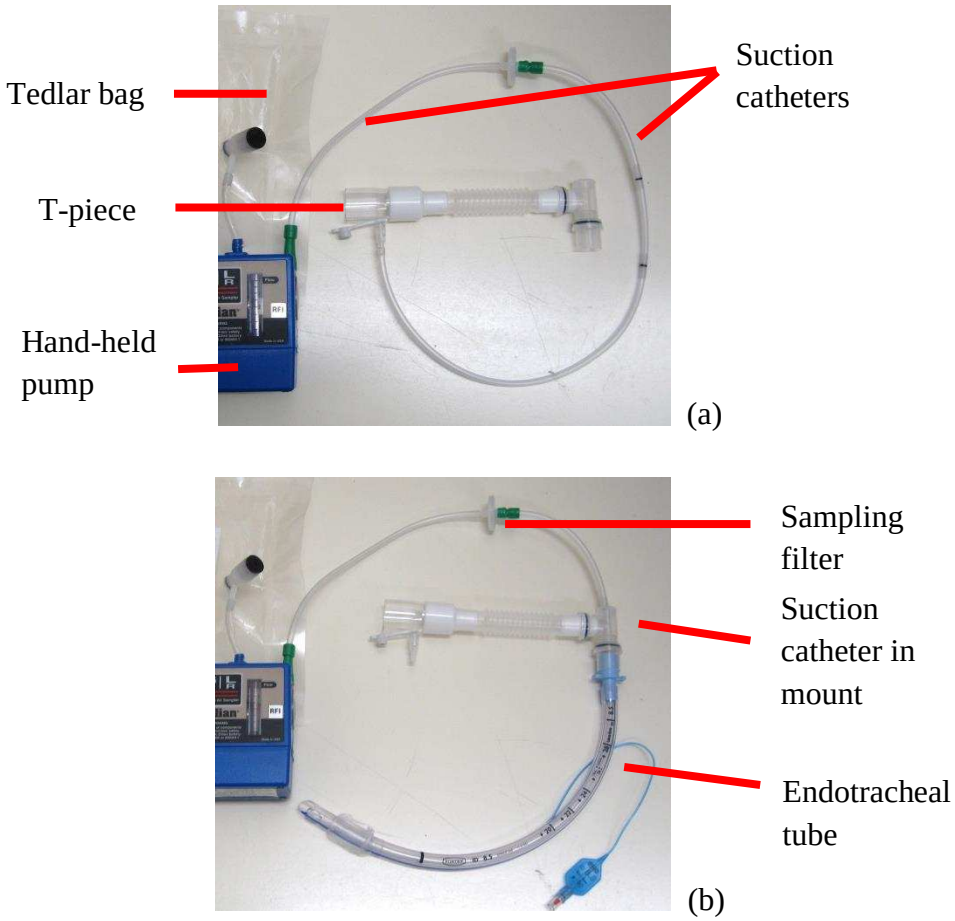


Figure 3-4. Breath sampling equipment attached to the T-piece (a) and inserted into the suction catheter mount (b).

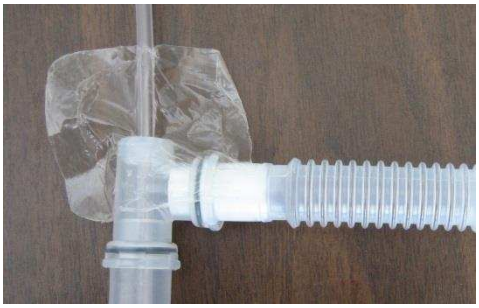


Figure 3-5. Suction catheter situated inside the mount, sealed with two Tegaderm™ film dressings.

Breathing manoeuvre

Breath samples were collected after a tidal breath and after a larger volume lung recruitment-style breath. Sampling location order was randomised by flipping a coin, however breath samples were always collected following tidal breathing first to minimise the number of times ventilator settings were altered

and ensure that delivery of a large volume breath that might recruit previously collapsed airways did not alter subsequent tidal volumes.

After a tidal breath, the expiratory pause button was held down for 4 sec to allow the collection of approximately 150 ml of end-expiratory breath into a Tedlar bag via the pump. The volume of the tidal breath was noted. The ventilator delivered several standard breaths before this process was repeated using another Tedlar bag.

Pressure-controlled lung recruitment-style breaths were delivered using Bi-Level mode. If necessary, the mode was changed and the inspiratory positive airway pressure (IPAP) and expiratory positive airway pressure (EPAP) adjusted to maintain the previous tidal volume. The IPAP was then set to 35 cmH_2O, the respiratory rate reduced to three breaths per minute, and at end-expiration after the recruitment-style breath, approximately 150 ml breath was collected over 4 sec during the natural pause before the next inspiratory breath. Recruitment-style breath volume was noted. Pressure and respiratory rate settings were returned to starting levels before the ventilator delivered the next breath. The ventilator delivered several standard tidal breaths before this process was repeated.

Direction of air flow during breath sample collection

A pneumotachometer (RSS 100, Hans Rudolph Inc, USA) was inserted into the respiratory circuit on the ventilator side of the T-piece to check the direction of flow during sample collection.

3.2.4. Infection control

The suction catheters and sampling filter were disposable and used for only one patient. The T-piece was decontaminated for re-use. Disinfectant wipes were used on the surfaces of the hand-held pump and Tedlar bags (Azo wipes, W. M. Bamford & Co Ltd, NZ). None of the cleaning procedures changed the VOC composition of samples.

3.2.5. Selected ion flow tube mass spectrometry

Volatile gas concentrations were measured by SIFT-MS, Voice200® (Syft Technologies Ltd, NZ), using SIM mode. The technique and ion reactions have been described in detail in Chapter 2. Each Tedlar bag was attached to the sampling capillary end cap of the heated inlet extension of the SIFT-MS and analysed for 30 sec.

3.2.6. Statistical analysis

Previous studies of breath testing in non-intubated and ventilated subjects have demonstrated CVs of 0-5.6%[168], however it is recognised that CVs in patients with disease are often higher than normal subjects[324]. As this the first study to explore the effects of breath sampling location, including directly from the airway, and of breathing manoeuvre on breath volatile concentrations in intubated and ventilated patients, the variability due to breath volume or sampling location were unknown. Sample size was therefore estimated from previous experience to allow the determination of clinically meaningful change for the purpose of more focussed future study design.

Means and percentage CVs of three end-exhaled breath samples were calculated for the four different breath sampling methods for each patient. Comparisons were made using Wilcoxon signed rank tests. Correlations were determined by Spearman's rank correlation (r_s). Bland-Altman plots were used to compare breath VOC concentrations using each method. Statistical analyses were performed using GraphPad Prism version 5.01 for Windows (GraphPad Software, USA). A value of $p<0.05$ was considered to be statistically significant.

3.3. Results

3.3.1. Subjects

Twenty non-diabetic patients (12 male, 8 female), median age 67.5 years (range 48-84 years), were recruited. Patient demographics, diagnosis and reason for admission to ICU can be seen in Table 3-2. Ethnicity data reflects that of the wider Christchurch population[325]. Medical concerns prevented large

volume breaths being delivered to two patients (patients 2 and 9). In two further patients (patients 8 and 14) it was not possible to collect direct airway breath samples due to significant air leak around the suction catheter with subsequent reduction in tidal volume. The pneumotachometer confirmed the direction of flow during breath sampling was from the patient and not the distal ventilator tubing.

Table 3-2. Patient demographics and ventilation data.

Subject	Age (years)	Gender	Ethnicity	Smoking status	Reason for admission to ICU	RR (breaths/min)	FiO_2	PEEP or IPAP/EPAP (cmH_2O)	$P_{et}CO_2$ (mmHg)
1	61	M	NZ European	N	Cardiac surgery	16	0.4	10	41
2	72	F	NZ European	N	Cardiac surgery	14	0.4	7.5	33
3	63	M	NZ European	-	Sepsis and ARDS	18	0.4	25/15	48
4	70	F	Caucasian	Ex	Pneumonia	18	0.3	27/10	29
5	69	F	NZ European	N	Cardiac surgery	16	0.4	5	31
6	66	M	NZ European	N	Cardiac surgery	16	0.4	5	35
7	78	M	NZ European	N	Cardiac surgery	12	0.4	5	42
8	75	F	NZ European	N	Cardiac surgery	16	0.3	5	22
9	61	M	NZ European	Ex	Pneumonia	20	0.95	25/12.5	36
10	64	F	NZ Maori	Y	Cardiac surgery	16	0.4	5	31
11	69	M	NZ European	N	Intracranial bleed	18	0.3	5	30
12	73	M	NZ Maori	Y	Vascular surgery	16	0.35	10	33
13	74	F	NZ European	N	Cardiac surgery	12	0.4	5	36
14	52	M	NZ European	Ex	Out of hospital cardiac arrest	16	0.3	5	38
15	74	F	NZ European	N	Cardiac surgery	16	0.4	5	34
16	63	M	NZ European	Ex	Cardiac surgery	12	0.6	10	39
17	48	M	NZ European	Y	Asthma	15	0.35	21/10	42
18	64	M	NZ European	Ex	Cardiac surgery	14	0.35	5	36
19	84	F	NZ European	Ex	Vascular surgery	16	0.6	5	25
20	65	M	NZ European	N	Cardiac surgery	16	0.45	5	42

M, male; F, female; N, Non-smoker; Y, smoker; Ex, ex-smoker; ARDS, acute respiratory distress syndrome; RR, respiratory rate; FiO_2, fraction of inspired oxygen; PEEP, positive end-expiratory pressure; IPAP, inspiratory positive airways pressure; EPAP, expiratory positive airways pressure; $P_{et}CO_2$, partial pressure of end tidal CO_2.

Table 3-3. Ventilation method, tidal volume and recruitment-style breath volume for the four breath collection methods.

Subject	Ventilation mode for tidal breathing	Breath from T-piece after tidal breath (method A)	Breath from airways after tidal breath (method C)	Breath from T-piece after recruitment-style breath (method B)		Breath from airways after recruitment-style breath (method D)	
		Tidal volume (ml)	Tidal volume (ml)	Tidal volume (ml)	Mean recruitment-style breath volume (ml)	Tidal volume (ml)	Mean recruitment-style breath volume (ml)
1	SIMV	500	500	475	1388	475	1033
2	SIMV	450	450	-	-	-	-
3	Bilevel	470	300	470	730	300	500
4	Bilevel	475	230	460	539	230	300
5	SIMV	480	330	480	1174	330	660
6	SIMV	500	365	500	1435	500	1136
7	SIMV	480	480	480	1404	480	1227
8	SIMV	420	-	405	778	-	-
9	Bilevel	460	350	-	-	-	-
10	SIMV	463	480	460	1326	460	700
11	SIMV	490	480	500	1534	480	985
12	SIMV	500	465	470	853	470	678
13	SIMV	435	434	425	1078	425	707
14	SIMV	430	-	520	1603	-	-
15	SIMV	473	450	420	1204	375	662
16	SIMV	570	600	550	988	550	883
17	Bilevel	540	440	580	1100	420	763
18	SIMV	440	430	470	1410	480	999
19	SIMV	450	450	445	1086	430	661
20	SIMV	480	480	510	1870	480	1375
	Median	474	450	473	1189	465	735

3.3.2. Breath volume

Individual patient breath volumes for each of the four sampling methods can be seen in Table 3-3. Tidal volume was significantly larger when samples were taken from the T-piece than directly from the airways (tidal breath p=0.01, recruitment-style breath p=0.008) (Figure 3-6). The reduction in tidal volume with direct airway sampling was due to air leaking around the suction catheter in the mount, despite sealing the area with Tegaderm film dressings, and resulted in a greater degree of inter-subject variation in mean breath volume when sampling at this location. Air leaks were more pronounced at higher positive end-expiratory pressure (PEEP) settings.

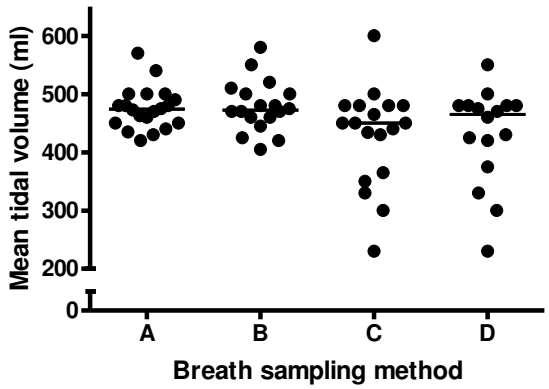


Figure 3-6. Individual mean patient tidal volumes delivered by the ventilator and group medians when using each breath collection method.

There was a significant difference between mean tidal breath volume and recruitment-style breath volume at each sampling location (T-piece p=0.0002, airway p=0.0005). When the IPAP required to maintain adequate tidal volume (during routine ventilation) was close to 35 cmH_2O, the recruitment-style breath volume was similar to the tidal breath volume. Unfortunately, increasing the ventilation pressure led to greater air leak around the suction catheter during direct airway sampling, attenuating the increase in breath volume and causing a significant difference between mean recruitment-style breath volume at the different sampling locations (p=0.0005). There was also greater inter-subject variation in mean recruitment-style breath volume compared to tidal volume at each sampling location (Figure 3-7).

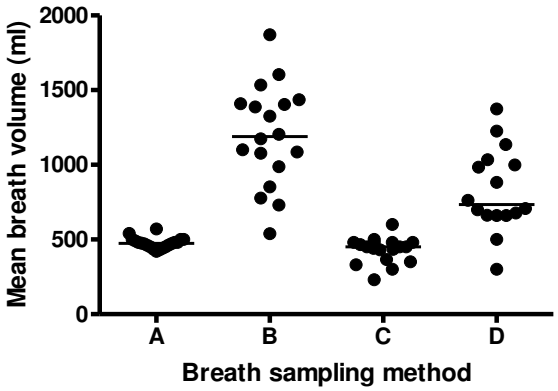


Figure 3-7. Individual mean patient breath volumes delivered by the ventilator and group medians when using each breath collection method.

3.3.3. Breath acetone concentration

Individual patient breath acetone concentration for each of the four sampling methods can be found in Appendix C. Group median exhaled acetone concentrations using each of the breath sampling methods, as well as reproducibility data, can be seen in Table 3-4.

Table 3-4. Median breath acetone concentration and intra-subject CV for breath acetone concentration using each breath sampling technique.

	Breath sampling method	Median acetone concentration (range) (ppb)	Median intra-method CV (IQR) (%)
A	T-piece after tidal breath	4060 (261–27800)	8.4 (3.9–11.4)
B	T-piece after recruitment-style breath	4005 (322–20500)	8.3 (5.7–13.1)
C	Airway after tidal breath	5500 (449–35367)	8.0 (4.1– 6.6)
D	Airway after recruitment-style breath	5250 (465–28800)	6.0 (4.7–10.8)

CV, coefficient of variation; IQR, interquartile range.

There was a strong correlation between an individual's breath acetone concentrations using each of the four breath sampling techniques (r_s>0.98 and p<0.0001 for each of the four analyses). Individual mean acetone concentration in breath samples directly from the airway was significantly higher than in samples from the T-piece (tidal breath p=0.004, recruitment-style breath p=0.0005). End-tidal breath samples collected from the T-piece had significantly higher mean acetone concentration than those collected following

a recruitment-style breath (p=0.03), however the difference in group median acetone concentrations was small (Figure 3-8). No relationship was seen between breath acetone concentration and breath volume when data from each sampling location was analysed (T-piece r_s= -0.01, p=0.95; airway r_s=0.26, p=0.13).

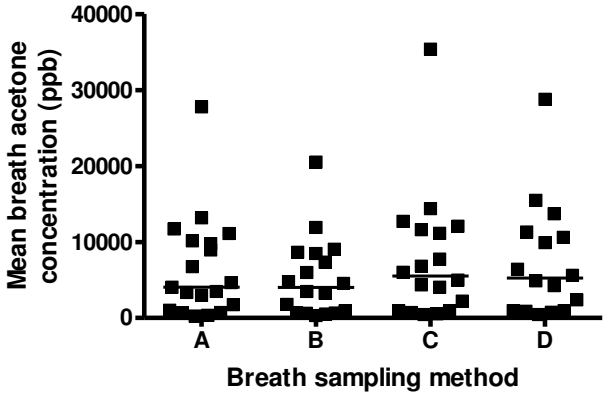


Figure 3-8. Individual mean patient end-exhaled acetone concentrations and group medians using each of the four breath sampling methods.

Bland-Altman plots show increased variability between sampling methods at higher breath acetone concentrations. In general, at acetone concentrations under 10000 ppb, there was only a small difference between methods (Figure 3-9).

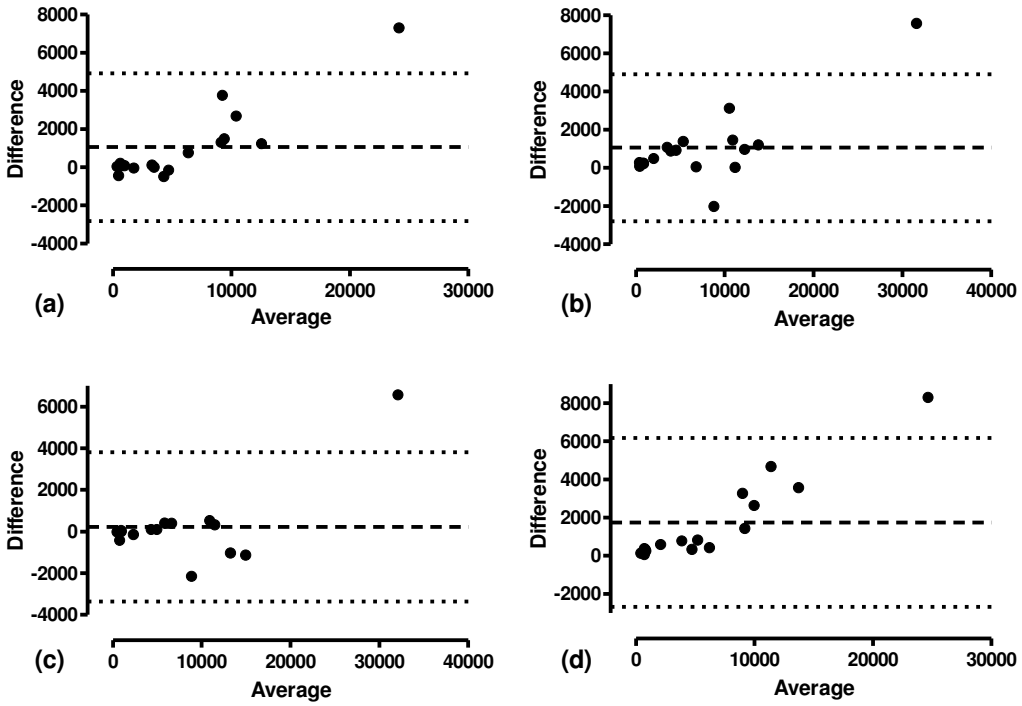


Figure 3-9. Bland-Altman plots comparing mean exhaled acetone concentrations (ppb) seen using each method (dashed line = bias, dotted lines = 95% limits of agreement): (a) T-piece after tidal breath minus T-piece after recruitment-style breath (bias 1056, -2812–4924), (b) airway after tidal breath minus T-piece after tidal breath (bias 1058, -2792–4908), (c) airway after tidal breath minus airway after recruitment-style breath (bias 221, -3366–3807), (d) airway after recruitment-style breath minus T-piece after recruitment-style breath (bias 1754, -2667–6175).

Breath acetone concentration following surgery

Twelve of the 20 subjects who provided breath sampled were admitted to the ICU following cardiac surgery (Table 3-5). Median end-tidal acetone concentration from T-piece samples in post-operative patients was higher than in patients who had not undergone surgery (6750 ppb, range 689–27800 ppb versus 867 ppb, range 261–8940 ppb) (p=0.01). There was no relationship between the duration of fasting and breath acetone concentration and no difference in breath acetone concentration between cardiac surgical patients who required cardiac bypass and those who did not.

Table 3-5. Cardiac surgical procedure and duration of fasting of post-operative patients enrolled.

Subject	Cardiac surgical procedure	Cardiac bypass?	Duration of fasting (hours)
1	AVR, CABG	Y	16
2	AVR, MVR	Y	16
5	CABG	N	13
6	CABG	N	11.5
7	AVR, CABG	Y	15.5
8	MV repair	Y	16
10	CABG	Y	14
13	Repair Type A dissecting aortic aneurysm	Y	20
15	CABG	N	17.75
16	CABG	N	10
18	AVR, Bentall procedure, CABG	Y	14.75
20	CABG	N	10.25

AVR, aortic valve replacement; Bentall procedure, composite graft replacement of aortic valve, aortic root and ascending aorta, with re-implantation of the coronary arteries into the graft; CABG, coronary artery bypass graft; MV, mitral valve; MVR, mitral valve replacement; N, no; Y, yes.

Corrected acetone concentrations

Median inspiratory acetone concentration corrected for losses due to the sampling apparatus was 149 ppb (range 87.0-427 ppb). Median corrected breath acetone concentrations can be seen in Table 3-6 below. The linear regression equation used for this correction is described in Chapter 2 of this thesis.

Table 3-6. Median corrected breath acetone concentration and intra-subject CV for breath acetone concentration using each breath sampling technique.

	Breath sampling method	Median acetone concentration (range) (ppb)	Median intra-method CV (IQR) (%)
A	T-piece after tidal breath	5198 (389-35248)	7.9 (3.9-13.1)
B	T-piece after recruitment-style breath	5128 (466-26008)	7.9 (5.6-12.8)
C	Airway after tidal breath	7020 (627-44826)	7.8 (4.1-16.5)
D	Airway after recruitment-style breath	6704 (647-36514)	5.9 (4.6-10.3)

CV, coefficient of variation; IQR, interquartile range.

3.3.4. Breath H_2S concentration

Individual patient breath H_2S concentration for each of the four sampling methods can be found in Appendix C. Group median exhaled H_2S concentrations using each of the breath sampling methods, as well as reproducibility data, can be seen in Table 3-7.

Table 3-7. Median breath H_2S concentration and intra-subject CV for breath H_2S concentration using each breath sampling technique.

	Breath sampling method	Median H_2S concentration (range) (ppb)	Median intra-method CV (IQR) (%)
A	T-piece after tidal breath	1.7 (0.7 - 4.2)	9.7 (7.3 – 12.7)
B	T-piece after recruitment-style breath	1.5 (0.7 - 3.9)	12.0 (8.3 – 16.4)
C	Airway after tidal breath	2.1 (0.7 - 4.7)	9.3 (6.7 – 15.5)
D	Airway after recruitment-style breath	2.0 (0.7 – 4.5)	9.8 (8.5 – 16.0)

CV, coefficient of variation; IQR, interquartile range.

There was a strong correlation between an individual's breath H_2S concentrations using each of the four breath sampling techniques (r_s>0.95 and p<0.0001 for each of the four analyses). There were small but significant differences in breath H_2S concentration between all four breath collection techniques. The concentration of H_2S in samples taken from the airways was higher than from the T-piece (after tidal breath p=0.009, after recruitment-style breath p=0.02), and in end-tidal samples compared to those collected after a recruitment-style breath (T-piece p=0.0006, airways p=0.0009) (Figure 3-10). No relationship was seen between breath H_2S concentration and breath volume when data from each sampling location was analysed (T-piece r_s= -0.08, p=0.62; airway r_s=0.07, p=0.71).

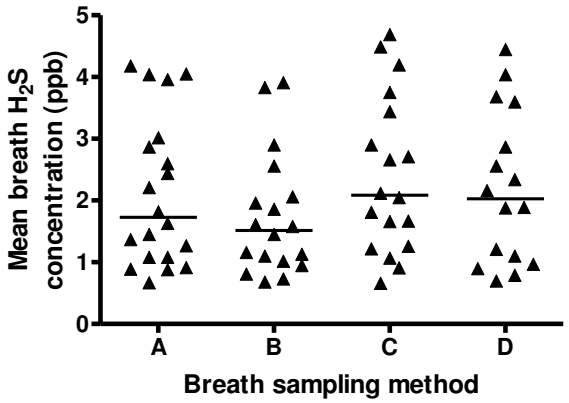


Figure 3-10. Individual mean patient end-exhaled H_2S concentrations and group medians using each of the four breath sampling methods.

Bland-Altman plots show a bias towards higher exhaled H_2S concentrations when sampling from the airways and using the end-tidal method; this did not change when the H_2S concentration measured increased (Figure 3-11).

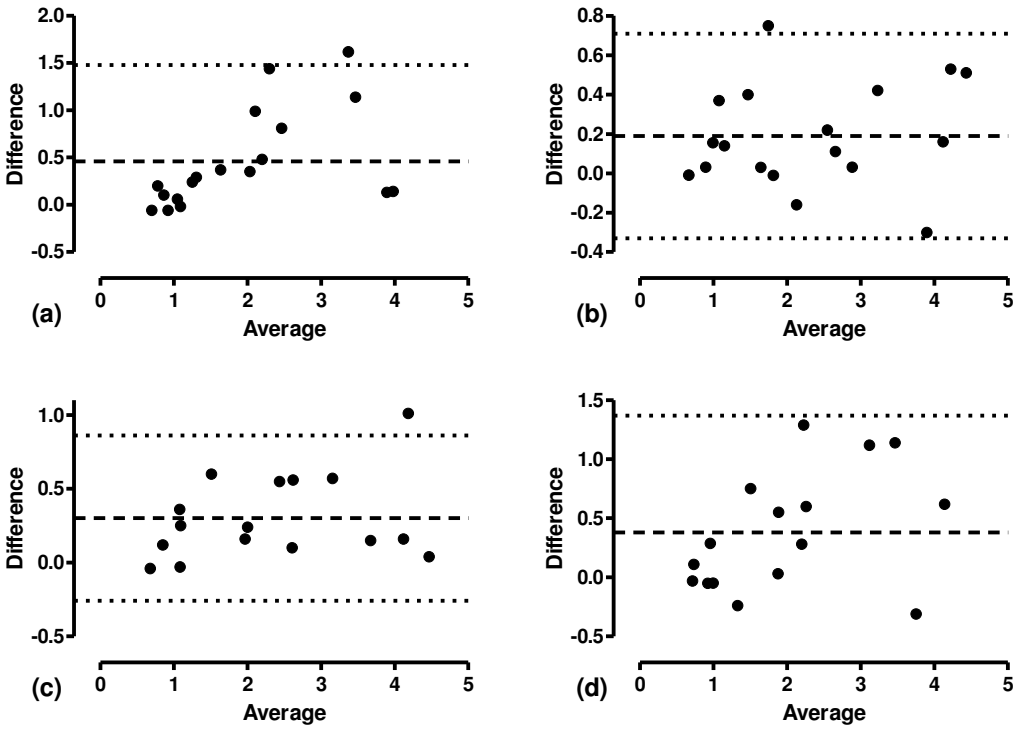


Figure 3-11. Bland-Altman plots comparing mean exhaled H_2S concentrations (ppb) seen using each method (dashed line = bias, dotted lines = 95% limits of agreement): (a) T-piece after tidal breath minus T-piece after recruitment-style breath (bias 0.46, -0.56–1.48), (b) airway after tidal breath minus T-piece after tidal breath (bias 0.19, -0.33–0.71), (c) airway after tidal breath minus airway after recruitment-style breath (bias 0.30, -0.26–0.86), (d) airway after recruitment-style breath minus T-piece after recruitment-style breath (bias 0.38, -0.61–1.37).

3.3.5. Breath HCN concentration

Individual patient breath HCN concentration for each of the four sampling methods can be found in Appendix C. Group median exhaled HCN concentrations using each of the breath sampling methods, as well as reproducibility data, can be seen in Table 3-8.

Table 3-8. Median breath HCN concentration and intra-subject CV for breath HCN concentration using each breath sampling technique.

	Breath sampling method	Median HCN concentration (range) (ppb)	Median intra-method CV (IQR) (%)
A	T-piece after tidal breath	1.7 (0.7–13.2)	11.8 (8.0–29.5)
B	T-piece after recruitment-style breath	1.2 (0.7–9.8)	14.5 (6.4–18.2)
C	Airway after tidal breath	2.1 (0.7–18.2)	12.8 (10.6–18.9)
D	Airway after recruitment-style breath	1.9 (0.7–14.1)	11.4 (3.6–21.8)

CV, coefficient of variation; IQR, interquartile range.

There was a strong correlation between an individual's breath HCN concentrations using each of the four breath sampling techniques (r_s>0.91 and p<0.0001 for each of the four analyses). Median breath HCN concentration was significantly higher in end-tidal samples compared to those after a recruitment-style breath at the T-piece (p=0.001). When samples were collected after a recruitment-style breath, the concentration of HCN was higher from the airways than the T-piece (p=0.0001). There was no significant difference in median HCN concentrations when comparing end-tidal breath samples at each location or the two breath manoeuvres when sampling directly from the airways (Figure 3-12). No relationship was seen between breath HCN concentration and breath volume when data from each sampling location was analysed (T-piece r_s= -0.07, p=0.67; airway r_s=0.19, p=0.30).

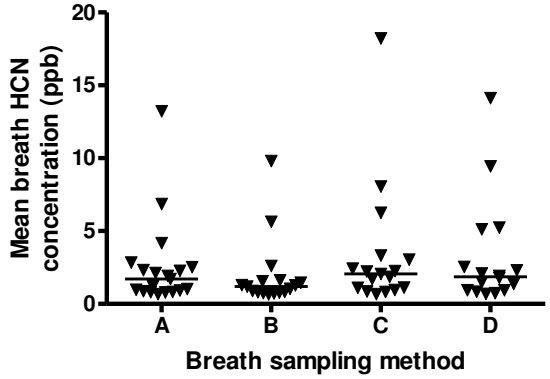


Figure 3-12. Individual mean patient end-exhaled HCN concentrations and group medians using each of the four breath sampling methods.

Bland-Altman plots show an increased variability between sampling methods at higher HCN concentrations. At lower HCN concentrations the sampling methods appear to provide similar results (Figure 3-13).

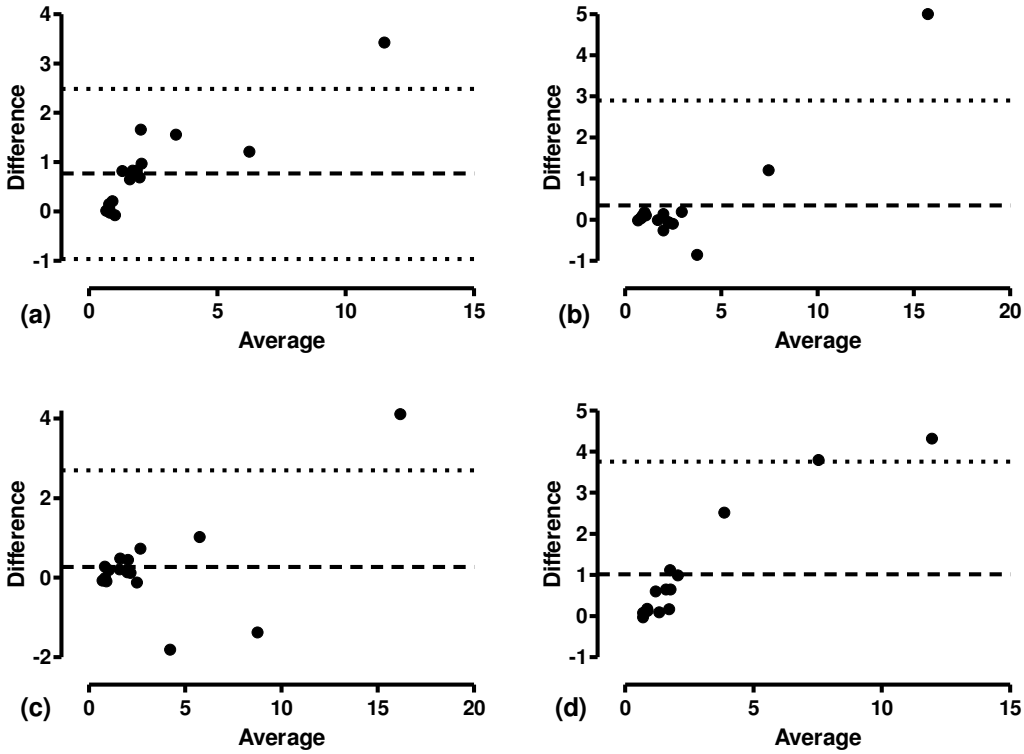


Figure 3-13. Bland-Altman plots comparing mean exhaled HCN concentrations (ppb) seen using each method (dashed line = bias, dotted lines = 95% limits of agreement): (a) T-piece after tidal breath minus T-piece after recruitment-style breath (bias 0.77, -0.96–2.49), (b) airway after tidal breath minus T-piece after tidal breath (bias 0.35, -2.20–2.90), (c) airway after tidal breath minus airway after recruitment-style breath (bias 0.27, -2.17–2.70), (d) airway after recruitment-style breath minus T-piece after recruitment-style breath (bias 1.02, -1.71–3.76).

3.3.6. Inspiratory volatile concentrations

Individual patient inspired VOC concentrations can be seen in Appendix C of this thesis. Group median concentrations and concentration ranges can be seen in Table 3-9 below. Relationships between inspiratory and exhaled breath concentrations were affected by the location and type of breath prior to sample

collection, therefore calculations for each of the breath sampling methods were required.

Table 3-9. Group median inspiratory volatile concentrations and ranges.

Volatile organic compound	Median inspiratory concentration (range) (ppb)
Acetone	71.7 (22.6-291)
H_2S	0.87 (0.42-1.62)
HCN	0.87 (0.66-1.30)

Acetone

Higher inspiratory acetone concentrations were seen when exhaled concentrations were higher, with a relationship seen between the two concentrations (r_s=0.50, p<0.0001). As the exhaled acetone concentration in 18 out of the 20 cases was at least one order of magnitude greater than the corresponding inspired acetone concentration, it is unlikely that inspired acetone concentrations had an effect on exhaled concentrations. It is more likely that higher breath acetone concentration led to greater contamination of the ventilator tubing due to adsorption.

Inspiratory samples were always obtained prior to collection of exhaled breath samples so that the collection apparatus did not become contaminated by higher breath compared to inspiratory concentrations.

Hydrogen sulphide

There was no relationship between inspired and exhaled H_2S concentrations when the results were analysed together or separately (T-piece after tidal breath r_s=0.15, p=0.54). Exhaled H_2S concentrations were significantly higher than inspired concentrations (T-piece after tidal breath: median exhaled concentration 1.7 ppb, median inspired concentration 0.87 ppb, p=0.0002).

Hydrogen cyanide

There was no relationship between inspired and exhaled HCN concentrations using breath collected from the T-piece after a tidal breath r_s=0.40, p=0.09. Exhaled HCN concentrations were significantly higher than inspired concentrations (T-piece after tidal breath: median exhaled concentration 1.7 ppb, median inspired concentration 0.87 ppb, p=0.0029).

Effect of the humidified circuit on inspired volatile concentrations

Humidification units were used in five of the 20 breathing circuits. This is fewer than would be expected in the ICU population; the majority of patients underwent elective surgery and were expected to be ready for extubation within several hours, therefore they were not felt to require humidified inspiratory air or oxygen by the ICU team.

The numbers in the humidified circuit group were low, however there did not appear to be any difference in inspiratory volatile concentration between breathing circuits containing a humidification unit or not (acetone $p=0.16$, H_2S $p=0.10$, HCN $p=1.00$).

3.3.7. External factors affecting measured breath volatile concentration

Closed suction catheter unit

When an intubated patient in the ICU has a communicable respiratory infection, it is necessary to replace the standard suction catheter mount with a closed suction catheter unit (KimVent: Turbo-Cleaning Closed Suction System for Adults, Kimberly-Clark, USA), to prevent droplet spread into the surrounding atmosphere. It was possible to replace one of the standard suction catheters with the closed suction catheter unit for breath sampling, as long as the valve was open (depressed) at the time of sampling (Figure 3-14). It was noticed, however, that breath acetone and HCN concentrations obtained via the closed suction catheter were much higher than expected, and decreased with each sample collected.

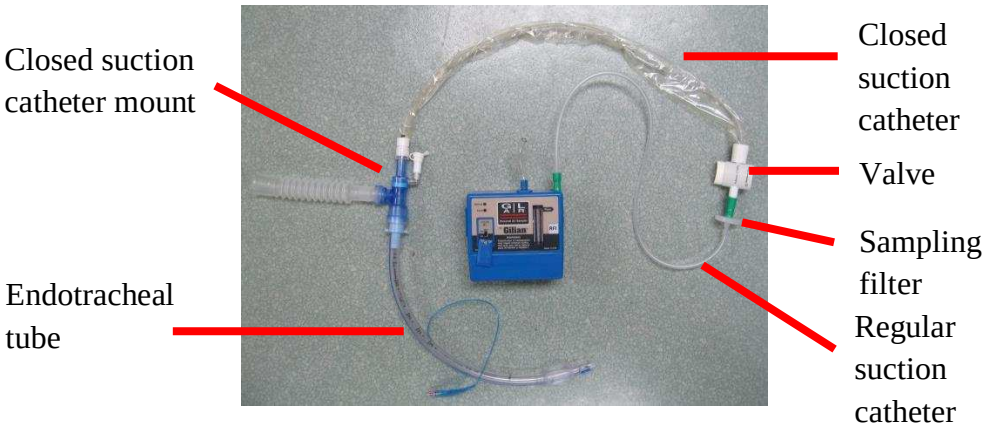


Figure 3-14. Breath sampling equipment attached to a closed suction catheter unit.

In an in vitro experiment, the headspace over a closed suction catheter unit was sampled after 30 min incubation at 40°C. Concentrations of acetone, H_2S and HCN were higher than background air levels (Table 3-10). Breath samples collected via the closed suction catheter unit were therefore discarded due to contamination by compounds emitted by the tubing itself. All other breath sampling equipment had been tested for the presence of volatiles at greater than atmospheric concentration prior to commencing the study (described in Chapter 2).

Table 3-10. Comparison of volatile concentrations in the headspace of the box (room air) before and after incubation of a closed suction catheter unit.

Volatile of interest	Headspace concentration (ppb)	
	Empty box	Box containing closed suction catheter
Acetone	19.7	3500
H_2S	0.7	5.2
HCN	1.1	38.6

Precursor ion stripping

At higher breath acetone concentrations, corresponding concentrations of breath H_2S and HCN also appeared to be higher (Figures 3-15 (a) and (b)). The increase in H_2S and HCN concentrations appeared to rise more steeply at concentrations of breath acetone over 10,000 ppb.

H_3O^+ precursor ion counts were examined in samples with very high acetone concentrations. In inspiratory samples, where acetone concentrations were

much lower, H_3O^+ counts were greater than 3,000,000 Hz. At acetone concentrations of 20,000 ppb, H_3O^+ counts were between 13,000 and 50,000 counts.

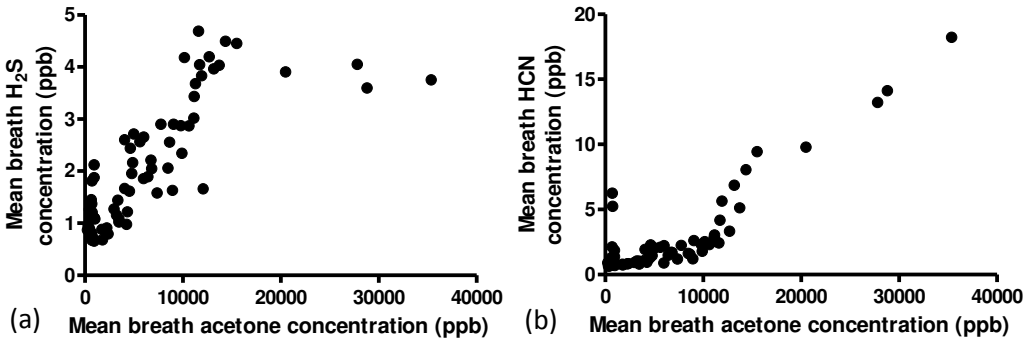


Figure 3-15. Individual data points show mean breath H_2S (a) and HCN (b) concentrations at matched acetone concentrations using all four sampling methods.

Salbutamol administration

Whilst collecting breath samples from a patient with asthma, salbutamol (Respigen™, Mylan New Zealand Ltd) was administered via a metered dose inhaler (MDI) through an adapter in the ventilator circuit adjacent to the T-piece. Acetone concentration in the samples after salbutamol administration was much higher than in previous samples, the concentration decreasing over subsequent samples. Both NO^+ and H_3O^+ precursor ions were depleted. Figure 3-16 shows the change in precursor ion counts in breath samples prior to and following salbutamol administration to the breathing circuit.

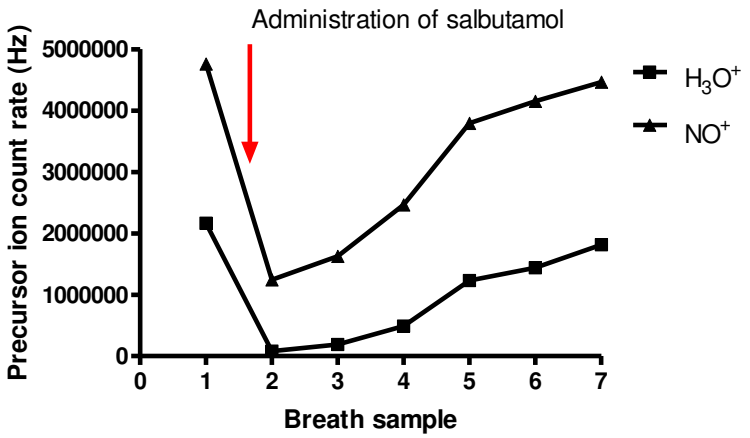


Figure 3-16. Reduction and subsequent increase in precursor ion counts following salbutamol administration via the breathing circuit.

To explore this phenomenon further, an in vitro experiment was performed, whereby salbutamol was sprayed into a glass jar and the headspace analysed. Initially it was not possible to analyse the sample at all due to a complete lack of precursor ions; the ion count gradually increased over time. At low precursor ion counts, the concentrations of compounds analysed were falsely elevated. In full mass scan mode using the H_3O^+ precursor ion, a large ion peak was seen at m/z 93 corresponding to the product of the reaction between ethanol and H_3O^+ ($(C_2H_6O^+)_2.H^+$), which is known to be a co-solvent in salbutalmol MDIs[326] (Figures 3-17 and 3-18).

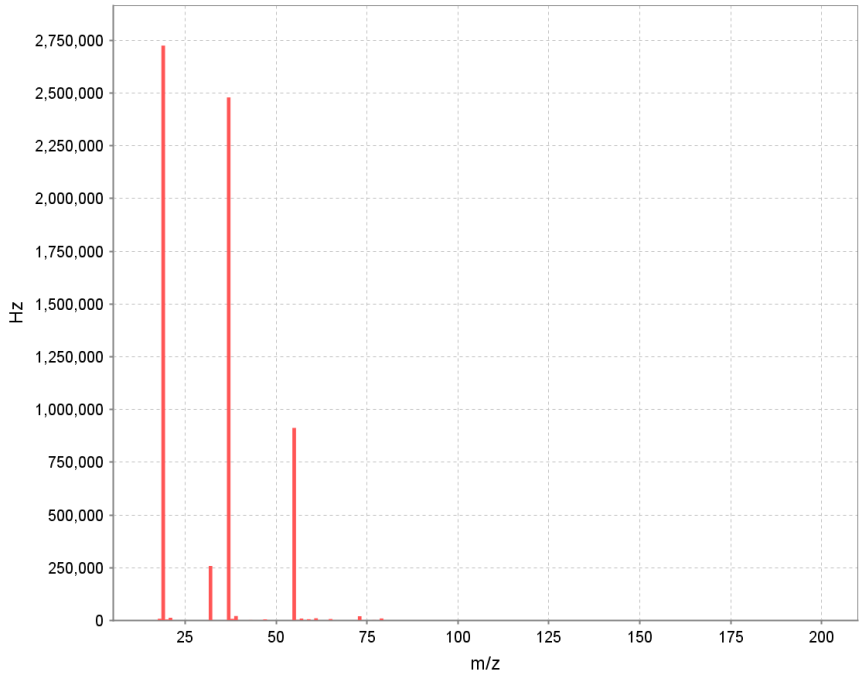


Figure 3-17. Mass spectrum of the empty glass sampling container analysed using the H_3O^+ precursor ion. The main peak at m/z 19 is the H_3O^+ precursor ion. The other two large peaks are the hydrated forms of the H_3O^+ precursor ion.

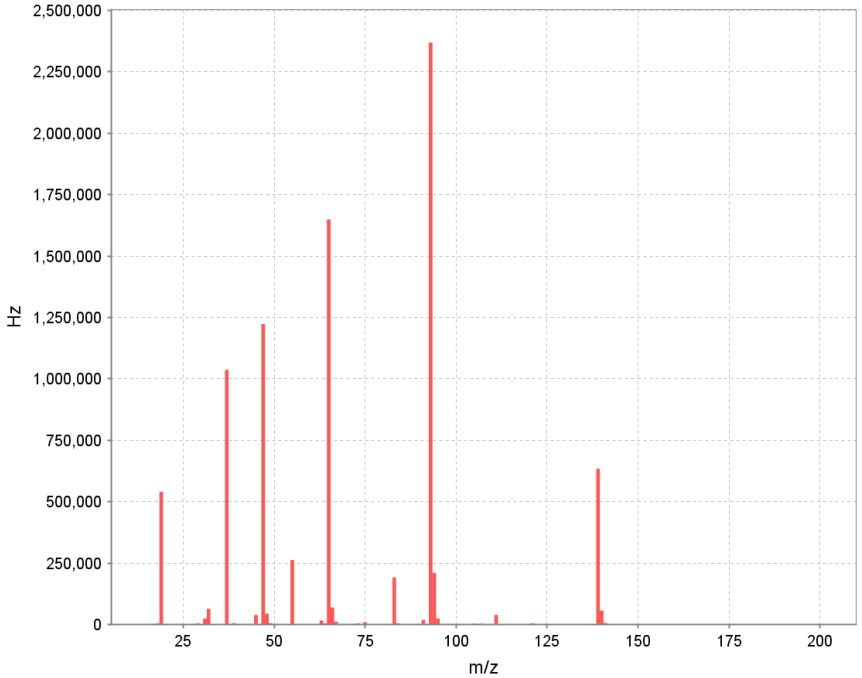


Figure 3-18. Mass spectrum of salbutamol sprayed into a glass sampling container analysed using the H_3O^+ precursor ion. Main peak at m/z 93 is the product of the reaction between ethanol and H_3O^+.

3.4. Discussion

This is the first study to explore the differences in VOC concentrations of breath samples obtained from two sampling sites following two breathing manoeuvres in intubated and ventilated patients in the ICU. End-tidal breath sampling from the T-piece produced less variability in breath volume, lower or comparable variability in volatile concentrations, similar mean breath volatile concentrations, and was technically the easiest to perform, therefore it was the favoured method. Equipment routinely available in the hospital setting was used in each of the breath sampling methods, enabling replication of this technique in clinical practice.

3.4.1. Breath sampling location

It is preferable to collect samples from as close to the airways as feasible to minimise dilution or contamination by components of the breathing circuit[7,147,166]. Analyte concentrations of breath samples collected directly from the airways were generally higher than those collected from the T-piece in the breathing circuit. This is likely to be predominantly due to increased dilution by dead space gas in samples from the T-piece. Unfortunately, despite sealing around the suction catheter in the mount when sampling from the airway, small air leaks required adjustments in PEEP to maintain airway pressure and tidal volume. Additionally, in a few patients, significant air leak around the suction catheter was observed and direct airway sampling was abandoned because tidal volumes could not be maintained adequately. In extreme cases this could have had detrimental consequences in certain patients on the ICU, for example those with severe pneumonia, when periods of reduced PEEP and tidal volume may lead to de-recruitment of airways and hypoxia. When sampling from the T-piece there was little reduction in PEEP, therefore no need to alter ventilator settings, making sampling from this location quicker, simpler and safer.

The suction catheter mount used in this study is widely available and designed to hold a suction catheter when removing secretions from the airway. A small amount of air leak is tolerated during this procedure, however the suction catheter must remain in the mount for longer when obtaining breath samples. It would be possible to design an attachment for the ventilator circuit that would

have a tighter seal to prevent air leaks, but this may well be expensive and not as freely available, thus the collection method could not be performed in routine clinical practice. If there was an alternative attachment readily available, sampling from the airway would be more attractive.

3.4.2. Breathing manoeuvre

Pressure-controlled breaths were used to deliver a recruitment-style breath to each patient, replicating a vital capacity breath. A similar technique is used routinely in the ICU to recruit collapsed airways and improve oxygenation and was favoured over volume-controlled large volume breaths due to lower risk of barotrauma[327]. In most cases, a recruitment-style breath of larger volume could be delivered when sampling from the T-piece than from the airway in the same person, as increased air leak around the suction catheter attenuated the increase in breath volume. Unfortunately, in one case, the recruitment-style breath volume delivered when sampling via the airway was smaller than the corresponding tidal breath volume when sampling via the T-piece. There was no added benefit in terms of repeatability of breath acetone concentration after the delivery of a recruitment-style breath rather than a tidal breath at the T-piece, and a modest improvement during airways sampling, however there were difficulties in sampling via this technique. Firstly, it was sometimes necessary to change the ventilation mode, which required knowledge of the ventilator set-up. Secondly, a wide range of recruitment-style breath volumes was produced, thus the change in breath acetone concentration due to change in breath volume was difficult to evaluate. Only small breath volume increases from tidal volume were possible when the IPAP required for on-going ventilation was close to 35 cmH_2O. Despite a significant difference in breath volume using each of the breathing manoeuvres the difference in breath VOC concentrations was small.

Variability in breath acetone concentration due to breath volume was less than that caused by other factors, notably surgical stress; therefore it was difficult to quantify the effect of breath volume on breath acetone concentration. As with other VOCs that are highly soluble, for example ethanol, larger breath volumes

appeared to cause a reduction in breath acetone concentration due to the "wash-in, wash-out" phenomenon[154].

3.4.3. Breath volume and flow rate

Unlike on-line studies, the use of an off-line method did not allow real-time evaluation of breath concentrations[106,207,209,210] or the ability to select a particular phase of the respiratory cycle of interest[209,210]. The timing of each breath sample at the very end of expiration did, however, enable the first portion of an exhalation to be delivered to the ventilator tubing as dead space gas. In comparison to off-line collection methods that require sample pre-concentration, analysis of breath samples by SIFT-MS is rapid and avoids potential sample loss or contamination during adsorption and desorption processes[328]. Results obtained using the end-tidal breath collection technique via a T-piece, with analysis as described, should also be comparable to on-line analysis techniques reporting end-exhaled breath concentrations. Sample losses due to the collection apparatus can be quantified and are discussed in Chapter 2 of this thesis. No other authors have published quantified losses due to the sampling method, either in on-line or off-line sampling.

In contrast to previous studies[159,168], there was no relationship between breath acetone concentration and breath volume. This could be a result of our sampling technique because, when collecting breath samples as described in this study, we could not predict residual volume and therefore 150 ml breath samples represent a different percentage of that volume in different patients. There was no relationship between breath H_2S or HCN concentrations and breath volume, which is consistent with previous studies[151,223].

Exhalation in intubated patients ventilated on controlled modes is passive and therefore it is not possible to control the exhalation flow rate. The flow rate of the hand-held pump during breath collection was constant at 1.8 L/min and sample collection was timed, thus ensuring a constant adequate volume of breath for analysis by SIFT-MS. The pump used could not run faster than this rate, however the flow rate of the suction units attached to the wall in the ICU was around 4.8 L/min. This suggests that higher pump flow rates would be suitable for breath sample collection as they would not cause lung trauma, as

long as they did not induce coughing, which would affect sample collection. Unlike breath concentrations of nitric oxide[172], the concentrations of breath acetone, H_2S and HCN do not appear to be flow rate dependent[151,168,223].

Ventilatory pattern[169] and cardiac output[1,2,97,176], as well as degree of pulmonary shunt[174,176], have been shown to affect breath volatile concentrations, and may have played a part in determining the measured breath VOC concentrations in this study. The effect of these variables, other than breath volume, on an individual's breath analyte concentrations was not assessed, as these variables did not change significantly over the duration of breath sampling and it was important that the patient's care was disrupted as little as possible by the breath sampling technique. Patients acted as their own controls, thus inter-subject changes due to the different breath sampling techniques were more important than inter-subject breath volatile concentrations.

3.4.4. Breath acetone concentration

Previous studies have shown a high degree of inter-person variation in breath acetone concentration[5,167,168], which is confirmed in this study. In this patient group other factors, for example degree of metabolic stress associated with sepsis and surgery, are likely to account for the higher degree of inter-person variability in breath acetone concentrations compared to intra-person variability as a consequence of the breath collection method. As mentioned previously, inter-person variation in breath acetone concentration was less important in this study as each patient acted as their own control when comparing each of the four breath collection methods.

The mode of ventilation (SIMV versus Bi-Level) was determined by ICU staff. In general, patients who had just returned from the operating theatre were ventilated on SIMV and those with respiratory compromise were ventilated on Bi-Level. Medical patients were fed via a nasogastric tube, not fasted, and had lower breath acetone concentrations. Post-operative patients underwent breath sampling within four hours of arriving in the ICU from the operating theatre and their high breath acetone concentrations are likely to be a combination of fasting[194,196,225] and metabolic stress caused by surgery[105].

3.4.5. Breath H_2S and HCN concentrations

Breath concentrations of H_2S and HCN in this study were very small; all observed H_2S concentrations were less than 5 ppb and less than 20 ppb for HCN. Differences due to sampling technique were, therefore, also very small and although statistically significant were probably not clinically significant. Only one patient was a current smoker, and although breath HCN concentrations are known to be raised due to cigarette smoking[191], it was not possible to assess the effect of smoking on breath HCN concentrations in this study.

High concentrations of endogenous compounds in breath, in this case acetone, can affect the calculated concentration of volatiles by increasing the number of reactions with precursor ions and depleting the precursor ion count rates. Product ion count rates of the lower concentration volatiles should not be affected, but the ratio of product ions to precursor ions is affected, leading to the generation of a higher than would be expected calculated concentration. It may, therefore, be necessary to look at product ion count rates and not calculated concentrations if a compound at a very high concentration is also present in the breath sample. It is possible that high H_2S and HCN concentrations seen in the breath of subjects with high acetone concentrations were due to precursor ion stripping, although it is also possible that those with higher breath acetone concentrations had greater levels of metabolic stress and inflammation and this was reflected in true increases in breath H_2S and HCN. Again, the absolute concentrations of H_2S and HCN were less important than changes in their concentrations due to different sampling methods, as each subject acted as their own control.

3.4.6. Effect of inspiratory on exhaled volatile concentrations

There was no relationship between inspired and exhaled volatile concentrations, and in all cases the median exhaled concentration was greater than the median inspired concentration. The inspired concentrations of H_2S and HCN were almost always greater than 25% of exhaled concentrations, however, and should therefore be treated with caution[2,8]. Scrubbing inspired air to remove VOCs of interest can be difficult, time consuming and not always

reliable[93,147], especially if it is being delivered via a ventilator. It was therefore not attempted in this study where it was not felt to be particularly important, as inspired VOC concentrations did not appear to interfere with breath concentrations. There did not appear to be any difference in inspired volatile concentrations when a humidification device was added to the breathing circuit.

3.4.7. Sample loss and contamination

It is possible that condensation of water vapour occurred in the Tedlar bags or other parts of the equipment at the time of breath collection due to using cold equipment. This may have altered observed sample concentrations, however samples were immediately incubated and analysed as soon as possible to minimise any such losses. The pump was flushed after use and other equipment disposable or cleaned to prevent contamination of future samples. The effect of the sampling apparatus on breath VOC concentrations is important so that it can be taken into account when comparing results from different studies using different sampling techniques, as previously described in Chapter 2 of this thesis.

In a previous study, chlorofluorocarbons (CFCs) present in inhalers used to deliver bronchodilator drugs produced characteristic peaks when measured by SIFT-MS in vitro and in vivo[107], which interfered with the quantification of substances with product ions at the same mass/charge ratios. Salbutamol MDIs, however, no longer contain CFCs as propellants and instead use hydrofluoroalkanes (HFAs) but can still cause problems when analysing breath samples. In order for the active compound to remain dispersed, ethanol must also be present as a co-solvent. The concentration of ethanol present in different preparations of salbutamol (Salamol® and Airomir®) has led to positive roadside alcohol breath tests[329] and two investigations show that after two puffs of salbutamol it is necessary to wait 3 min in order to pass the alcohol breath test[330,331]. It is likely that the high concentration of ethanol present in the Respigen™ formulation of salbutamol in this case depleted the precursor ions by reacting with them, affecting the concentration of analytes measured. It would therefore be prudent to wait at least 10 min after a subject

has used inhaled medication before performing breath tests, which includes collecting breath samples from a ventilator circuit.

3.4.8. Sample size

One final limitation of the study was the sample size. There was a higher degree of inter-subject variability in breath volatile concentration, particularly acetone, than expected; some patients having much higher acetone concentrations than reported in previous studies. This meant that intra-subject changes in volatile concentration due to breathing manoeuvre and location were smaller than inter-subject differences caused by patient factors. Importantly, patients acted as their own controls, which enabled differences for each of the four sampling methods to be seen. A larger sample size may have helped to separate further the differences in volatile concentrations due to sampling techniques.

3.4.9. Summary

Although measured analyte concentrations were slightly lower than with direct airway sampling, there was comparable intra-subject reproducibility when sampling from the T-piece. End-tidal breath sampling via the T-piece was the simplest technique and was able to be successfully carried out in all patients, so this method of sampling is recommended. In subsequent parts of this thesis, the method of end-tidal breath collection via a T-piece will be employed. It is important to be aware that high concentrations of analytes, even if they are not of interest in terms of quantification, can affect the way in which VOC concentrations are calculated by SIFT-MS, and these should be minimised as much as possible.

4. Off-line end-exhaled breath testing in healthy anaesthetised patients

4.1. Introduction and aims

Ideally, when choosing a group of subjects to act as controls, consideration should be paid to the ability to collect and analyse all breath samples using the same technique to minimise the number of variables. There is no suitable control group of intubated and ventilated patients in the ICU with which to compare patients with critical illness, as respiratory, cardiac or neurological failure, including that induced by drugs, may all affect breath volatile concentrations. Intubated and ventilated patients undergoing surgical procedures constitute a group of healthy individuals from whom baseline concentrations of exhaled volatiles could be collected. By sampling following intubation, but prior to the start of a surgical procedure, breath VOC concentrations will not be affected by surgery itself.

Anaesthesia machines are designed for rebreathing of exhaled gases, including volatile anaesthetic agents, unlike ventilators in the ICU that vent exhaled gases to the atmosphere and deliver fresh medical air/oxygen directly into the breathing circuit on every inspiratory breath. The recycling of exhaled breath into the inspiratory limb of the anaesthesia ventilator allows the concentration of anaesthetic volatiles to be controlled, so that equilibrium can be reached between breath and plasma concentrations. The oxygen content of the gas mix is altered by adding as much medical oxygen as necessary and CO_2 is removed from the breathing circuit by soda lime granules. When breath sampling in the operating theatre, it is necessary to use higher inspired gas flow rates than sometimes used during anaesthesia to prevent rebreathing of endogenous volatiles, which may affect measured breath concentrations. The use of total intravenous anaesthesia (TIVA) not only prevents potential contamination with anaesthetic volatiles, but also allows the gas flow rate to be increased without using large quantities of anaesthetic gases, which are not only expensive but have been argued to be damaging to the environment[332,333].

It was the intention in this study to develop an off-line sampling technique for use in anaesthetised intubated patients, comparable to the end-tidal breath collection technique via a T-piece described in Chapter 3 of this thesis, using equipment readily available in the hospital environment. As with the method developed in the ICU, the physical location of the SIFT-MS device necessitated an off-line discontinuous technique. Acetone was selected as a model compound to validate the breath collection technique as it is present in human breath in large quantities and can be easily and accurately measured using SIFT-MS[168]. Whilst altering the anaesthesia machines so that they behaved like ICU ventilators, it was necessary to investigate the effects of breath sampling from either side of the filter in the breathing circuit, altering the inspiratory gas flow rate, and the position of the adjustable pressure limiting (APL) valve on inspiratory and exhaled acetone concentrations, as well as the reproducibility of these measurements. The final aim was to provide control concentrations of acetone, H_2S and HCN for comparison with breath samples obtained from intubated and ventilated patients in the ICU.

4.2. Methods

4.2.1. Subjects and anaesthesia

The study procedures were approved by the Upper South A Regional Ethics Committee, New Zealand. Twenty-six elective and semi-elective, non-diabetic, adult patients awaiting various surgical procedures at Christchurch Hospital, for which endotracheal intubation was indicated, consented to take part. Patients were recruited during October and November 2011. Immediately prior to the surgical procedure, subjects performed spirometry and peripheral venous blood was obtained and sent to Canterbury Health Laboratory for analysis of fasting glucose, ketones (acetone, beta-hydroxybutyrate), renal function (urea, creatinine) and inflammatory marker status (WCC and CRP). The sample for acetone testing was immediately put on ice with subsequent laboratory analysis of the headspace by gas chromatography. The analysis of H_2S concentration in blood samples was carried out at Canterbury University, Christchurch, New Zealand, using a protocol described by Zhang et al.[334] as there is no commercially available assay. Blood samples had to be prepared for storage at

Christchurch Hospital before transfer to Canterbury University in batches; whole blood samples were centrifuged and the plasma immediately frozen at -25°C, with subsequent freezing at -80°C. Past medical history, drug history, duration of fasting prior to surgery and planned surgical procedure were noted for each subject.

The breath sampling technique required little change to standard anaesthetic practices; patients were pre-oxygenated before induction of anaesthesia with propofol and an opiate chosen by the anaesthetist. A paralysing agent was given before intubation. Two different anaesthesia machines were used in the operating theatres; Aisys Carestation (GE Healthcare/Maddison, Helsinki) and ADU Carestation (Datex-Ohmeda/GE Healthcare, Helsinki). Each patient was ventilated using a circle breathing system. Ventilator settings were determined by the anaesthetist, commonly a tidal volume of 500 ml and respiratory rate of 10 breaths per minute. Unless otherwise stated, the inspiratory gas flow rate was set at 6 L/min and the APL valve was in the fully open position. End-tidal CO_2 was monitored continuously along with all other routine monitors. Data was collected on ventilatory parameters (FiO_2, respiratory rate and tidal volume).

4.2.2. Exhaled breath collection apparatus

A schematic of the apparatus is shown in Figure 4-1. A T-piece (oxygen enrichment attachment, Respironics Inc, USA) was inserted into the breathing circuit at the ventilator gas outlet to facilitate inspiratory gas sampling (Figures 4-2(a) and (b)). Another T-piece, connected to an adaptor (Care Medical Ltd, NZ), was inserted between the endotracheal tube and breathing circuit filter (Inmed Manufacturing Sdn Bhd, Malaysia) (Figure 4-3)). As described in Chapter 3, two suction catheters with a sampling filter between them were connected at one end to the side port of the T-piece using the cut-off tip of a 1 ml syringe and at the other end to a hand-held pump.

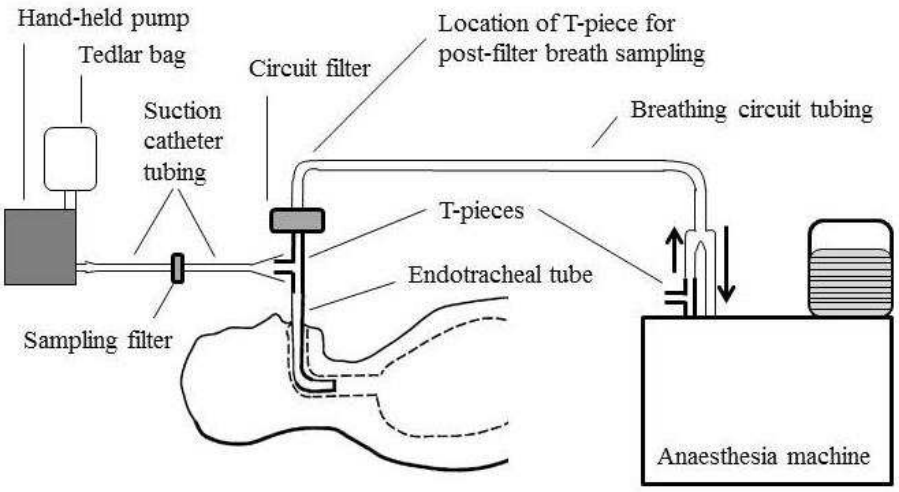


Figure 4-1. Schematic of the experimental setup for pre-filter breath sampling. Arrows show direction of gas flow.

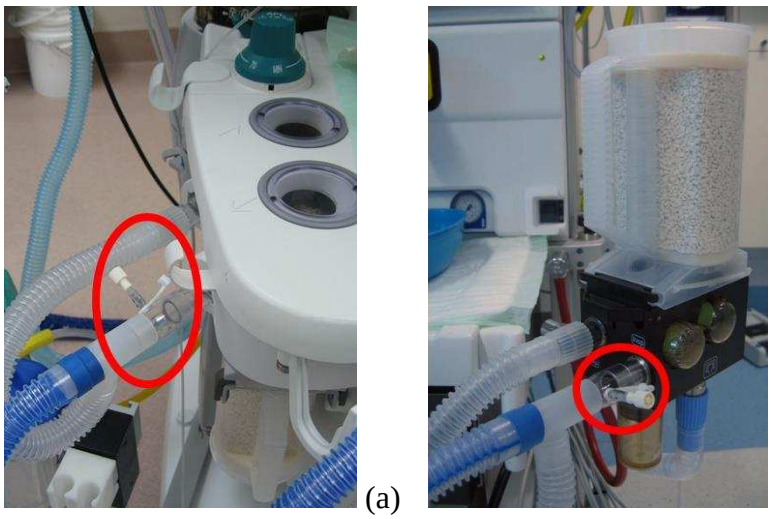


Figure 4-2. Photographs show a T-piece (circled) within the inspiratory limb of the breathing circuit; (a) Aisys Carestation and (b) ADU Carestation anaesthesia machines.

Figure 4-3. Photograph showing the T-piece and adaptor (circled) adjacent to the endotracheal tube (right) and circuit filter (left).

4.2.3. Exhaled breath collection

The apparatus was assembled as above. The ventilator was switched off at the end of expiration, timed by observing the position of the bellows, for 4 sec whilst a sample of approximately 150 ml of breath was collected into a 1 L transparent Tedlar® bag (SKC Inc, USA) via the pump. The ventilator was switched back on and several standard breaths were delivered. The process was then repeated until three samples had been collected into separate Tedlar bags.

All breath samples were obtained prior to the start of the surgical procedure and incubated at 40°C prior to analysis. All but one set of samples were analysed within 30 min of collection by SIFT-MS. A pneumotachometer (RSS 100, Hans Rudolph Inc, USA) was inserted into the respiratory circuit to confirm the direction of flow during sample collection was from the patient and not the distal breathing circuit. The pump was flushed by running through room air for at least 2 min between patients.

4.2.4. The effect of the breathing circuit filter

To investigate whether the filter located in the breathing circuit had any effect on the concentration of acetone detected in breath samples, triplicate end-expiratory breath samples were collected, as described above, from T-pieces located on each side of the circuit filter. Pre-filter sample collection was carried out in 22 patients and post-filter sample collection in seven patients, including three patients for whom samples were collected from both sides of the filter.

4.2.5. The effect of altering the inspiratory gas flow rate

In all patients the flow rate was set at 6 L/min during the sampling process, however, to explore whether varying the inspiratory gas flow rate would affect inspiratory and/or exhaled acetone concentrations the effects of four different flow rates were investigated. In two patients, different flow rates were investigated by collecting an inspiratory gas sample and triplicate end-expiratory breath samples. For patient A, the flow rates tested were 2 L/min, 6 L/min, 10 L/min, and 15 L/min. For patient B, the same flow rates, but in descending order, were tested. A 2 min equilibration period was used between each different flow rate.

4.2.6. The effect of the adjustable pressure limiting (APL) valve

The APL valve is adjusted during manual ventilation; closing the valve allows the build-up of pressure and inflation of the manual ventilation bag, as well as delivery of the contents of the bag to the patient. When fully open, no gas is allowed to build up in the circuit and any excess gas is vented to ventilator scavenging system. The valve can be positioned partially or fully open, or closed (Figure 4-4). During mechanical ventilation it is not important whether the APL valve is open or closed as it is no longer part of the ventilation circuit, however it is routinely fully opened. In order to obtain end-expiratory breath samples as described above, the ventilator had to be switched off, diverting the breathing circuit to the manual ventilation bag. Because of this, the position of the APL valve during sampling becomes relevant, and closing the value might be expected to cause unquantifiable dilution of the sample. To confirm that closing the valve would lower the concentration in the sample, breath was collected with the valve fully open (usual position during mechanical ventilation) and partially closed (a position chosen by the anaesthetist).


(a)

(b)

Figure 4-4. Photographs display the location of the adjustable pressure limiting (APL) valve (red circle) in the Aysis Carestation (a) and ADU Carestation (b) anaesthesia machines.

4.2.7. Infection control

The suction catheters and sampling filter were disposable and used for only one set of samples. The T-piece and adapter were decontaminated for re-use. Disinfectant wipes were used on the surfaces of the hand-held pump and Tedlar bags (Azo wipes, W. M. Bamford & Co Ltd, NZ). None of the cleaning procedures changed the VOC composition of samples.

4.2.8. Selected ion flow tube mass spectrometry

Breath volatile concentrations were measured by SIFT-MS, Voice200® (Syft Technologies Ltd, NZ) as described in Chapter 2.2.6. of this thesis. Bag samples were attached to the heated inlet extension of the SIFT-MS and analysed for 30 sec.

4.2.9. Statistical analysis and calculations

There was limited previous work on which to base a calculation of sample size in order to determine differences in breath volatile concentrations between controls (patients in this investigation) and patients in the ICU (subsequent investigations in this thesis). No other breath studies in the operating theatre examine the volatiles of interest here along with off-line analysis without pre-concentration of samples. Intra-subject, inter-day and intra-day CVs for exhaled acetone in a previous study of non-intubated and ventilated healthy

volunteers were between 0% and 5.6%[168]. The sample size chosen was equivalent to that of the first investigation in this thesis, looking for clinically significant change to plan future studies.

Means and percentage CVs of three end-exhaled breath samples for each patient were calculated. Comparisons were made using Mann-Whitney U tests for unpaired and Wilcoxon signed rank tests for paired data. Correlations were determined by Spearman's rank correlation coefficient (r_s). Statistical analyses were performed using GraphPad Prism version 5.01 for Windows (GraphPad Software, USA). A value of $p<0.05$ was considered to be statistically significant.

Inspired and exhaled acetone concentrations were adjusted for losses caused by the sampling apparatus using the linear regression equation formulated in Chapter 2.3.2. Due to very low concentrations observed, H_2S and HCN concentrations were not adjusted and measured concentrations are displayed.

4.3. Results

4.3.1. Patients

Nine male and 17 female patients were recruited in total, median age 60 years (range 22-91 years). Patient demographics and ventilator settings are shown in Table 4-1. Interpretable spirometry results were available for 17 patients. Of these, 15 were normal and two showed an obstructive pattern (one mild, one moderately severe[335]). Two patients were current smokers and five confirmed ex-smokers. Ethnicity data reflects that of the wider Christchurch population[325]. All patients had normal fasting glucose, renal function and inflammatory marker status. Median duration of fasting prior to breath sampling was 12 hours (range 6.5-17.8 hours). Following breath collection, patients underwent a variety of surgical procedures; twelve laparoscopic procedures, six open abdominal or pelvic procedures and seven others. Breath samples were collected prior to commencement of the operation, so no influence on breath biomarkers as a result of operation type could be determined.

Table 4-1. Subject characteristics and ventilation data.

Subject	Gender	Age (years)	Ethnicity	Smoking status	Surgical procedure	FEV_1 (L) (% predicted)	FVC (L) (% predicted)	FEV_1/FVC (%)	Tidal volume (ml)	RR (breaths per min)	$P_{et}CO_2$ (mmHg)
1	m	44	NZ European	N	Abdominal wall	4.86 (116)	5.94 (115)	82	500	10	42
2	m	64	NZ European	N	Open abdominal	3.52 (111)	3.86 (95)	91	500	12	43
3	m	79	NZ European	Ex	Open abdominal	2.92 (112)	3.61 (103)	81	500	10	39
4	m	54	NZ European	N	Hand surgery	3.64 (99)	4.61 (100)	79	500	10	34
5	m	29	NZ European	N	Open abdominal				550	10	38
6	m	66	NZ European	N	Laparoscopy	3.88 (124)	5.49 (136)	71	450	10	37
7	m	69	NZ European	N	Laparoscopy	2.58 (80)	3.29 (78)	79	500	10	40
8	m	64	NZ European	Ex	Open abdominal	3.50 (103)	4.63 (106)	76	500	12	36
9	m	29	NZ European	Ex	ENT	3.62 (86)	4.74 (95)	76	500	10	38
10	f	40	NZ European	N	Laparoscopy	2.66 (92)	3.29 (99)	81	500	10	35
11	f	40	NZ European	N	Laparoscopy				400	10	36
12	f	34	Maori	Smoker	Laparoscopy				550	10	40
13	f	57	Polynesian	N	Open pelvic	1.65 (79)	2.28 (92)	72	450	10	43
14	f	41	Maori	N	Laparoscopy				500	10	40
15	f	69	NZ European	Ex	Hand surgery				425	8	38
16	f	48	African	N	Laparoscopy				500	12	34
17	f	43	Maori	Smoker	Laparoscopy	2.99 (98)	3.73 (106)	80	550	10	39
18	f	91	NZ European	N	Breast surgery	0.85 (52)	1.39 (63)	61	450	10	40
19	f	62	NZ European	N	Breast surgery	1.17 (54)	2.34 (90)	50	500	10	38
20	f	88	NZ European	N	Breast surgery				400	10	42
21	f	46	NZ European	N	Laparoscopy	3.00 (108)	4.07 (126)	74	500	10	33
22	f	73	NZ European	N	Open abdominal				500	10	32
23	f	65	NZ European	Ex	ENT	1.42 (84)	2.66 (129)	53	450	10	39
24	f	22	NZ European	N	Laparoscopy				450	10	45
25	f	26	Asian	N	Laparoscopy	2.88 (87)	3.80 (100)	76	500	10	35
26	f	37	Caucasian	Ex	Laparoscopy	3.23 (101)	3.83 (104)	84	500	12	33

Ex, ex-smoker; N, non-smoker; ENT, Ear, Nose and Throat surgery; FEV_1, forced expiratory volume in one second; FVC, forced vital capacity; Tidal volume delivered by the ventilator; RR, respiratory rate; $P_{et}CO_2$, partial pressure of end-tidal CO_2.

4.3.2. The effect of the breathing circuit filter

Median CV for breath acetone concentration in samples collected from the patient side of the filter was lower than in samples taken from the distal side (pre-filter CV 8.3% (IQR 6.9–14.5%) n=22, post-filter 10.1% (IQR 6.8–14.1%) n=7).

Data was available for three patients who had samples collected from both sides of the biological filter, making it possible to compare the absolute change in concentration. Acetone concentrations were higher when measured on the patient side of the filter (Table 4-2).

Table 4-2. Difference in mean exhaled breath acetone concentration between samples taken from either side of the biological filter in the respiratory circuit.

Patient	Mean acetone concentration (ppb)		Change in acetone concentration	
	Patient side of filter	Anaesthesia machine side of filter	ppb	%
2	303	205	98	32
3	386	366	20	5
13	157	65	92	59

The reason for the change in acetone concentration caused by the breathing circuit filter was explored in greater detail in the laboratory. Test gases of 100% relative humidity were produced using dilution apparatus (Syft Technologies Ltd, NZ), a permeation chamber (Dynacalibrator Model 150, VICI Metronics, USA) and permeation tubes of acetone (Kin-tek, USA). Gas mixtures containing different concentrations of acetone were individually passed into the SIFT-MS instrument for quantification of acetone, then passed through an identical filter to those used in the operating theatre breathing circuits and the acetone concentration again analysed directly via SIFT-MS (Figure 4-5). The concentration of acetone once it had been passed through the filter was initially lower than the pre-filter acetone concentration, but increased over 15 sec to a plateau equivalent to the pre-filter concentration. The filter had a dead-space volume of 50 ml. We therefore surmise that the reduction in acetone concentration across the filter observed in patients was due to a dilutional/dead space effect rather than absorption across the filter.

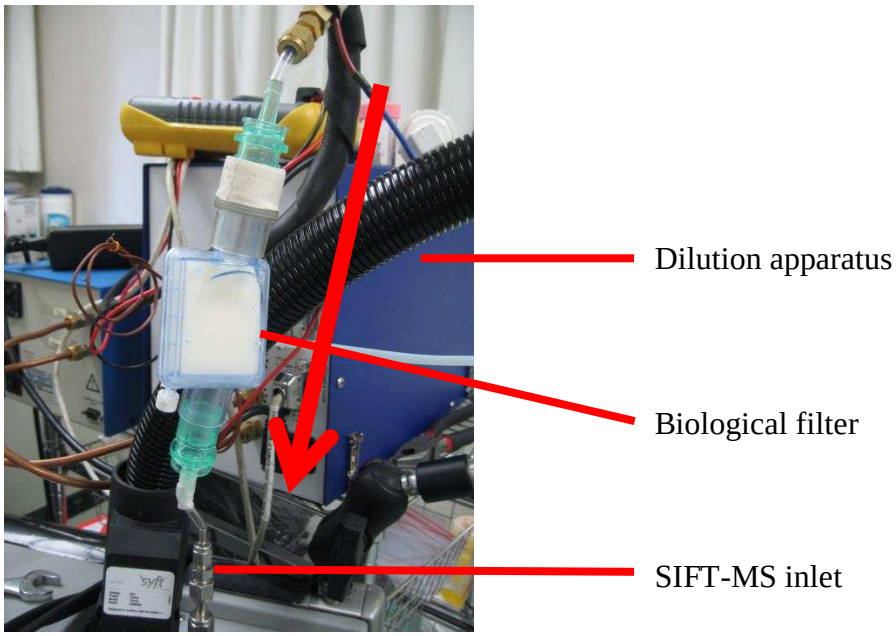


Figure 4-5. Photograph of the filter in-line between the dilution apparatus containing a permeation tube of acetone and the SIFT-MS instrument. The direction of gas flow is marked by an arrow.

4.3.3. The effect of different anaesthesia machines on inspired and exhaled volatile concentrations

Ventilator settings were chosen by the anaesthetist, delivering tidal volumes of 400-550 ml at respiratory rates of 8-12 breaths per minute. There was no relationship between the anaesthesia machine used and tidal volume or respiratory rate.

Of the two different types of anaesthesia machine present in the operating theatres, the Aysis Carestation was used in 61.5% (n=16) of cases and the ADU Carestation in 38.5% (n=10).

Acetone

Median inspired acetone concentration for all 26 patients was 151 ppb (range 90-577 ppb). Acetone concentration of the inspired gas from the ADU Carestation was higher than from the Aysis Carestation (medians 284 ppb and 134 ppb, respectively; p=0.0002) (Figure 4-6). Despite the differences in inspired acetone concentration associated with the different anaesthesia machines, there was no significant difference in exhaled acetone concentration

in subjects ventilated by the different devices (medians: ADU 630 ppb, Aysis 513 ppb; p=0.95). In addition, there was no relationship between acetone concentrations of inspired gas and exhaled breath in the 22 patients in whom pre-filter samples were collected (r_s=0.33, p=0.14).

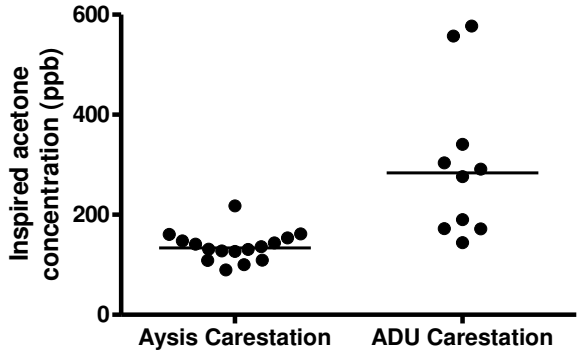


Figure 4-6. Individual data points and column medians show the concentration of acetone in the inspired gas of the two main anaesthesia machines.

Hydrogen sulphide and hydrogen cyanide

Median inspired H_2S concentration for all 26 patients was 1.26 ppb (range 0.53–6.83 ppb). H_2S concentration of the inspired gas from the ADU Carestation was higher than that from the Aysis Carestation (medians 1.95 ppb and 0.92 ppb, respectively; p=0.0078). There was no difference in exhaled H_2S in subjects ventilated by the different anaesthesia machines.

Median inspired HCN concentration for all 26 patients was 0.77 ppb (range 0.44–6.41 ppb). There was no significant difference between inspired HCN concentrations collected from each model of anaesthesia machine (medians: ADU 0.72 ppb, Aysis 0.81 ppb; p=0.51). There was no difference in exhaled HCN in subjects ventilated by the different anaesthesia machines.

4.3.4. The effect of altering the inspiratory gas flow rate

Despite a large variation in inspired concentration (Figure 4-7), the exhaled acetone concentration was not affected by altering the flow rate (Table 4-3). For the two patients tested the median (inter-flow) CV for expired acetone concentration across the four different flow rates (5.6%) was comparable to the median CV for the triplicate samples collected at a single flow rate (6.6%).

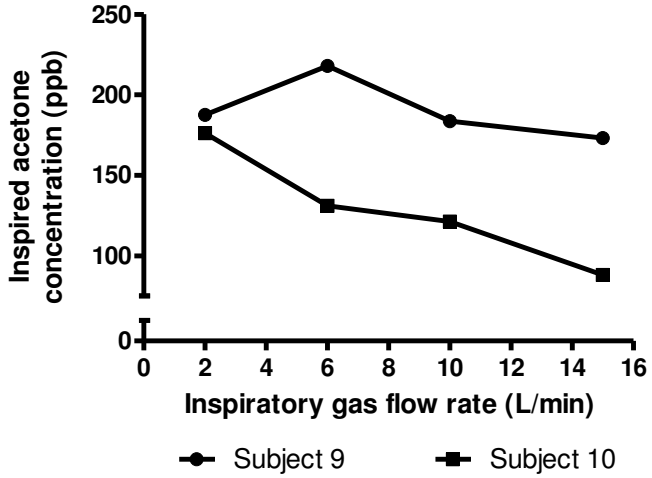


Figure 4-7. Acetone concentration of inspiratory gas at each flow rate.

Table 4-3. Mean inspired and exhaled acetone concentrations at different inspiratory gas flow rates.

Inspiratory gas flow rate (L/min)	Mean exhaled acetone concentration (ppb)	
	Subject 9	Subject 10
2	998	364
6	946	341
10	967	365
15	923	322

4.3.5. The effect of the APL valve

In the three patients tested (subjects 23, 25 and 26), exhaled acetone concentration was higher when the APL valve was fully open compared to partially closed. With the valve open, the mean concentration was 1389 ppb, compared to 783 ppb with the valve closed. There was a median 37% reduction in acetone concentrations between the two valve positions.

4.3.6. Analyte concentrations in exhaled breath

The pneumotachometer was used to visually confirm that samples were collected from the patient and there was no significant back flow from the ventilator circuit. Median breath volatile concentrations and reproducibility data are presented in Table 4-4.

Table 4-4. Median inspired and exhaled breath concentrations and median intra-subject CVs of VOC concentrations in healthy anaesthetised patients (n=22).

Analyte		Median exhaled breath concentration (range) (ppb)	Median intra-subject CV (IQR) (%)
Acetone	Measured	537 (157-5163)	9.2 (7.6-16.0)
	Calculated	738 (257-6594)	8.3 (6.9-14.5)
H_2S	Measured	1.00 (0.71-2.49)	10.0 (8.2-14.8)
HCN	Measured	0.82 (0.60-1.51)	6.8 (3.8-10.5)

CV, coefficient of variation; IQR, interquartile range.

Acetone

There was a relationship between exhaled breath acetone concentration and both plasma acetone (r_s=0.80, p<0.0001) (Figure 4-8) and plasma beta-hydroxybutyrate concentrations (r_s=0.55, p=0.0075) (Figure 4-9). There was no relationship between breath acetone and blood glucose concentrations (r_s= -0.31, p=0.17). Duration of fasting did not correlate with breath acetone, plasma acetone, plasma beta-hydroxybutyrate or blood glucose concentrations.

The patient with the highest breath and plasma ketone levels was male, with no significant past medical history or regular medications. He was not diabetic, as confirmed by his normal fasting glucose concentration, and did not fast significantly longer than other subjects (15 hours compared to a median of 12 hours), however he showed a more marked ketotic response to fasting. If his data is excluded, the relationships between breath acetone and plasma acetone (r_s=0.77, p<0.0001) and plasma beta-hydroxybutyrate concentrations are not affected (r_s=0.49, p=0.025).

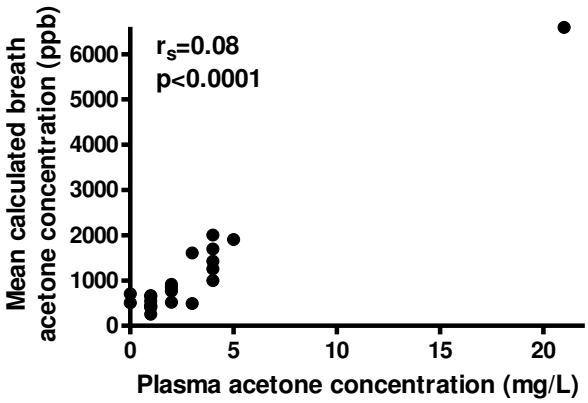


Figure 4-8. Individual data points show the relationship between mean calculated exhaled breath acetone and plasma acetone concentrations.

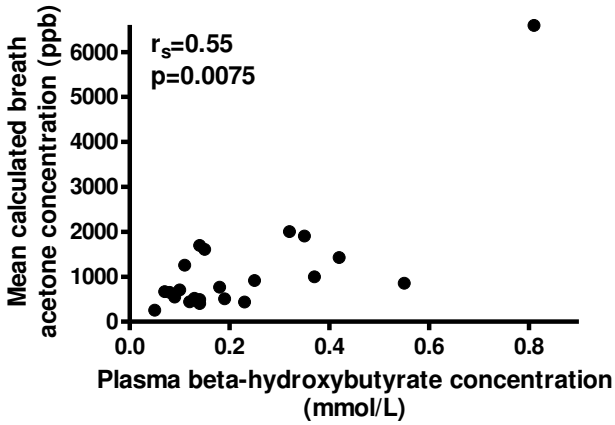


Figure 4-9. Individual data points show the relationship between mean calculated exhaled breath acetone and plasma beta-hydroxybutyrate concentrations.

There was no significant correlation between the concentrations of acetone in inspired gas and exhaled breath (n=22, r_s=0.14, p=0.54). Breath acetone concentration did not correlate with tidal volume delivered by the ventilator (n=22, r_s= -0.23, p=0.29), FVC (n=15, r_s=0.32, p=0.24) or FEV_1 (n=15, r_s=0.15, p=0.60). There was no relationship between breath acetone concentration and height (r_s=0.21, p=0.34), weight (r_s=0.075, p=0.74) or body mass index (BMI) (r_s= -0.14, p=0.54). There was no relationship between breath acetone concentration and age (r_s=0.04, p=0.87).

Exhaled breath acetone concentrations measured in men were higher than those in women, however this difference was not statistically significant (medians

1046 ppb and 738 ppb, respectively; p=0.36). There was no significant difference in tidal volumes delivered by the ventilator to men and women (median 500 ml in both groups, p=0.31), however there was a difference between the FVC values in the two groups (median 4.61 L male and 3.59 L female, p=0.05).

Hydrogen sulphide and hydrogen cyanide

It was not possible to calculate corrected HCN concentrations at the low measured concentrations seen, and although intra-subject CVs for H_2S concentrations were improved by correcting for losses due to the apparatus, the results are likely to be unreliable at such low concentrations, where any minor error in analysis is exaggerated.

There was no significant difference between breath concentrations and inspired gas concentrations of either analyte (H_2S p=0.12, HCN p=0.24), and for this reason there was a relationship between the concentrations of both volatiles in inspired gas and exhaled breath (H_2S r_s=0.59, p=0.004; HCN r_s=0.52, p=0.014).

Due to the very low concentrations of these volatiles, it was not possible to accurately determine the effects of the breathing circuit filter, inspired gas flow rate and ALP valve position on breath concentrations. No interpretation could be made about the effect of age or gender on breath H_2S and HCN concentrations as a result of the very low analyte concentrations and relationship between inspired and exhaled concentrations.

Median venous H_2S concentration for all 26 patients was 12.8 μmol/L (range 6.1–42.3 μmol/L). There was no relationship between breath and plasma H_2S concentrations (n=22, r_s= -0.056, p=0.80), and no relationship between breath H_2S or HCN concentrations and WCC or CRP blood results.

4.4. Discussion

This is the first description of a single end-exhalation breath sampling method analysed by SIFT-MS in anaesthetised, intubated and ventilated patients. While this method was designed for use with SIFT-MS, it is applicable to other

techniques, including PTR-MS, where off-line sampling of these breath volumes is possible, or in combination with SPME for GC-MS analysis. The importance of exploring the influences of flow rate, sampling site and other aspects of ventilator mechanics, on volatile concentration, has been demonstrated. By doing this and establishing a defined protocol, analyte concentrations in breath can be repeatedly and reproducibly measured in samples from anaesthetised patients and compared with samples obtained from patients ventilated in the ICU. Few authors have reported CVs for sample collection in the intubated patient, however the CVs for this technique and subsequent analysis are of comparable magnitude to others reported for the repeat on-line analysis of acetone[168,221].

It was important in this study that the method of breath sample collection did not interfere with standard anaesthetic procedures. This was deliberate, to ensure smooth sample collection, and high compliance from operating theatre staff. Additionally, it was important that the apparatus used to collect the samples was the same as that used in other parts of this thesis and readily available in the hospital environment. For these reasons, items common to the operating theatre and ICU were used (suction catheters, oxygen enrichment attachment T-pieces), which could be disposed of or decontaminated after use, protecting biological safety of the patient and all staff.

4.4.1. Inspiratory gas flow rate and APL valve

During the method development phase, to avoid possible contamination of breath samples with anaesthetic volatile gases and allow higher gas flows through the circuit to prevent rebreathing of organic volatiles, TIVA (i.e. propofol and an opiate) was used. This enabled a method of breath collection comparable to that used in the ICU to be performed, but did limit the study to a specific group of operative procedures and therefore a particular group of patients for which TIVA was appropriate; generally laparoscopic and gynaecological surgery. There is no reason why this method of breath collection and analysis could not be applied when inhaled anaesthetic gases are used if the effects of the anaesthetic volatiles were properly quantified and did not interfere with the quantification method[336]. However, this should be

approached with caution as anaesthetic flow rates are frequently delivered at less than 1 L/min, therefore the effects of this on all VOCs of interest would need prior investigation.

Analyte concentrations could be reliably and reproducibly measured in samples obtained using the default 6 L/min inspiratory gas flow rate on these anaesthesia machines, with the APL valve open as is routine during mechanical ventilation. An inspiratory gas flow rate of 6 L/min was greater than each patient's minute volume so should have prevented rebreathing in the anaesthesia machine. When the soda lime granules, used to absorb CO_2 from the breathing circuit, were removed from the anaesthesia machine at this flow rate, no increase in the partial pressure of end-tidal CO_2 was seen, thus indicating no significant rebreathing of CO_2. APL position can be variable, especially if the same anaesthetist is not used for a whole study, i.e. one person's partially opened may be different from the next. In this regard, stipulating that the APL valve must be "fully open" simplifies the procedure and minimises potential variation.

As has been shown, altering the flow rate or the position of the APL valve has the potential to affect both the inspired gas and measured breath concentrations. To achieve reproducible results a consistent well described method should be used for all patients in a study population.

4.4.2. Composition of breath samples and timing of sample collection

The composition of breath at end-exhalation is presumed to represent that of alveolar gas[147,166]. By switching the ventilator off at the end of a tidal exhalation, VOCs contained within the subsequent breath sample collected should be equivalent to end-exhaled concentrations, thus the introduction of additional devices into the respiratory circuit (i.e. fractionating devices[212]) can be avoided. End-exhaled acetone concentrations reported here are comparable to results of non-diabetic subjects in other studies[167,168,204] and those of anaesthetised patients via a continuous breath sampling technique in the operating theatre[106]. That the concentrations measured are comparable to other techniques where end-exhaled breath is known to be collected suggest that the

technique described here does target alveolar breath. However this method is not without limitations; it is not possible to identify other parts of the breath if analysis of a different expiratory phase is desired.

Other discontinuous sampling techniques have used CO_2 monitoring or temperature to judge the end-expiratory phase, since these devices are generally already in the breathing circuit. Continuous end-tidal CO_2 monitoring was used in the operating theatre, the trace being displayed on the anaesthesia machine monitor. Capnographs have an inherent delay between the patient's alveoli and the analyser interface, and also due to the computation method, thus it could not reliably be used for the timing of breath collection.

Some discontinuous techniques require an additional intermediate step such as the use of a gas tight syringe to remove sample and then application of the sample onto SPME or TD tube. This can introduce additional absorption or condensation problems. The technique described here involves collecting the sample into the bag that is analysed, so sample loss is limited to the method of breath collection, particularly as samples were analysed almost immediately after collection. Additionally, rapid analysis can be achieved by SIFT-MS despite using this discontinuous technique. Results are readily available as analysis takes only a few minutes when lengthy sample preparation with SPME or TD is not required.

The presence of an endotracheal tube may reduce anatomical dead space by bypassing the upper airways, however the observed concentration of highly soluble VOCs in breath may also be reduced by avoiding an area of gas exchange. One benefit of the presence of an endotracheal tube is the elimination of breath sample contamination by mouth reservoirs of H_2S[149,262,292] and HCN[150,151]. Breath H_2S and HCN concentrations in the current study are similar to previous studies involving nasally exhaled breath[150,151,262], where nasally exhaled breath concentrations were only slightly higher than ambient air.

As sampling took place prior to the start of the surgical procedure, any possible changes in VOC concentration associated with the stress of surgery[105,106] were avoided.

4.4.3. Location of breath sample collection

It is advisable to take samples from the patient side of the filter within the respiratory circuit to avoid any unquantified analyte loss. The dead-space of the filter is one third the volume of each breath sample collected and because it is purged with fresh gas on every inspiratory breath, sample dilution may occur. In a study of the differences in exhaled isoflurane (an anaesthetic gas) concentration at different positions within the respiratory circuit of mechanically ventilated patients, the authors concluded there was no significant difference between the concentrations of exhaled isoflurane measured at various sites in the expiratory limb of the breathing circuit, however there was no filter present in the breathing circuit[6]. The best place to monitor inspired gas concentrations was as close to the ventilator as possible[6], however a subsequent study in the operating theatre has shown that rebreathing of VOCs can occur around the biological filter in the breathing circuit[337] (discussed below).

4.4.4. Inspired volatile concentrations

It is evident from the results of this and other studies that different anaesthesia machines, machine settings, and hospital environments[184] alter inspired volatile concentrations. We observed a large discrepancy between inspiratory acetone and H_2S concentrations in the different anaesthesia machines, the cause of which is unclear. It may be the result of volatiles being absorbed or released by components of the breathing system within the anaesthesia machines, differing gas concentrations in the oxygen supply of different areas of the hospital, or another difference in the operating theatre set-up.

In a recent study of exhaled isoprene in anaesthetised patients[337], high concentrations of inspiratory isoprene collected on the patient side of the breathing circuit filter were felt to be due to the rebreathing nature of the anaesthesia machine and contamination from exhaled VOC liberated from the breathing circuit filter on each inspiratory breath. Inspiratory samples were collected from the gas outlet of the anaesthesia machine in this study, before it had passed through the circuit filter, eliminating any effect of rebreathing around the filter. Inspiratory samples were not collected from the patient side

of the filter. The correlation between inspired and exhaled concentrations of H_2S and HCN may be due to the previously described influence of inspired concentrations of some VOCs[147,180,337], but no relationship was seen between inspired and exhaled acetone concentrations at the relatively high inspiratory flow rate used in this study. It is difficult to know whether a relationship between inspiratory and exhaled acetone concentrations would have been present if the inspiratory flow rate was lower and/or both types of sample had been collected from the same location, as in the study by Hornuss et al.[337]. Inspired concentrations from this location may more accurately reflect true inspired VOC concentrations.

As a result of the lack of correlation between inspiratory and exhaled acetone concentrations, standardising the air supply or limiting this study to a single operating theatre and type of anaesthesia machine, which would have made it unnecessarily difficult to recruit participants, was not attempted. Correcting for inspired gas concentrations by simply subtracting them from exhaled concentrations does not take into consideration the complexity of the relationship, which has led some authors to recommend that care should be taken when interpreting breath volatiles if the inspired concentration is greater than 5%[8] or 25% of the exhaled concentration[2], which would apply to both H_2S and HCN in this study. As has been discussed in Chapters 1.3.6. and 3.4.6. of this thesis, inspired air can be scrubbed to remove VOCs, but this can be problematic and unreliable[93,147] so was not attempted.

4.4.5. Breath acetone concentration

As has been noted in other studies[167,168,205], there was a difference in breath acetone concentration between men and women, though in this study it did not reach statistical significance. The cause for the observed difference is unknown; differences in metabolic demand have been proposed by some authors[167], and others have suggested gender differences in lung volume, which are important in the forced exhalation methods used to obtain breath samples[168]. In this study, the median tidal volume delivered by the ventilator was the same for both male and female subjects, removing any gender variation in exhaled breath volume. There was no correlation between breath

acetone and either FEV_1, FVC, height, weight or BMI. Despite this, our study shows a small gender difference in exhaled breath acetone concentration, which may be the result of a larger airway surface area for exchange of acetone in men or imply that breath acetone concentration is related to metabolic factors rather than exhaled breath volume.

In mechanically ventilated patients, it is even more important than in awake, spontaneously breathing patients to understand the relationship between breath and blood VOC concentrations as there is a greater chance of contamination of the inspired gas[8]. Several studies have reported a relationship between exhaled acetone concentration and blood acetone, beta-hydroxybutyrate or glucose concentrations in serial measurements in both diabetic and non-diabetic populations[195,226,240,243]. A relationship between breath acetone and plasma acetone concentrations was observed, comparable to results seen in studies of fasted healthy subjects[230,232], and a positive correlation between breath acetone and plasma beta-hydroxybutyrate concentrations, previously seen during glucose tolerance tests[240] and the ingestion of ketogenic meals[195]. No significant relationship was seen between exhaled acetone and blood glucose concentrations in this study, similar to a previous study of individuals with Type 2 diabetes[205]. In contrast, two studies looking at breath acetone and blood glucose concentrations in healthy volunteers during a glucose tolerance test[225,240] and a study of subjects with Type 1 diabetes[243] revealed positive relationships between breath acetone and blood glucose concentrations.

When drawing comparisons with other studies it is important to note that our study involved fasting healthy individuals; patients with diabetes were excluded due to known effects of diabetes mellitus on breath acetone concentration[226,242]. No significant difference in breath acetone concentration has been found between individuals with Type 1 diabetes and healthy controls[226], however those with Type 2 diabetes appear to have significantly higher breath acetone concentration[242]. It is possible that relationships of breath acetone concentration with plasma measurements may be affected by the abnormal glucose metabolism of individuals with diabetes, and the absence of significant change in glucose concentration in healthy fasted individuals despite marked changes in breath acetone concentration. We did not control for

dietary factors because all patients were fasted for more than six hours, however fasting itself has been shown to cause an increase in breath acetone concentrations in non-diabetic subjects[167,194,196].

Patients in this study were starved for a considerable amount of time prior to surgery, up to 17.8 hours, which is greater than the six hours required according to North American[338] and UK[339] guidelines. This is often because elective surgical patients are fasted from midnight before surgery, with possible delays building up over the day in the operating theatre. Clear fluids are permitted up to two hours before surgery. Interest in the utility of pre-operative carbohydrate-rich drinks has led to studies reporting attenuation in pre-operative thirst, hunger, anxiety and general discomfort[340], post-operative nausea and vomiting[341], and earlier return of gut function following colorectal surgery[342]. Pre-operative oral carbohydrate treatment also reduces the development of immediate post-operative insulin resistance[343]. The evidence has led to a British Consensus Guideline recommending the consideration of pre-operative carbohydrate-rich drinks 2-3 hours before induction of anaesthesia for elective surgical procedures in adult non-diabetic patients with normal gastric emptying[344]. Patients in this study showed signs of pre-operative ketosis due to fasting, which may have been improved by the administration of carbohydrate-rich fluid.

4.4.6. Breath H_2S and HCN concentrations

Breath concentrations of H_2S and HCN were not significantly different from inspired gas concentrations in this study, however it was anticipated that breath volatile concentrations, other than acetone, would not be elevated because subjects were healthy and did not have significant systemic or pulmonary inflammation or infection. Due to very low H_2S and HCN concentrations measured, it was not possible to correct concentrations for losses due to the sampling apparatus using the linear regression equations in Chapter 2.3.2. At such low concentrations, corrected results for these two volatiles were likely to be unreliable; any minor error in analysis is exaggerated and the linear regression line is not as reliable when volatile concentrations are around their limit of detection in humid gas by SIFT-MS. The concentrations of exhaled

H_2S and HCN appeared to be influenced by inspired concentrations. In contrast, a recent investigation of six healthy volunteers found no relationship between inspired gas (room air and medical air supply) and nasally exhaled breath HCN concentrations[151]. The higher breath HCN concentrations in that study may reflect the upper airways contribution to breath HCN concentration eliminated here by the presence of an endotracheal tube.

There was no relationship between breath and blood H_2S concentrations, however it is still unclear whether H_2S detected in breath is a result of systemic or airway production[223], or mouth contamination[149,292]. Blood H_2S concentrations have been reported for two patients with sepsis[261], and in small numbers of patients with COPD[287-289] and asthma (reviewed[254]), as well as healthy controls, however there is no agreed normal range for this compound or commercial assay available.

The concentrations of these VOCs in illness will be explored in subsequent chapters of this thesis, when breath concentrations are predicted to be higher than inspired gas concentrations if, like NO, they are produced in the airways[94].

4.4.7. Limitations and appropriateness of baseline volatile concentrations

It is recommended that spirometry is performed after measurements of NO concentration in breath[94], as the exhalation manoeuvres appear to temporarily reduce NO concentrations[345-347]. Repeated spirometric manoeuvres caused a reduction in NO that lasted for less than one hour in a group of asthmatics and healthy subjects[346] and up to eight hours in a group of allergic asthmatics[345]. By performing spirometry 30-60 min prior to breath sample collection in this study, it is possible that VOC concentrations were altered, however there is no data available for the effects of spirometry on VOCs other than NO.

Breath samples were taken at any time of the day, which may have had an impact on breath volatile concentrations, however it is unclear whether diurnal variation in breath acetone concentration, for example, is related to timing of meals or another cause. It is known that inter-subject variability is greater than inter-day variability of breath acetone concentration[167,168]. In most other studies

of breath acetone there has been no attempt to link breath and blood levels, whereas in this study we could see that breath acetone concentrations correlated with blood concentrations.

For this group of anaesthetised patients to provide baseline breath volatile concentrations they needed to be healthy, without conditions known to affect breath volatile concentrations, but also be representative of the population they would be compared to. As previously mentioned, patients with diabetes were excluded due to known difficulties in interpreting breath acetone concentrations[226,242]. The exclusion of patients with significant lung disease and smoking was attempted, however in reality it was not fully possible due to the greater need to identify patients in whom TIVA would be appropriate and where theatre staff were happy to be involved in the study. Cigarette smoke contains numerous volatile organic compounds[188], although of the three VOCs of interest in this study only HCN from cigarette smoke can be detected in human breath[190]. For this reason non-smokers were recruited if possible to reduce any possible contamination of breath samples, however they would not have smoked for several hours prior to breath sample collection, reducing the level of potential contamination. Including two smokers and two patients with obstructive spirometry improved the relevance of any results to the whole population, as patients admitted to the ICU will not all be non-smokers with normal lung function. Although children were excluded from the study, subjects were recruited over a wide age range which makes them more representative as a control group.

4.4.8. Summary

This off-line method for single end-exhalation breath collection with SIFT-MS analysis in intubated, ventilated patients is repeatable and reproducible. The study highlights the importance of exploring variation in volatile concentrations associated with local theatre and ICU environments and equipment. Patient numbers in some parts of the study are small, making exact assessment of the impact of components of the breathing circuit or changes in anaesthesia machine set-up difficult. However, it is still possible to conclude that it is important to keep all settings the same in order to make accurate

comparisons between different patient samples. A suitable method can be established with set parameters to minimise variation introduced by the sampling methods and therefore reproducibly measure VOC concentrations.

The sampling method presented here is comparable to that described for breath sampling in intubated patients in the ICU. The ease of this sampling method lends itself to serial measurements of breath volatiles to monitor changes during an operation or to test treatment algorithms in the operating theatre or ICU. Breath VOC concentrations obtained via this method are comparable to previous studies and can, therefore, be used as a reference point in any intubated patient in whom it is not possible to collect a baseline breath sample. This breath collection and analysis technique is suitable for further investigation of these and other breath volatiles in the operating theatre, for example to assess the effects of surgical procedures.

5. Breath acetone analysis in critical illness

5.1. Introduction and aims

Non-invasive breath acetone monitoring in the ICU could provide rapid, frequent or continuous assessment of physiological changes occurring as a consequence of critical illness. Breath acetone has been investigated as a marker of ketosis[195,244], and metabolic stress in patients with heart failure[245] and during surgery[105,106]. It is produced by decarboxylation of acetoacetate mainly during lipolysis and is excreted in breath[227,228]. Beta-hydroxybutyrate is also produced from acetoacetate; the direction of this reaction is dependent upon the redox state of the cells, therefore the relationship between the relative quantities of acetoacetate, acetone and beta-hydroxybutyrate is not constant[348].

For VOCs like acetone that are produced by metabolic pathways, it is important to understand the relationship between their concentrations in exhaled breath and the systemic circulation in order for them to be useful[8]. The high variability seen in repeated measurements of acetone concentration in plasma compared to breath means the monitoring of breath acetone concentration may be more reliable than plasma measurements[115].

Sepsis is a major cause of mortality in the ICU, due to haemodynamic compromise and multi-organ failure[10-13]. Activation of the inflammatory cascade is known to cause "stress hyperglycaemia", which is common in patients with critical illness in the absence of pre-existing diabetes mellitus[12]. Glucose control appears to be important in the prevention of unnecessary morbidity and mortality in this situation[65,69,76,81].

The aims of this part of the study were to explore any changes in breath acetone concentration over time in two groups of intubated and ventilated patients in the ICU; patients with stress hyperglycaemia and those with new pulmonary infiltrates on chest radiograph. Variation of breath acetone concentration with insulin administration and feeding was examined, with the ultimate aim of assessing whether it could be used to guide insulin or feeding regimes. Breath acetone was investigated as a marker of pulmonary infection versus other causes of pulmonary infiltrates on chest radiograph. The utility of

breath acetone as a marker of metabolic stress and therefore illness severity and clinical outcomes was explored, comparing it to other validated tests and tools. The relationships between breath acetone and arterial acetone, beta-hydroxybutyrate and glucose concentrations were examined, along with the effects of factors known to influence breath acetone concentration, including breathing pattern (described in Chapter 1).

5.2. Methods

5.2.1. Subjects

Study procedures were approved by the Upper South A Regional Ethics Committee, New Zealand. Between October 2011 and April 2012, consecutive critically ill non-diabetic adult patients requiring mechanical ventilation on a controlled mode in the ICU, Christchurch Hospital, were enrolled if they had either or both of the following: stress hyperglycaemia requiring insulin therapy as per local protocol[82] and/or new pulmonary infiltrates on chest radiograph. Baseline sampling was performed prior to insulin administration and/or within 24 hours of intubation or development of infiltrates if already intubated. Patients, or their next of kin, provided written consent prior to sample collection. In addition, if the patient had not given their own consent prior to inclusion, and regained adequate cognitive functioning, they confirmed their consent to participate in retrospect.

Each patient was ventilated using a Nellcor Puritan Bennett™ ventilator system (USA). Some ventilator circuits included a humidifier (Fisher & Paykel MR 730 Respiratory Humidifier, NZ). Ventilator modes and settings were determined by ICU staff and not altered for the purpose of breath collection.

Breath and contemporaneous arterial blood samples were taken at enrolment (between 07:00 and 17:00) and daily thereafter. Subsequent samples were taken between 07:00 and 13:30 unless the patient was spontaneously breathing or not present on the ICU, for example in the operating theatre or radiology department. Arterial, rather than venous, blood samples were collected as this is routine practice in the ICU and was non-invasive due to the presence of an arterial line for continuous BP monitoring. Arterial blood gas samples were

processed using a blood gas analyser in the ICU. Other blood samples were sent to Canterbury Health Laboratory immediately for processing; the sample for acetone testing was sent on ice and the headspace analysed by GC. In several patients, contemporaneous arterial and central venous blood samples were drawn to look for differences in acetone concentration as a consequence of its excretion in breath. Sampling was discontinued if the patient no longer required controlled ventilation, was extubated, underwent tracheostomy or died.

The following data was collected for each patient: diagnosis and reason for admission, number of days on the ICU, age, gender, ethnicity and smoking status. Note was made of physiological parameters at the time of breath collection, as well as feeding, current medication and disease severity scores. The requirement for continuous veno-venous haemodiafiltration (CVVHDF) and microbiological and radiological results were recorded. All information was updated on a daily basis at the time of breath sample collection.

5.2.2. Inspiratory air sampling

All methods utilised a T-piece (oxygen enrichment attachment, Respironics Inc, USA) inserted into the breathing circuit at the ventilator gas outlet, or humidifier outlet, for inspiratory gas sampling.

5.2.3. Exhaled breath collection

End-tidal breath collection was performed via a T-piece as described in Chapter 3.2.3. of this thesis. The volume of the tidal breath was noted. The ventilator delivered several standard breaths before this process was repeated in triplicate using separate Tedlar bags. All inspiratory and exhaled breath samples were incubated at 40°C and analysed within 40 min of collection by SIFT-MS. The pump was flushed by running through room air for at least 2 min between patients.

5.2.4. Infection control

The suction catheters and sampling filter were disposable and used for only one set of samples. The T-piece was decontaminated for re-use. Disinfectant wipes were used on the surfaces of the hand-held pump and Tedlar bags (Azo wipes,

W. M. Bamford & Co Ltd, NZ). None of the cleaning procedures changed the VOC composition of samples.

5.2.5. Selected ion flow tube mass spectrometry

Breath acetone concentration was measured by SIFT-MS, Voice200® (Syft Technologies Ltd, NZ), using SIM mode with the NO^+ reagent ion (described in Chapter 2). Each Tedlar bag was attached to the sampling capillary end cap of the heated inlet extension of the SIFT-MS and samples were analysed for 30 sec.

5.2.6. Calculations and statistical analysis

No previous studies examine the change in breath volatile concentrations over time in patients in the ICU. A previous study by Schubert et al.[8] explored the relationships between inspiratory and venous acetone concentrations, ventilatory and cardiac parameters on breath acetone concentration in 46 patients in the ICU using an off-line sampling technique with sample pre-concentration. Breath H_2S and HCN concentrations have not been studied in this patient group. The sample size chosen should allow assessment of the relationship between breath volatile concentrations and their inspiratory and blood concentrations, as well as the influence of ventilatory and cardiac variables.

Inspired and exhaled acetone concentrations quoted have been corrected for losses due to the sampling apparatus (linear regression equations are presented in Chapter 2).

Means and percentage CVs of each set of three end-tidal breath samples were calculated. Comparisons between groups were made using Mann Whitney U tests for unpaired and Wilcoxon signed rank tests for paired data. Correlations were determined by Spearman's rank correlation (r_s). In some cases, only data from days 1 and 2 were compared so that data from patients enrolled for many more days did not skew the results. As the units of measurement were different, a modified Bland-Altman plot was used to compare breath acetone and arterial acetone measurements as percentages of their maximum concentration[208]. Statistical analyses were performed using GraphPad Prism

version 5.01 for Windows (GraphPad Software, USA). A value of $p<0.05$ was considered to be statistically significant.

The ratio of inspiratory acetone as a percentage of exhaled acetone concentration ((C_{insp}/C_{exp})*100) was calculated with a mean and standard error of the mean (SEM). Acetone elimination rate was calculated using the following equation: $C_{exp}*(V_T*RR)$.

Alveolar dead space ratio (V^{alv}_D/V_T) was estimated using the modified Bohr equation: (V^{alv}_D/V_T) = ($PaCO_2 - P_{et}CO_2$) / $PaCO_2$. Alveolar-arterial oxygen gradient (A-a gradient) was calculated by using the formula (FiO_2 * (atmospheric pressure – water pressure) – ($PaCO_2$/0.8)) – PaO_2, when atmospheric pressure and water pressure at sea level are 760 mmHg and 47 mmHg, respectively (Christchurch Hospital Heliport is at 17 m elevation[349]).

SIRS criteria were met when two or more of the following were present: (1) temperature >38°C or <36°C, (2) heart rate >90 beats per minute, (3) respiratory rate >20 breaths per minute or $PaCO_2$ <32 mmHg, (4) white blood cell count >12000 or <4000/μl or >10% immature forms[14].

5.3. Results

5.3.1. Patient demographics and health status

Thirty-four consecutive patients admitted to the ICU, Christchurch Hospital, who fulfilled the inclusion criteria were enrolled, however data from two patients was subsequently excluded from analysis as consent from their next of kin was revoked. Results from 32 patients were analysed; 20 male and 12 female, median age 61.5 years (range 26–85 years). Demographic data can be seen in Table 5-1. Thirty patients were NZ European (93.75%), one patient was Asian and one patient was of Maori descent. Ethnicity data reflects that of the wider Christchurch population[325]. Thirteen patients were current smokers, eight were ex-smokers an eleven were non-smokers.

Twenty-eight patients had new pulmonary infiltrates on chest radiograph and 19 patients were hyperglycaemic requiring insulin therapy. It was not possible

to obtain breath and blood samples prior to administration of insulin in eight of the 19 patients with hyperglycaemia.

At enrolment, 22 patients fulfilled the criteria for SIRS[14], 17 of whom had clinical or microbiological evidence of infection and therefore fulfilled criteria for sepsis[14]. Twelve patients had pneumonia; two of these patients did not have sepsis. Positive microbiological results were obtained from eight of the twelve patients with pneumonia; five patients had positive legionella DNA PCR tests (Legionella longbeachae was cultured from one of these patients), two patients had tracheal aspirates with moderate to heavy growth of Haemophilus influenzae and Streptococcus pneumoniae, and one patient grew Group B Streptococcus on blood culture. Of the other patients with sepsis, one had Staphlococcus aureus line sepsis, five had intra-abdominal infection (including one with alpha haemolytic streptococcus cultured from blood and intra-abdominal fluid), one had urosepsis and one patient grew Klebsiella pneumoniae in their blood, urine and tracheal aspirate.

At enrolment, 22 patients fulfilled PO_2/FiO_2 ratio criteria for ARDS[21] (nine mild, eleven moderate, two severe) and six patients had pulmonary oedema.

Samples were collected for a median of three days (range 1–8 days). Sampling was most frequently discontinued due to development of spontaneous breathing (65.6%), which prevented breath sampling by the method described. The next most common reasons for discontinuation of sampling were death (18.8%), extubation (12.5%) and tracheostomy (3.1%).

A variety of medications were administered on a daily basis, all of which were chosen by the ICU team depending upon the patient's medical condition and could not, therefore, be controlled for. All patients were sedated using a combination of intravenous propofol and fentanyl according to local protocol.

Table 5-1. Patient demographics, inflammatory status, physiology and ventilation mode at enrolment.

Subject	Gender	Age (years)	SIRS	Sepsis	Infiltrates	Insulin	Sampling days	Ventilator mode	Breath acetone (ppb)	PO_2/FiO_2	$P_{et}CO_2$ (mmHg)	Alveolar dead space
1	m	61	3	y	y*	#	3	Bi-Level	11375	54.7	35	0.54
2	m	66	3	y	y*	#	5	Bi-Level	1130	101.4	-	-
3	m	81	3	y		y	2	Bi-Level	1785	150.0	22	0.19
4	f	71	3	y	y*		2	SIMV	3054	167.5	31	0.30
5	f	60	4	y	y		1	SIMV	763	220.0	41	0.11
6	m	51	1	n	y		1	SIMV	1793	225.7	40	0.15
7	m	85	3	y	y*		3	Bi-Level	701	222.5	43	0.22
8	m	74	0	n	y	#	3	SIMV	2565	226.7	29	0.42
9	m	62	4	y	y*		2	Bi-Level	722	165.8	52	0.31
10	f	77	3	n	y		2	SIMV	853	363.3	33	0.27
11	f	78	0	y	y*	#	2	Bi-Level	2265	284.4	27	0.36
12	f	42	3	n	y		1	SIMV	839	168.3	53	0.17
13	f	40	2	y	y*		6	SIMV	1598	101.3	43	0.26
14	m	67	2	y	y	y	3	SIMV	515	72.0	40	0.07
15	m	46	1	n	y*	y	3	Bi-Level	1750	323.3	38	0.19
16	m	54	2	y	y*	#	8	SIMV	1332	118.3	33	0.38
17	m	60	2	n	y	y	2	Bi-Level	2379	168.3	34	0.29
18	m	81	2	y	y*	y	5	SIMV	1191	141.8	43	0.15
19	f	55	3	y	y*	#	5	Bi-Level	6696	245.7	32	0.24
20	f	53	1	n		y	2	SIMV	1201	260.0	27	0.27
21	f	59	3	y	y	y	3	SIMV	2590	217.5	31	0.52
22	m	46	2	y	y	y	8	Bi-Level	1266	146.0	43	0.17
23	m	38	2	n	y		3	SIMV	1569	336.7	26	0.41
24	m	37	1	n	y*		6	SIMV	259	116.0	57	0.23
25	m	74	0	n		y	4	SIMV	2227	240.0	37	0.14
26	m	64	3	n	y	#	6	Bi-Level	753	152.0	32	0.16
27	m	83	3	n	y	#	5	SIMV	260	202.0	42	0.29
28	f	83	2	y	y		2	SIMV	4236	385.7	37	0.14
29	f	26	3	y	y		2	Bi-Level	333	426.7	46	0.04
30	m	39	1	n		y	1	Bi-Level	1147	348.0	40	-0.14
31	m	67	2	n	y		2	Bi-Level	4366	108.3	50	0.43
32	f	85	1	n	y	y	2	SIMV	2354	358.3	43	0.19

SIRS (systemic inflammatory response syndrome): 0-1, no SIRS; 2-4, SIRS. Sepsis: n, criteria for sepsis not fulfilled; y, criteria for sepsis fulfilled. Infiltrates, new pulmonary infiltrates on chest radiograph; y, yes; n, no; *, pneumonia present; #, requiring insulin but no pre-insulin samples obtained. SIMV, synchronised intermittent mandatory ventilation. Breath acetone, mean breath acetone concentration. PO_2/FiO_2, arterial partial pressure of oxygen divided by the fraction of inspired oxygen; $P_{et}CO_2$, partial pressure of end-tidal CO_2; alveolar dead space ratio calculated by $(P_aCO_2-P_{et}CO_2)/P_aCO_2$.

5.3.2. Inspired and exhaled acetone concentrations

A pneumotachometer was used to visually confirm that samples were collected from the patient and there was no significant back flow from the ventilator circuit.

Median inspired acetone concentration was 110 ppb (range 73-412 ppb) for all samples obtained. Median breath acetone concentration of all samples collected was 853 ppb (range 162-11375 ppb). There was a high degree of inter-subject variation in breath acetone concentration. There was a relationship between inspired and exhaled acetone concentrations (r_s=0.39, $p<0.0001$), however exhaled acetone concentrations were between 1.4 and 100 times greater than their corresponding inspired concentrations. Mean (C_{insp}/C_{exp})*100 was 11.8% (SEM ± 1.4).

Median intra-subject CV for breath acetone concentration of all breath samples collected was 8.51% (IQR 5.8-12.4%). There was no relationship between intra-subject CV for breath acetone concentration and alveolar dead space or lower PO_2/FiO_2, indicating that the degree of ventilation-perfusion mismatch and impairment in gas transfer did not increase the variability of breath acetone concentration in the three consecutive breath samples collected for each patient daily. There were changes in alveolar dead space fraction and PO_2/FiO_2 over time, however there was no relationship between changes in these measurements and changes in mean breath acetone concentration.

In general, there was a reduction in breath acetone concentration over time (Figure 5-1). Increases in breath acetone concentration were associated with surgery, reduction or discontinuation of feeding, increase in gastric aspirate volume (indicating a reduction in gut motility and/or malabsorption of enteral feed), general deterioration in condition (which may be an indicator of a reduction in gut motility and/or malabsorption of enteral feed) and imminent death. Changes in breath acetone concentration over time mirrored changes in arterial acetone concentration, although the relationship between breath and arterial concentrations was not constant.

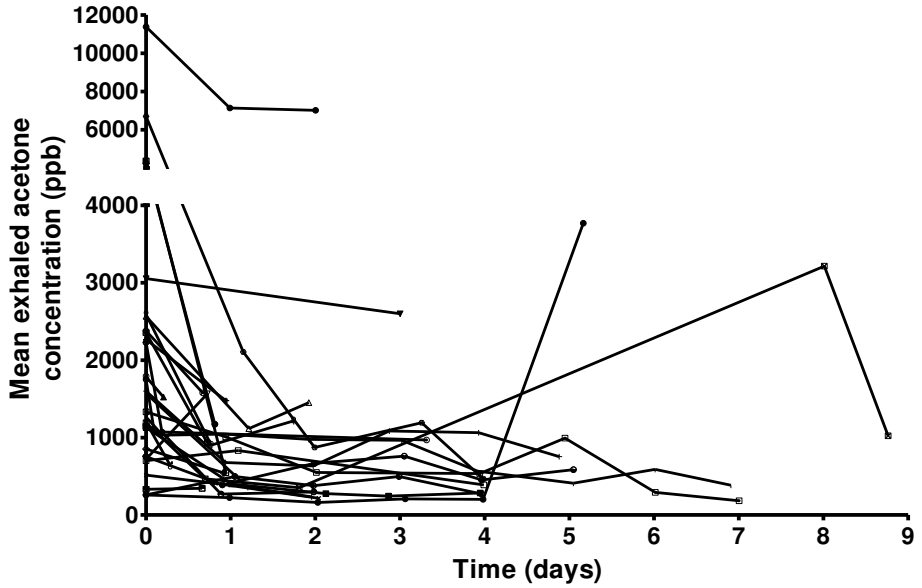


Figure 5-1. Variation in breath acetone concentration for each patient over time (lines join each sampling event for an individual patient).

5.3.3. Pulmonary dead space and shunt

Physiological dead space and pulmonary shunt could not be calculated as it was not possible to measure the partial pressure of mixed exhaled CO_2 or mixed venous CO_2 (patients did not require pulmonary artery catheters for routine management). A-a gradient was used as an estimate of combined shunt and dead space ventilation.

As expected, there was a reciprocal relationship between alveolar dead space and PO_2/FiO_2 ratio (r_s= -0.32, p=0.015). There was a positive relationship between exhaled acetone concentration and alveolar dead space (r_s=0.42, p=0.017), but not with PO_2/FiO_2 ratio or A-a gradient. Calculated alveolar dead space was greater in patients with ARDS than those who did not meet criteria for ARDS (patients with pulmonary oedema excluded) (medians: ARDS 0.25, no ARDS 0.04; p=0.039), as would be expected in this condition[153].

There was a significant difference in PO_2/FiO_2 ratio between men and women (medians: men 159, women 253; p=0.022), men had poorer lung function, but this did not result in a difference in breath acetone concentration (medians: men 1299 ppb, women 1932 ppb; p=0.32).

5.3.4. Relationship between central venous and arterial acetone and glucose concentrations

A total of ten matched central venous and arterial blood samples were taken from two patients, revealing identical acetone concentrations irrespective of blood sampling location.

Central venous blood glucose samples were paired with arterial glucose samples in four patients, a total of 20 matched samples; mean difference 0.1 mmol/L (arterial greater than central venous concentration), range arterial glucose 1.7 mmol/L less than to 2.2 mmol/L greater than central venous concentration.

5.3.5. Relationship between breath acetone and arterial ketones

There was a strong relationship between breath and arterial acetone concentrations in the first two sets of samples collected from each patient (r_s=0.64, p<0.0001) (Figures 5-2 and 5-3). The modified Bland-Altman plot shows that as the normalised average concentration of acetone increases the difference between the two measurements (breath and arterial) increases. There was a stronger correlation between breath and arterial acetone concentrations in patients with pneumonia than in all patients (r_s=0.78, p<0.0001). The relationship between breath acetone and arterial beta-hydroxybutyrate concentrations (r_s=0.52, p<0.0001), was similar to the relationship between arterial acetone and arterial beta-hydroxybutyrate concentrations (r_s=0.56, p<0.0001). Neither relationship was changed by subtracting inspired from exhaled acetone concentration.

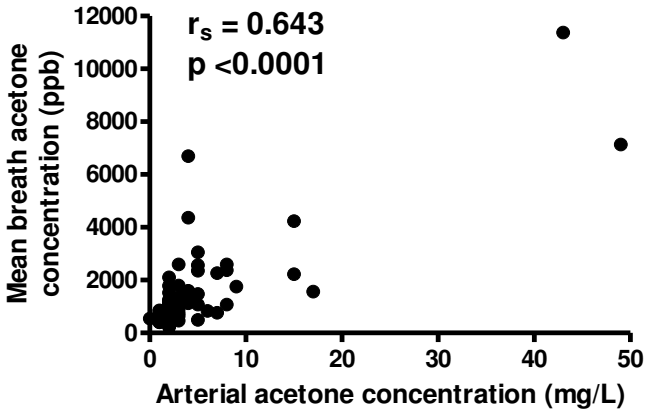


Figure 5-2. Individual data points of the relationship between breath and arterial acetone concentrations in the first two sets of samples collected from each patient.

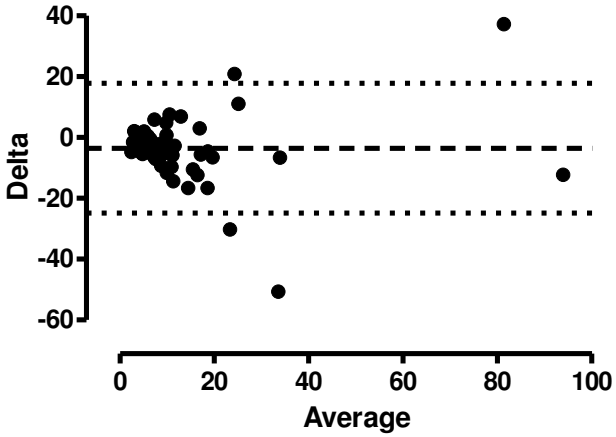


Figure 5-3. Modified Bland-Altman plot displaying the relationship between breath and arterial acetone concentrations as percentages of their maximal value. Average = average value of the normalised breath and arterial acetone concentrations; delta = difference between the normalised breath acetone concentration and the normalised arterial acetone concentration. Bias was -3.58 (dashed line) with 95% limits of agreement of -24.9 and 17.8 (dotted lines).

Removing the results from the patient with the highest breath and arterial ketone concentrations did not alter the relationships between breath acetone and arterial ketone concentrations. The Bland-Altman plot, however, showed a reduction in bias and an increase in the 95% limits of agreement (-37.4–42.2), i.e. the variability between the two measurements of acetone concentration appeared to increase.

5.3.6. Relationship between breath acetone and arterial glucose

There was no significant difference in breath acetone concentration between hyperglycaemic and euglycaemic patients. There was no relationship between breath acetone and arterial glucose concentrations in the first two sets of samples collected from each patient, either in all patients or in the group of hyperglycaemic patients. Figure 5-4 shows the relationship between exhaled acetone and arterial glucose concentrations at enrolment (a) and in the next set of samples taken (b). The graphs highlight that some patients remained ketotic, signified by high breath acetone concentration, despite normal, or near normal, arterial glucose concentration.

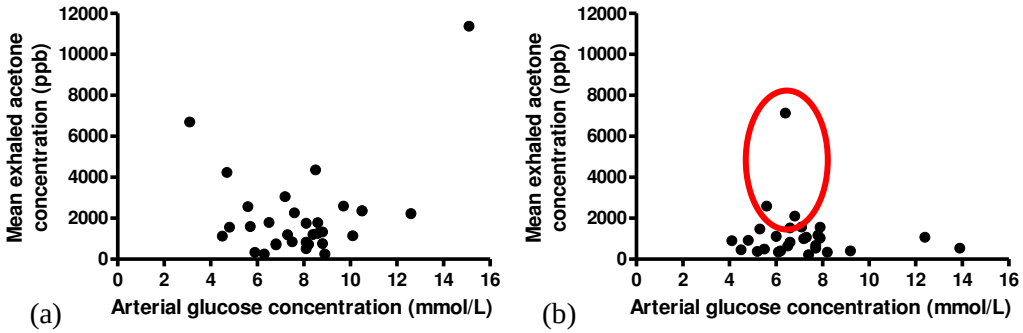


Figure 5-4. Individual data points of the relationship between breath acetone and arterial glucose concentrations at enrolment (a) and the next sample collection (b). The red circle indicates patients with high breath acetone concentration with normal, or near normal, arterial glucose concentration.

5.3.7. The effects of insulin and feeding on breath acetone concentration

There was a significant reduction in breath acetone concentration between pre-insulin and post-insulin samples in patients with hyperglycaemia (medians: pre-insulin 1767 ppb, post-insulin 980 ppb; p=0.002) (Figure 5-5). There was no clear relationship between breath acetone concentration and modelled insulin sensitivity in the group of patients receiving exogenous insulin (Figure 5-6).

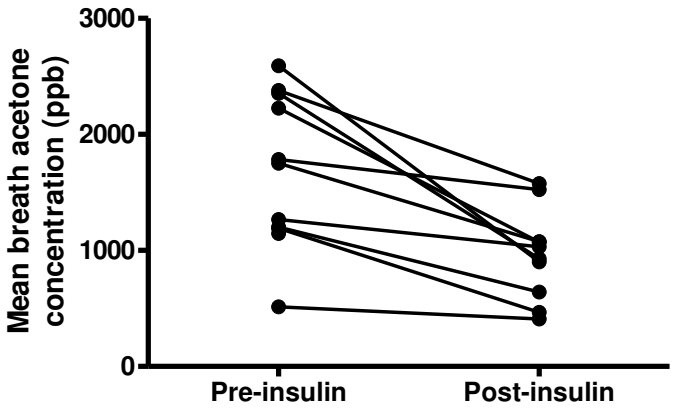


Figure 5-5. Individual data points show the change in mean breath acetone concentration after insulin administration in patients with hyperglycaemia, in whom pre-insulin and post-insulin samples were obtained (n=10).

There was a reciprocal relationship between breath acetone concentration and the percentage of estimated feed requirement administered hourly in all patients (r_s= -0.39, p<0.0001) and when subjects requiring insulin were analysed separately (n=19, r_s= -0.36, p=0.0018). This may be explained by fasting causing ketosis and elevated breath acetone concentration[167,194,196].

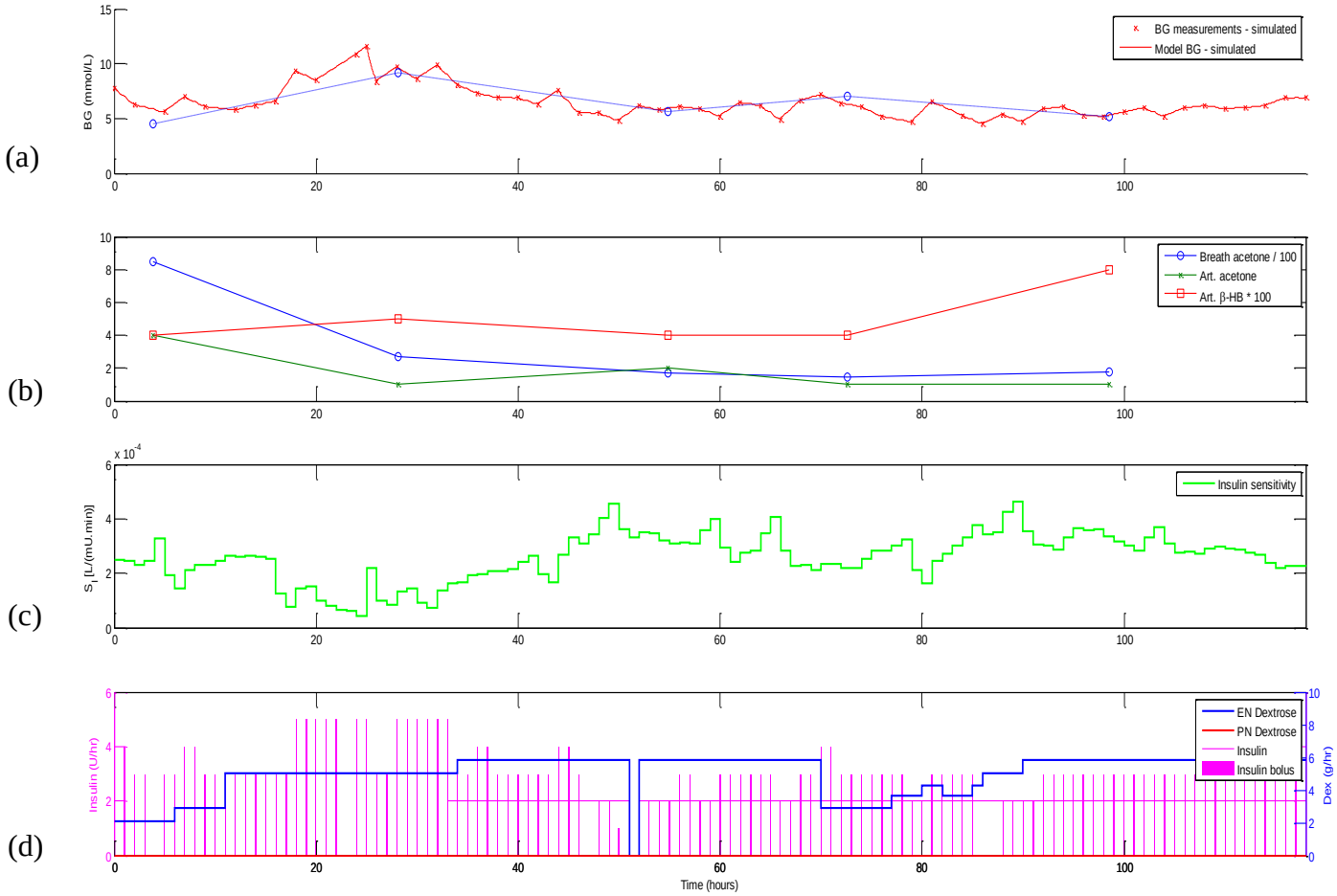


Figure 5-6. Graphical comparison of (a) hourly (red) versus daily (blue) plasma glucose concentration, (b) breath acetone (blue) and plasma ketone concentrations (acetone green, beta-hydroxybutyrate red), (c) modelled insulin sensitivity and (d) enteral dextrose (blue) and insulin (purple) administration over time in a patient with stress hyperglycaemia.

5.3.8. Relationship between breath acetone and clinical parameters

When looking at patients with pulmonary infiltrates only (n=28), there was no significant difference in breath acetone concentration or acetone elimination rate between patients with pneumonia, SIRS, sepsis or ARDS and those without these diagnoses. Therefore, breath acetone concentration could not be used to distinguish between these conditions.

Initial breath acetone concentration as a marker of metabolic stress did not correlate with mortality in the ICU or during hospital stay, validated scores of illness severity (APACHE II score[23] and SOFA score[26]), or the requirement for renal replacement therapy. Initial breath acetone concentration was not predictive of number of ventilator days, length of ICU or hospital stay.

There was no relationship between breath acetone concentration and tidal breath volume, respiratory rate, minute ventilation, heart rate or systolic blood pressure (SBP) on a daily basis. There was no significant difference in SBP between patients receiving ionotropic or vasopressor drugs and those who were not; no difference in breath acetone concentration was seen between patients in these two groups indicating that these drugs had no effect on exhaled acetone concentration.

5.3.9. Breath acetone in the ICU, pre-operative and post-operative settings

Day 1 breath acetone samples in the ICU cohort were significantly higher than breath acetone concentrations in healthy fasted anaesthetised patients prior to surgery (medians: ICU 1451 ppb, pre-operative patients 812 ppb; $p=0.038$) (patient cohort described in Chapter 4; comparison of patient demographics see Appendix D). There was also a significant difference in breath acetone concentration between pre- and post-operative patients (median post-operative patients 8603 ppb; $p<0.0001$) (n=13, post-operative patients described in Chapter 3) and patients in ICU compared to post-operative patients ($p<0.0001$) (Figure 5-7).

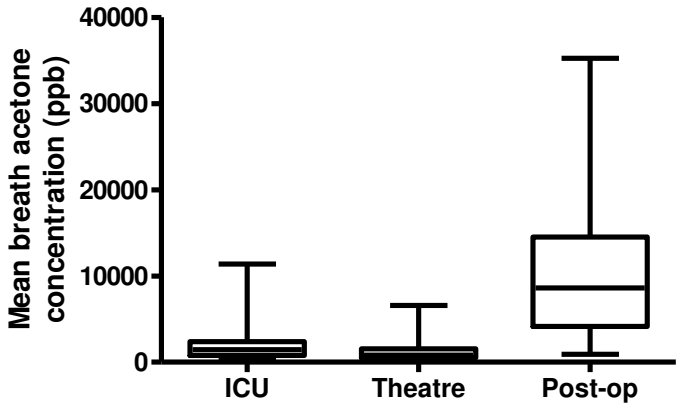


Figure 5-7. Box and whiskers plots compare mean breath acetone concentrations of patients' first ICU samples, healthy anaesthetised patients and patients returning to the ICU following surgery. Whiskers show the range of values, medians are indicated on boxes.

Only one patient in the ICU had breath and blood samples taken pre- and post-operatively, showing an increase in both breath and arterial acetone concentrations (Figure 5-8). Post-operative breath and blood samples were collected 4 hours after the surgical procedure (rotator cuff surgery). The patient had been admitted due to pneumonia following a road traffic accident and had been fed via a nasogastric tube since admission to ICU; feeding was stopped for five hours due to surgery.

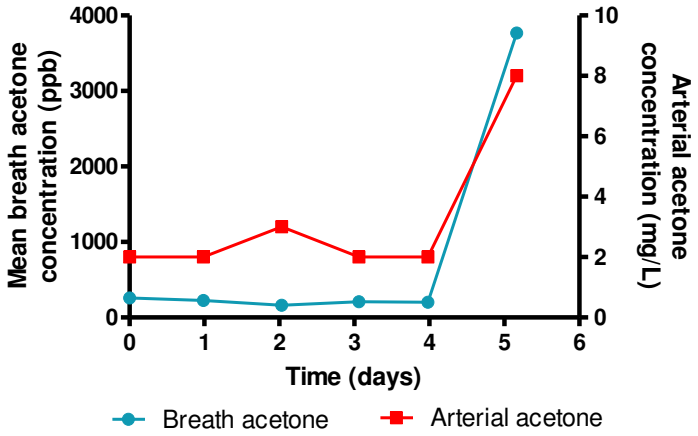


Figure 5-8. Individual data points show mean breath acetone and arterial acetone concentrations collected daily on the ICU for a single patient. Surgery was performed on day 5, 4 hours prior to sample collection.

5.4. Discussion

Using a single breath end-tidal breath collection method, a reliable and repeatable method of breath collection for analysis of acetone concentration by SIFT-MS has been presented. The technique is simple to perform and uses equipment readily available in the ICU environment, thus making it attractive for use in routine clinical practice. Disruption to a patient's ventilation pattern was minimised as much as possible and arterial blood samples were collected from an arterial cannula in situ for continuous BP monitoring and routine blood sampling.

5.4.1. Measuring ketone concentrations

The concentration of acetone in arterial samples at room temperature is not stable as it rapidly moves into the gas phase[230]. Spontaneous decarboxylation of acetoacetate also occurs[230,348], therefore blood samples must be kept cold until analysis, which can be a problem. It has been suggested that the variability of repeated measurements of blood acetone concentration in single blood samples is higher than that of breath samples, indicating that breath acetone concentration may be a more reliable measurement[115]. Due to the problems with blood acetone measurement and with a reasonable correlation between breath and arterial acetone concentrations in this study, it can be concluded that breath acetone may be used as a surrogate for systemic acetone concentrations.

The detection of ketone bodies other than acetone in breath is not possible as they are not excreted in this way[227,228], but it is possible to detect acetoacetate and beta-hydroxybutyrate in blood. Dipstick tests (described below) are occasionally used to detect the presence of acetoacetate, however an exact measurement of the concentration is not possible with this method, and due to its volatile nature, laboratory testing involves the same problems as acetone analysis. Beta-hydroxybutyrate can be measured in the laboratory, but results are not available as rapidly as breath results via SIFT-MS, and on point of care instruments[229], however these are often not available in the ICU and are not truly non-invasive.

Urinary ketone measurement

An alternative non-invasive method of ketone detection involves using a urine dipstick. Relying on the reaction of acetoacetate with nitroprusside, a semi-quantitative measurement of ketosis is possible, however the tests cannot identify beta-hydroxybutyrate in the sample, and can only detect acetone if glycine is added to the test strip[229]. Beta-hydroxybutyrate is the most abundant ketone body and its formation from acetoacetate is increased in many conditions present in the ICU, for example severe hypoxia, hepatic ischaemia and multi-organ failure, thus reducing the urine dipstick's ability to detect severe ketosis[229]. Urinary ketone concentrations may not reflect blood levels due to hydration levels, urinary volume, and renal haemodynamics[348]. Urinary testing in patients with critical illness is impossible if the patient is anuric; in this study that would have applied to seven patients and 19 daily measurements.

Arterial versus central venous acetone and glucose concentrations

No difference was seen between central venous and arterial acetone concentrations in the small number of patients sampled. Blood acetone levels were reported to whole numbers and all but 8 values were single integers. This did not provide enough sensitivity to enable conclusions to be drawn about the effect on blood concentration of the excretion of acetone in breath. A small difference was noted between contemporaneous arterial and central venous glucose concentrations; in some cases arterial acetone concentration was higher and in other patients central venous concentration was higher. Discrepancies were generally very small and may have been true differences or might have been caused by sampling or analysing errors.

5.4.2. Relationship between breath acetone and arterial ketone concentrations

The relationship between breath and arterial acetone concentrations seen in this and other studies[8,115,232] may enable breath acetone to be used as a non-invasive marker of ketosis in intubated and ventilated patients in the ICU. The modified Bland-Altman plot, however, seems to indicate that the relationship changes as

acetone concentration increases. In the study by Reichard et al.[232] the ratio of plasma to breath acetone in fasted subjects was smaller when the plasma concentration was above 1 mg/L when compared to lower plasma concentrations, which applies to almost every sample in this study.

The relationship was not changed by using breath concentration after subtracting inspired acetone concentration. The correlation between inspired and exhaled acetone concentrations seen is unlikely to have affected exhaled concentrations as, on average, the inspiratory concentration was only 11.8% of the exhaled concentration. Inspired gas samples were collected as close to the ventilator outlet as possible to minimise contamination, however, as described in Chapter 4.4.4., it is possible that rebreathing occurred around the filter in the breathing circuit, thus inspiratory gas sampling from the same location as exhaled breath sampling may more accurately quantify true inspired VOC concentrations. In all cases the ventilator, breathing circuit and breath collection apparatus were the same, so limiting the differences in acetone released from these components in different individuals.

The relationship between breath acetone and arterial beta-hydroxybutyrate concentrations was not as strong but still significant. Our results were very similar to the relationship seen in subjects fed a ketogenic diet[195]. The relationship between the different ketone bodies is dependent on the redox state of the cells, therefore the relationship between breath and arterial acetone concentrations can be expected to be stronger than that between breath acetone and other arterial ketone concentrations[348].

5.4.3. Causes of changes in breath acetone concentration

This is the first study to collect daily breath and blood samples for up to eight days from patients in the ICU. Changes in breath acetone concentration were seen over time, with a general trend towards reduction in acetone concentration with treatment and feeding. The reduction in breath acetone concentration over time was not constant, with a more rapid decrease in the first 48 hours, which may have reflected the fact that patients who had rapid clinical improvement started to breathe spontaneously earlier and therefore did not provide many

breath samples. Patients who were enrolled for a greater length of time were likely to be more unwell and may have shown less rapid improvement in breath acetone concentration over time.

Unfortunately, because many factors appear to affect breath acetone concentration, including degree of lipolysis, breathing pattern[169], dead space ventilation and pulmonary shunt[176], cardiac output[97] and airway perfusion, some of which cannot be controlled for, interpretation of results remains difficult.

Each patient was ventilated using a mode, tidal volume and respiratory rate set by the ICU staff, so it was possible that different patients' breathing patterns may have led to differences in breath acetone concentration[169]. Relationships between respiratory rate, tidal breath volume, or minute ventilation and breath acetone concentration was not observed, however, so these variables were not felt to have a strong influence on breath acetone concentration in this study.

It was not possible to measure cardiac output or pulmonary shunt in this study as patients did not have a pulmonary artery catheter in situ. Alveolar dead space ventilation was calculated instead of total dead space ventilation as mixed expiratory CO_2 could not be measured. There was no relationship between the change in alveolar dead space and breath acetone concentration over time, which indicates that factors other than alveolar dead space fraction were more important in determining breath acetone concentration.

In a study by Schubert et al.[8], the degree of pulmonary shunt was greater in patients with sepsis but dead space ventilation was not different between groups. A difference in the degree of alveolar dead space but not A-a gradient was observed in patients with ARDS versus no ARDS in this thesis, however the study population was different to that of Schubert et al.[8]. Here, patients with pulmonary infiltrates and/or insulin requirement were enrolled, and samples could only be collected from patients requiring mandatory ventilation, so spontaneously breathing ventilated patients who had less severe illness were excluded. The benefit was that samples were taken very early in a patient's ICU stay, before aggressive treatment had been instigated, therefore patients

should have had the severest metabolic derangement making any changes in breath concentration more obvious. It is possible that if a less unwell group of patients were included as a control, then differences between patients with and without sepsis, SIRS and ARDS would have been greater. ARDS guidelines have recently been updated[21] so, in comparison to previous studies, the group of ARDS patients described in this study included those with less severe lung injury, previously termed acute lung injury[350]. Moving this sub-group of patients to the non-ARDS group did not, however, change the results of the analysis.

5.4.4. Breath acetone and pneumonia

It was not possible, in this study, to use breath acetone concentration or acetone elimination rate to distinguish between pneumonia and other causes of pulmonary infiltrates, or ARDS and those with less severe lung injury. This is in agreement with a study by Schubert et al.[8], who found no difference in breath acetone concentration between septic and non-septic patients. They did, however, find a significant difference in mixed venous and arterial blood acetone concentrations between septic and non-septic patients, which was not observed here.

In the sub-group of patients with pneumonia, there appeared to be a stronger relationship between breath and arterial acetone concentrations. The explanation for this is unclear. Eight of the 12 patients had positive microbiological findings for Legionella species, H. influenzae and S. pneumoniae. Unlike P. aeruginosa ,which produces characteristic compounds, including hydrogen cyanide[314,316,351] and 2-aminoacetophenone[111], there is no data on VOCs produced by Legionella species and little data for H. influenzae and S. pneumoniae. Studies of VOCs produced by bacteria in various culture media[128,351-353] show individual patterns related to different species, and that most produce small quantities of acetone, although only one these studies included samples containing the bacteria detected in our study[353]. Acetone was frequently detected in the headspace above various bacterial cultures on agar plates, although seldom at significantly higher concentrations than background levels produced by heated culture media[354]. It is possible that acetone was

produced in the lungs by pathogenic bacteria in this study, however it is unlikely to have reached high enough concentrations to be detected in single breath samples without pre-concentration[355].

5.4.5. Breath acetone and surgery

Metabolic stress during surgery[105,106] and in patients with heart failure[245] has previously been described. Comparison between patients in the ICU and anaesthetised patients before the start of a surgical procedure with post-operative patients in the ICU shows that something other than duration of fasting causes elevation of breath acetone concentrations. The single patient in the ICU who underwent a surgical procedure had significantly higher breath and arterial acetone concentrations post-operatively compared to relatively stable concentrations pre-operatively. This patient's post-operative breath acetone concentration was not as high as that of some post-cardiac surgical patients, however the surgical procedure was shorter in duration and less complicated, therefore likely to generate less of a metabolic response. No different medications were used during the operation and there was no appreciable change in ventilator or cardiovascular parameters to explain the difference. In order to further investigate the rise in acetone concentration due to surgery, it would be necessary to monitor a group of patients undergoing a range of surgical procedures pre-, peri- and post-operatively.

5.4.6. Breath acetone and arterial glucose concentrations, feeding and insulin

There was no relationship between breath acetone and blood glucose concentrations in these non-diabetic subjects. Starvation and ketogenic meals increase breath acetone concentration[194-196], with a rise in breath acetone concentration seen in our patients when feed volume was reduced, feeding was stopped, or the patient was not absorbing their feed adequately. There is no data to suggest that enteral feed itself contains or produces acetone (or another compound with an identical m/z ratio that would interfere with its analysis by SIFT-MS), or that feed sitting in the stomach of patients with high gastric aspirates alters measured breath acetone concentration. In fact, the presence of

a cuffed endotracheal tube is likely to reduce contamination of breath samples by gastric contents, for example ethanol, which may be released by food itself or by the activity of gut bacteria[201]. Increased feed volume appeared to reduce breath acetone concentration, which is in agreement with studies in healthy subjects who were fasted and then fed[194,225]. Feeding in the ICU is administered continuously, therefore any changes in breath acetone concentration are likely to occur gradually, without diurnal variation seen in normal subjects eating meals[167,168].

There was a significant reduction in breath acetone concentration between pre- and post-insulin samples in hyperglycaemic patients, which is likely to be due to a combination of treatment with insulin and feeding. There is no formal agreement around several areas of feeding in the ICU, mainly when feeding should be started and the carbohydrate content and composition of the feed. Overfeeding can be detrimental, not only due to the increased risk of aspiration if gastric emptying is delayed, but it may also contribute to hyperglycaemia if it is present. Despite high glucose requirements due to accelerated protein catabolism[84], lower calorie feeding may improve ICU and hospital outcomes[88,89].

The results of this study highlight that some patients remained ketotic despite normal, or near normal, glucose concentrations, which may be because insufficient time had elapsed following insulin treatment to see a reduction in breath acetone concentration, or may reflect a group of patients with a high degree of inflammation and catecholamine release driving the ketogenic pathway and promoting insulin resistance[12,245]. These patients may also be ketotic due to relative starvation and require more carbohydrate feeding. In patients receiving insulin treatment in this study, reductions in breath acetone concentration were not associated with improvements in insulin sensitivity, implying not only that the relationship between glucose, ketones and insulin in critical illness is complex, but also that many factors may affect breath acetone concentration.

To explore further whether breath acetone concentration could track the degree of ketosis and aid the modulation of feeding and insulin regimes in this group of patients, the frequency of breath acetone sampling must be increased or a continuous sampling method should be employed.

5.4.7. Limitations of the breath sampling technique

In this study, representative breath samples were collected using a single breath collection technique as each patient was ventilated on a controlled mode without spontaneous breathing, so an almost identical tidal volume was delivered with each breath. A problem with this method compared to other breath collection methods was that breath sampling had to be discontinued when the patient's condition improved enough for spontaneous assisted ventilation. If the pump ran faster, it may have been possible to collect a sufficiently large end-tidal breath sample for analysis, though if the respiratory rate was too fast there would not have been adequate time to collect enough breath from a single exhalation before the initiation of inspiration and either mixed breath or multiple breath samples would have been necessary.

5.4.8. Summary

In this study, it was possible to accurately and reliably measure breath acetone concentrations in intubated and ventilated patients in the ICU using SIFT-MS. A relationship was observed between breath acetone and arterial acetone and beta-hydroxybutyrate concentrations that may enable breath acetone to be used as a surrogate for blood concentrations. The administration of insulin and feeding caused a significant reduction in breath acetone concentration in patients with stress hyperglycaemia, and the reduction in feed volume and difficulty absorbing feed resulted in increased breath acetone concentration. The relationship between breath acetone, feed volume and insulin administration needs further investigation before breath acetone can be used to aid the modulation of feeding regimes and insulin therapy.

6. Exhaled hydrogen sulphide and hydrogen cyanide in the Intensive Care Unit

6.1. Introduction and aims

Hydrogen sulphide (H_2S) and hydrogen cyanide (HCN) may be breath markers of inflammation and/or infection, but their biological roles are still uncertain. It is not yet known whether these VOCs may be useful in the ICU setting, where early accurate detection of pulmonary infection and subsequent administration of appropriate treatment may alter outcomes[28,39].

H_2S has been proposed as the third gaseous inflammatory mediator after carbon monoxide and NO[253]. The role of H_2S in the inflammatory cascade is not completely clear; it appears to influence leucocyte function and the release of cytokines and chemokines[248,264,265,334]. It may also modulate the activity of NO (reviewed[248]). HCN is produced by neutrophils[308,309] and appears to be associated with the respiratory burst of phagocytosis[310]. It is also produced by some bacteria in vitro, notably Pseudomonas species[311,316], and appeared to be seen in the breath of P. aeruginosa infected children with CF[110].

If these VOCs are produced by metabolic pathways, an understanding of the relationship between their concentrations in exhaled breath and the systemic circulation is important[8], however, if like NO they are produced in the airways, breath concentrations will not reflect plasma concentrations (reviewed[152]).

The aims were to investigate the measurement of H_2S and HCN in ICU patients with new pulmonary infiltrates on CXR and to correlate any changes in concentration over time with clinical parameters. The effects of factors known to cause variation in breath volatile concentrations, for example breathing pattern, were examined, as well as any relationship between breath and arterial concentrations. The possibility of distinguishing between pneumonia and other causes of pulmonary infiltrates on CXR using breath H_2S and HCN concentrations was explored.

6.2. Methods

6.2.1. Subjects

Patient selection, ventilator set-up, sampling and data collection are described in Chapter 5.2.1. Only patients with new pulmonary infiltrates were included.

6.2.2. Blood samples

Arterial blood gas samples were processed using a blood gas analyser in the ICU. Routine blood samples were sent to Canterbury Health Laboratory for processing straight away; samples for HCN testing were immediately put on ice and the headspace analysed by gas chromatography. The analysis of H_2S concentration in blood samples was carried out as described in Chapter 4.2.1. In several patients, arterial and central venous blood samples were drawn to look for differences in volatile concentrations.

6.2.3. Inspiratory air sampling

The process is described in Chapter 5.2.2.

6.2.4. Exhaled breath collection

End-tidal breath collection was performed via a T-piece as described in Chapter 3.2.3. of this thesis. The volume of the tidal breath prior to breath sample collection was noted. Several standard breaths were delivered by the ventilator before this process was repeated in triplicate using separate Tedlar bags. All inspiratory and exhaled breath samples were incubated at 40°C and analysed within 40 min of collection by SIFT-MS.

6.2.5. Infection control

The suction catheters and sampling filter were disposable and used for only one set of samples. The T-piece was decontaminated for re-use. Disinfectant wipes were used on the surfaces of the hand-held pump and Tedlar bags (Azo wipes, W. M. Bamford & Co Ltd, NZ). None of the cleaning procedures changed the VOC composition of samples.

6.2.6. Selected ion flow tube mass spectrometry

Breath H_2S and HCN concentrations were measured by SIFT-MS, Voice200® (Syft Technologies Ltd, NZ), using SIM mode with the H_3O^+ reagent ion (described in Chapter 2). Each Tedlar bag was attached to the sampling capillary end cap of the heated inlet extension of the SIFT-MS and samples were analysed for 30 sec.

6.2.7. Calculations and statistical analysis

Measured H_2S and HCN concentrations are quoted in this chapter, as volatile concentrations were too low to accurately correct for losses due to the sampling apparatus (linear regression equations developed in Chapter 2.3.2.).

Statistical analyses were performed using GraphPad Prism version 5.01 for Windows (GraphPad Software, USA) as discussed in Chapter 5.2.6. A value of $p<0.05$ was considered to be statistically significant. Calculations were also performed as described in Chapter 5.2.6., including A-a gradient, SIRS scores and ratios of inspiratory H_2S and HCN as percentages of exhaled concentrations.

6.3. Results

6.3.1. Patient demographics and health status

The patients enrolled in the study are described in detail in Chapter 5.3.1. In this part of the study, results from 28 patients with pulmonary infiltrates on CXR were included; 17 male (60.7%), 11 female (39.3%), median age 61.5 years (range 26-85 years). Twelve patients were current smokers and 16 were non-smokers (six ex-smokers).

The pneumotachometer was used to visually confirm that samples were collected from the patient and there was no significant back flow from the ventilator circuit.

6.3.2. Breath hydrogen sulphide

Inspiratory and exhaled H_2S concentrations

Median exhaled H_2S concentration was 0.96 ppb (range 0.22-5.12 ppb) for all samples obtained. The degree of respiratory failure did not appear to affect breath H_2S concentration; there was a weak relationship between exhaled H_2S concentration and alveolar dead space ratio (r_s=0.04, p=0.04), but not with PO_2/FiO_2 (r_s= -0.01, p=0.98) or A-a gradient (r_s= -0.11, p=0.57). Median intra-subject CV for exhaled breath H_2S concentration for all samples collected was 9.97% (IQR 6.8-14.6%), which was not affected by the alveolar dead space ratio (r_s=0.05, p=0.65) or lower PO_2/FiO_2 (r_s= -0.15, p=0.15).

Median inspiratory H_2S concentration for all samples collected was 0.91 ppb (range 0.18-4.70 ppb). There was a relationship between inspiratory and exhaled H_2S concentrations (r_s=0.83, p<0.0001) (Figure 6-1) with no significant difference between inspiratory and exhaled concentrations (p=0.47). Mean (C_{insp}/C_{exp})*100 was 99.7% (SEM ± 4.9).

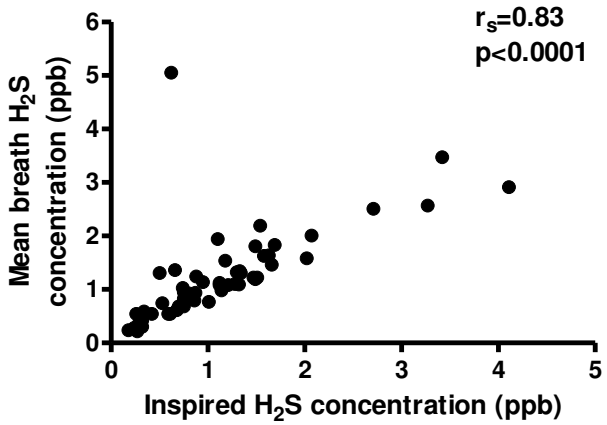


Figure 6-1. Individual data points of the relationship between inspired and mean exhaled H_2S concentrations (all samples).

Changes in breath H_2S concentration over time

There was generally very little change in breath H_2S concentration over time (Figure 6-2). One of the two patients who had breath H_2S concentrations greater than 5 ppb underwent a surgical procedure and will be discussed in Section 6.3.4. The other was a patient admitted to the ICU following an out of

hospital cardiac arrest with evidence of pulmonary oedema. There was no evidence of infection and no change in clinical condition over the three days of sampling to account for changes in breath H_2S concentration. At the time of elevation of breath H_2S concentration, corresponding breath acetone concentration was not significantly elevated (less than 2000 ppb except for one patient following surgery when breath acetone concentration was 3767 ppb).

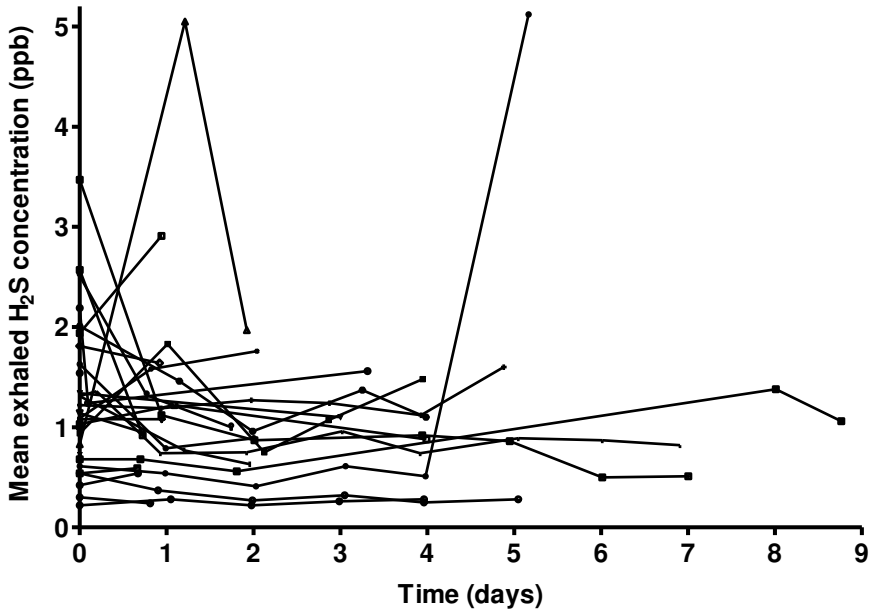


Figure 6-2. Variation in breath acetone concentration for each patient over time (lines join each sampling event for an individual patient).

Relationship between breath and blood H_2S concentrations

Median arterial H_2S concentration of all samples was 11.5 μmol/L (range 1.1-37.7 μmol/L). There was no relationship between breath and arterial H_2S concentrations in the first two samples taken from each patient (r_s=0.22, p=0.12) (one missing blood sample due to processing errors, total 52 samples). This was not altered by analysing patients with two or more SIRS criteria, sepsis and pneumonia separately. The modified Bland-Altman plot (Figure 6-3) shows a high degree of variability between normalised breath and arterial H_2S concentrations irrespective of the absolute concentration, confirming there is no relationship between the two types of sample concentration.

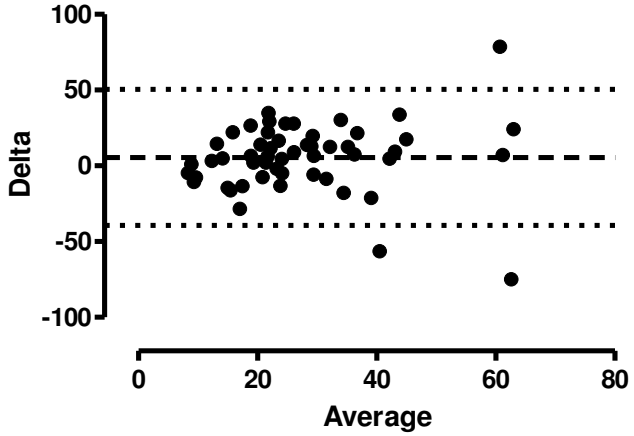


Figure 6-3. Modified Bland-Altman plot displaying the relationship between breath and arterial H_2S concentrations as percentages of their maximal value. Average = average value of the normalised breath and arterial H_2S concentrations; delta = difference between the normalised breath H_2S concentration and the normalised arterial H_2S concentration. Bias was 5.51 (dashed line) with 95% limits of agreement of -39.4 and 50.4 (dotted lines).

Relationship between arterial and central venous H_2S concentrations

A total of ten matched arterial and central venous blood samples were taken from two patients. Mean difference between matched samples was 1.0 μmol/L (arterial concentration higher than central venous concentration), range 2.3 μmol/L when central venous concentration is higher and 8.2 μmol/L when arterial concentration is higher. The relationship between arterial and central venous concentrations can be seen in Figure 6-4.

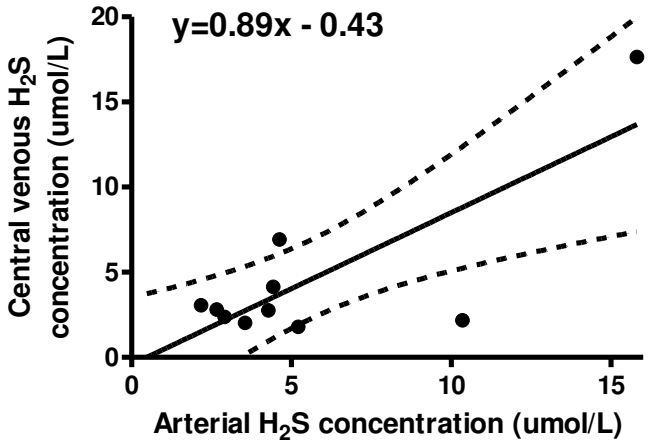


Figure 6-4. Graph showing the relationship between arterial and central venous H_2S concentrations. Individual data points are plotted. Solid line represents the linear regression line (equation shown), dotted lines show 95% confidence interval. R^2=0.62.

Relationship between H_2S concentration and clinical factors

There was no correlation between breath or arterial H_2S concentration and age (breath r_s= -0.12, p=0.56; arterial r_s=0.25, p=0.20). There was no difference in initial breath or plasma H_2S concentration between men and women (breath p=0.56, arterial p=0.78) and there were no relationships seen between breath H_2S concentration and tidal volume (r_s= -0.02, p=0.88), respiratory rate (r_s= -0.13, p=0.21), minute ventilation (r_s= -0.07, p=0.47), mean arterial pressure (r_s=0.07, p=0.51) or heart rate (r_s=0.06, p=0.57) on a daily basis.

There was no significant difference in breath or arterial H_2S concentrations in patients with SIRS compared to those without SIRS (breath p=0.43, arterial p=0.79), sepsis compared to those without sepsis (breath p=0.73, arterial p=0.63), and patients with pneumonia compared to those with another cause for pulmonary infiltrates on CXR (breath p=0.32, arterial p=0.66). Neither breath nor arterial H_2S concentrations displayed relationships with commonly used blood markers of infection and inflammation: WCC (breath r_s= -0.08, p=0.58; arterial r_s=0.00, p=0.99), CRP (breath r_s= -0.18, p=0.19; arterial r_s=0.00, p=1.00).

6.3.3. Breath hydrogen cyanide

Inspiratory and exhaled HCN concentrations

Median breath HCN concentration was 0.76 ppb (range 0.31-11.53 ppb) for all samples obtained. The degree of respiratory failure did not appear to affect breath HCN concentration; a weakly positive relationship was observed between exhaled HCN concentration and alveolar dead space ratio (r_s=0.39, p=0.047), but not with PO_2/FiO_2 (r_s= -0.13, p=0.51) or A-a gradient (r_s=0.00, p=1.00). Median intra-subject CV for exhaled HCN concentration was 8.53% (IQR 4.9-11.2%) for all samples collected; variability was not increased by increased alveolar dead space ratio (r_s=0.11, p=0.33) or lower PO_2/FiO_2 (r_s=0.03, p=0.78).

Median inspiratory HCN concentration for all samples collected was 0.67 ppb (range 0.28-2.45 ppb). There was a relationship between inspiratory and exhaled HCN concentrations (r_s=0.66, p<0.0001) (Figure 6-5), with no significant difference between inspiratory and exhaled concentrations (p=0.07). Mean (C_{insp}/C_{exp})*100 was 88.0% (SEM ± 2.7).

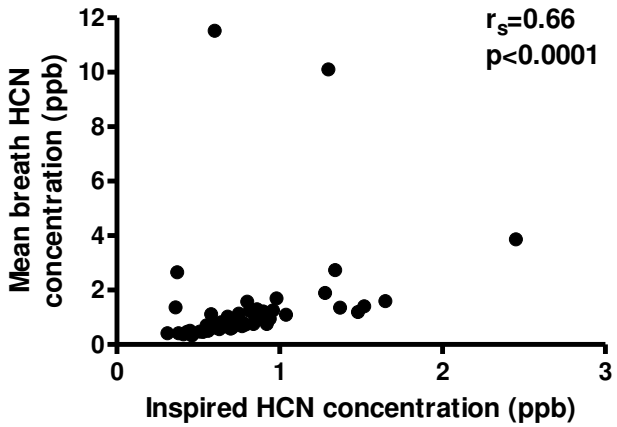


Figure 6-5. Individual data points of the relationship between inspired and mean exhaled HCN concentrations (all samples).

Changes in breath HCN concentration over time

There was little change in breath HCN concentration over time for each individual patient (Figure 6-6). The highest breath HCN concentration at enrolment (10.1 ppb) was taken from a smoker. The concentration remained

high the next day before falling. The patient with the next highest initial HCN concentration (3.9 ppb) was admitted following an out of hospital cardiac arrest associated with pulmonary oedema. There was no evidence of infection and despite a reduction in breath HCN concentration there was a general deterioration in clinical condition resulting in death. The other two patients who displayed increases in breath HCN concentration that can be seen on the graph, were admitted following motor vehicle accidents and the development of pneumonia (Haemophilus influenzae and Streptococcus pneumoniae isolated from tracheal aspirates of both patients). Both patients showed gradual clinical improvement over time and breath HCN concentration did not reflect changes in other markers of inflammation and infection (WCC and CRP).

No patients had positive microbiological samples for Pseudomonas species or other bacteria known to produce HCN[311,316].

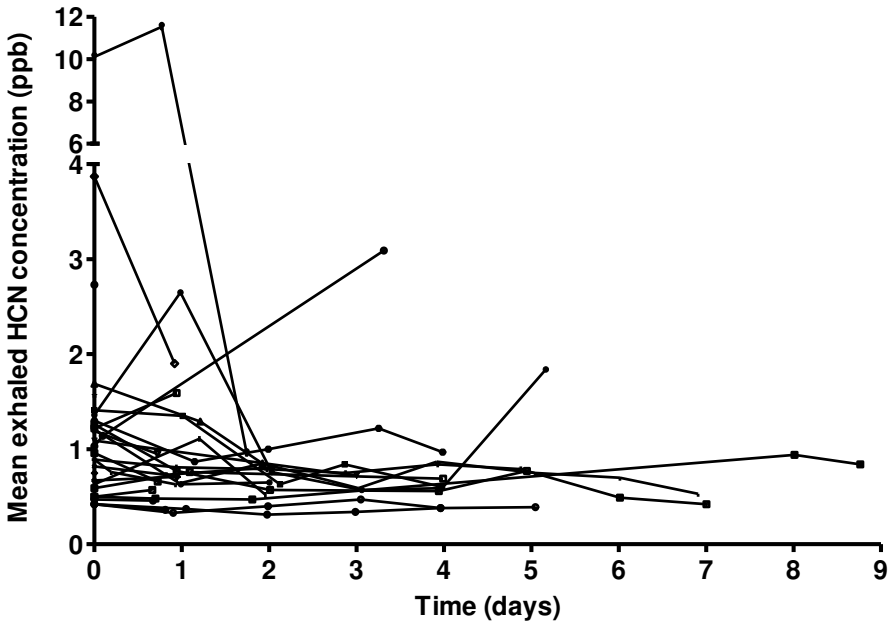


Figure 6-6. Variation in breath HCN concentration for each patient over time (lines join each sampling event for an individual patient).

Relationship between breath and blood HCN concentrations

Median arterial HCN concentration across all blood samples was 2.6 μmol/L (range 0-15 μmol/L). Blood samples from the first 15 patients were not analysed for HCN concentration, therefore results were available for 17

patients. Only eight of the 58 blood samples (13.8%) contained an HCN concentration greater than the normal range (<8 μmol/L). There was a change in the laboratory reporting of blood HCN concentrations during the study, therefore 37 samples were reported to whole numbers and 21 samples were reported to two significant figures.

There was a weakly positive relationship between breath and arterial HCN concentrations in the first two samples taken from each patient (r_s=0.39, p=0.041), which appeared to be stronger in patients with sepsis compared to all patients (n=7, r_s=0.93, p=0.0067). The modified Bland-Altman plot (Figure 6-7) shows that as the average of the normalised concentrations increases, the difference between breath and arterial concentrations increases.

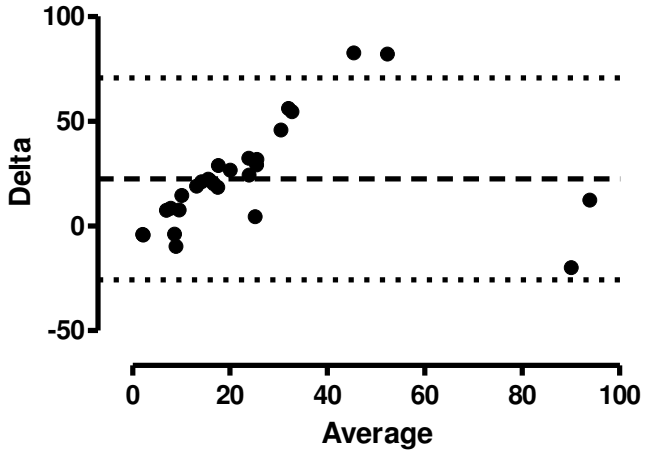


Figure 6-7. Modified Bland-Altman plot displaying the relationship between breath and arterial HCN concentrations as percentages of their maximal value. Average = average value of the normalised breath and arterial HCN concentrations; delta = difference between the normalised breath HCN concentration and the normalised arterial HCN concentration. Bias was 22.45 (dashed line) with 95% limits of agreement of -25.7 and 70.6 (dotted lines).

Relationship between arterial and central venous HCN concentrations

A total of ten matched arterial and central venous blood samples were taken from two patients. Mean difference between matched samples was 0.1 μmol/L (arterial concentration higher than central venous concentration), range 1

μmol/L with either concentration higher than the other. Figure 6-8 shows the relationship between arterial and central venous concentrations.

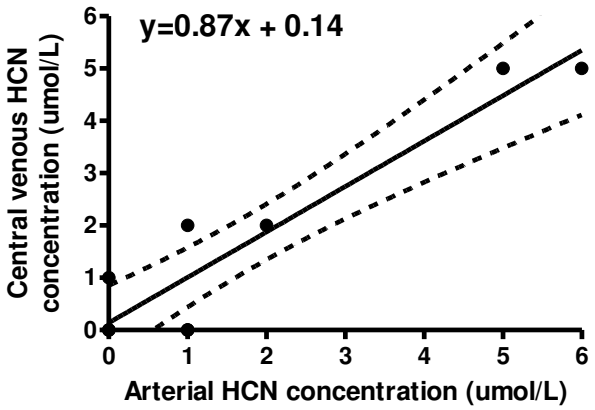


Figure 6-8. Graph showing the relationship between arterial and central venous HCN concentrations. Individual data points are plotted. Solid line represents the linear regression line (equation shown), dotted lines show 95% confidence interval. R^2=0.88.

Relationship between HCN concentration and clinical factors

There was no difference in initial breath or plasma HCN concentration between men and women (breath p=0.67, arterial p=0.35) and no relationship between initial breath or plasma HCN concentration and age (breath r_s= -0.24, p=0.21; arterial r_s= -0.51, p=0.60), tidal volume (r_s= -0.16, p=0.88), respiratory rate (r_s= -0.15, p=0.14), mean arterial pressure (r_s=0.039, p=0.71) or heart rate (r_s= -0.010, p=0.92) on a daily basis. There was a weak relationship between breath HCN concentration and minute volume (r_s= -0.22, p=0.028).

There was a difference between breath HCN concentration in patients with SIRS compared to those without SIRS (p=0.0068), however the difference in group median concentrations was small (SIRS group 0.82 ppb and no SIRS group 1.57 ppb, respectively). There was no difference in breath HCN concentration between patients with sepsis and those without sepsis (p=0.53), and patients with pneumonia and those with another cause for pulmonary infiltrates on CXR (p=0.59). No significant difference in arterial HCN concentrations was seen between patients with SIRS and those without SIRS (p=1.00), patients with sepsis compared to those without sepsis (p=0.48), and

patients with pneumonia compared to those with another cause for pulmonary infiltrates on CXR (p=0.89). Neither breath nor arterial HCN concentrations displayed strong relationships with widely used and validated blood markers of infection and inflammation: WCC (breath r_s= -0.20, p=0.15; arterial r_s=0.35, p=0.011), CRP (breath r_s= -0.20, p=0.16; arterial r_s= -0.092, p=0.51).

6.3.4. The effect of smoking on H_2S and HCN concentrations

There were 13 smokers (40.6%) and 19 non-smokers (59.4%) in the ICU cohort; 12 smokers (42.9%) and 16 non-smokers (57.1%) with pulmonary infiltrates on CXR. There were no significant differences in reason for admission to ICU between smokers and non-smokers; five of 12 smokers (41.7%) and seven of 16 non-smokers (43.8%) had pneumonia, two of 12 smokers (16.7%) and three of 16 non-smokers (18.8%) were treated for infection from another source.

There was no significant difference in initial breath or arterial H_2S concentrations between smokers and non-smokers (breath: smokers 1.18 ppb, non-smokers 1.09 ppb, p=0.85; arterial: smokers 10.16 μmol/L, non-smokers 10.44 μmol/L, p=0.24).

There was no difference in exhaled HCN concentration of initial samples between smokers and non-smokers (smokers 1.11 ppb, non-smokers 0.93 ppb, p=0.63), but initial arterial HCN concentration was significantly higher in smokers than non-smokers (smokers 9.0 μmol/L, non-smokers 3.7 μmol/L, p=0.047) (Figure 6-9).

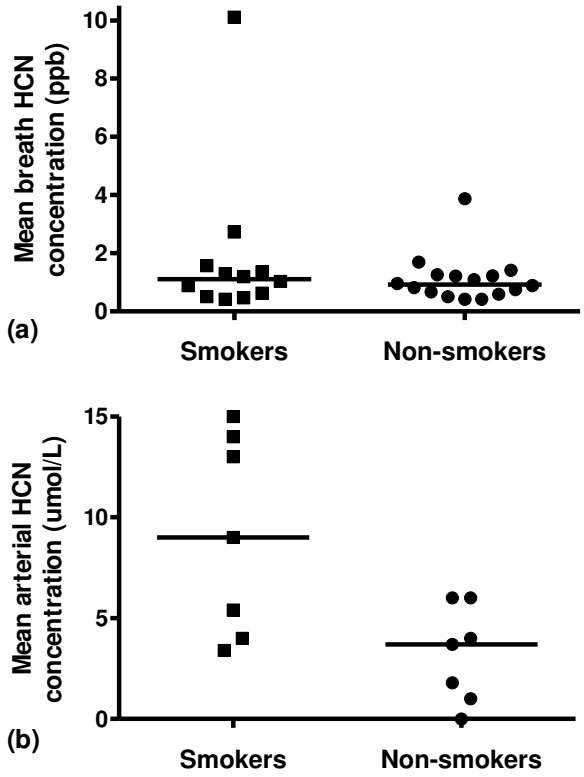


Figure 6-9. Individual data points and medians to compare initial breath (a) and arterial (b) HCN concentrations between smokers and non-smokers ((a) n=28, (b) n=14).

6.3.5. Breath H_2S and HCN in the ICU, pre-operative and post-operative settings

There was an overlap in the range of exhaled H_2S concentrations in each cohort of patients, with concentrations in the pre-operative (from Chapter 4) and ICU groups being lower than that of the post-operative group (from Chapter 3) (medians: ICU 1.12 ppb, pre-op 1.00 ppb, post-op 2.6 ppb). Median post-operative exhaled HCN concentration was greater than in the other two cohorts (medians: ICU 0.99 ppb, pre-op 0.82 ppb, post-op 2.28 ppb), and the variation in breath HCN concentrations was greater in both ICU groups compared to the pre-operative group. A significant difference between exhaled H_2S and HCN concentrations in post-operative patients compared to both pre-operative patients (H_2S $p<0.0001$, HCN $p<0.0001$) and those with critical illness in the ICU (H_2S $p=0.0004$, HCN $p=0.0011$) was observed. There was no difference between exhaled H_2S and HCN concentrations in pre-operative controls and

ICU patients (H_2S p=0.57, HCN p=0.39) (Figure 6-10). A comparison of patient demographics between ICU patients and healthy anaesthetised controls can be seen in Appendix D.

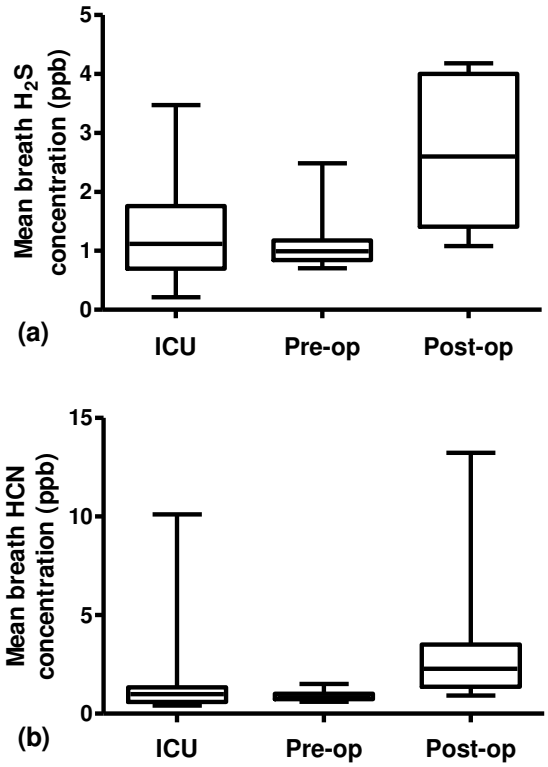


Figure 6-10. Box and whiskers plots comparing exhaled volatile concentrations of initial breath samples from ICU patients, healthy anaesthetised patients (pre-op) and patients returning to the ICU following surgery (post-op); (a) exhaled H_2S concentration and (b) exhaled HCN concentration. Whiskers show the range of values and boxes display the median and interquartile range.

One patient in the ICU cohort had breath and blood samples taken before and 4 hours after rotator cuff surgery. The patient was admitted to the ICU with pneumonia requiring invasive ventilation following a road traffic accident, his clinical condition improving throughout his ICU stay. An increase in breath, but not arterial, H_2S and HCN concentrations was observed following surgery (Figure 6-11); the rise in H_2S concentration was more marked than that for HCN. Of note, a concurrent increase in arterial and exhaled acetone concentrations was also seen. Acetone results are presented in Chapter 5.3.9.

and discussed in Chapter 5.4.5. The potential effects of high breath acetone concentration on the calculation of breath H_2S concentration is discussed in Chapter 3.4.5.

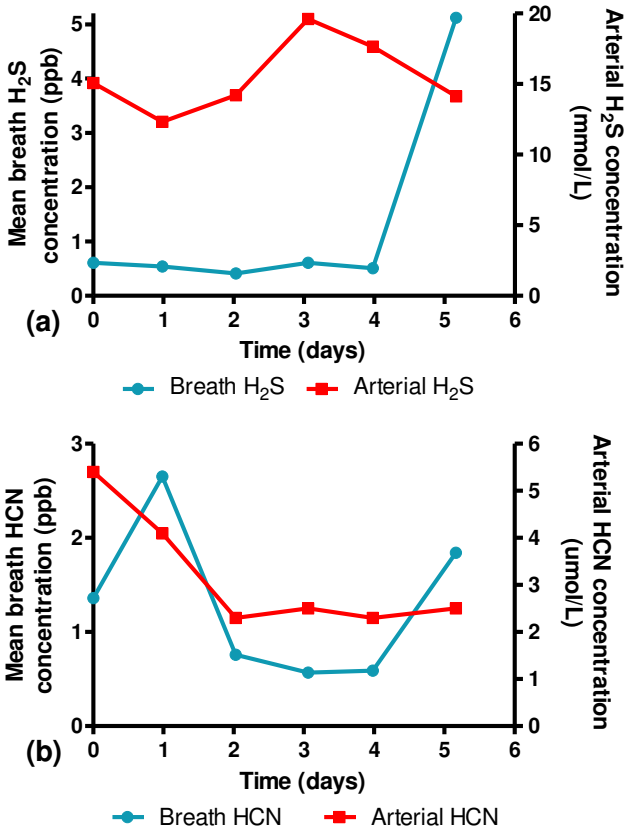


Figure 6-11. Individual data points show mean breath and arterial (a) H_2S and (b) HCN concentrations collected daily on the ICU for a single patient. Surgery was performed on day 5, 4 hours prior to sample collection.

6.3.6. Plasma H_2S in the ICU and pre-operative settings

There was no significant difference between blood H_2S concentrations in healthy pre-operative patients and the first sample collected for all patients in the ICU (p=0.11). In fact, median blood H_2S concentration was higher in pre-operative patients than ICU patients (12.8 umol/L versus 10.5 µmol/L, respectively).

6.4. Discussion

This study appears to be the first to collect breath and blood samples from intubated and ventilated patients in the ICU for analysis of H_2S and HCN concentrations. There was no significant difference in breath volatile concentrations between patients with pulmonary infection and other causes of pulmonary infiltrates on CXR. Using the breath collection and analysis method described, it is, therefore, not possible to use breath H_2S or HCN for the diagnosis or monitoring of pneumonia in critical illness.

6.4.1. The relationship between inspiratory and exhaled breath concentrations

Inspiratory and exhaled H_2S and HCN concentrations were similar, with relationships observed between the two sets of values. This makes further interpretation of any results difficult. As illustrated by other authors, care should be exercised when interpreting breath volatile concentrations in cases where the inspired volatile concentration is greater than 5%[8] or 25% of the exhaled concentration[2]. There are several factors that may have led to a relationship between inspired and exhaled volatile concentrations, however, it is important to remember that due to the lack of previous research into these volatiles in breath it was not known whether they would be produced in the lungs due to local inflammation, emitted by bacteria in the lungs, excreted via the lungs due to their presence in the systemic or bronchial circulation, or not detected at all.

Firstly, inspired concentrations of some VOC have previously been shown to influence expired concentrations[147,180,337], thus it is possible that exhaled breath concentrations here merely reflect inspired volatile concentrations. Inspired concentrations in this study are very low, far lower than those commented on in the articles mentioned above, and a recent study has shown that in healthy, spontaneously breathing adults, low atmospheric inspired HCN concentrations did not affect exhaled concentrations[151], arguing against this level of inspired HCN affecting exhaled concentrations. It has already been shown that the apparatus used for breath sampling did not emit compounds at m/z peaks

relating to H_2S or HCN (Chapter 2.3.2.), but it is possible that re-breathing of volatiles occurred around the breathing circuit filter. This phenomenon was described in a study in the operating theatre, where fresh gas passing through the filter on inspiration released volatiles from the breathing circuit filter that had accumulated during expiration[337]. It could not have occurred in all patients in this study, however, because a breathing circuit filter was not always present.

Secondly, inspired and exhaled volatile concentrations may have been in equilibrium because very little or no H_2S or HCN is present in human breath in the setting of critical illness. This is backed up by the lack of difference between healthy subjects in the operating theatre setting (Chapter 4) and in the ICU. Furne et al.[262] demonstrated an atmospheric H_2S concentration of 1.6 ppb and exhaled breath concentrations around 0.33 ppb above atmospheric concentrations. Despite analysing oral breath samples, after a breath hold with the mouth open to prevent contamination, H_2S concentrations are comparable with this study. Inspired and nasally exhaled H_2S concentrations were also similar to those of a study of healthy subjects by Dummer[223] (median inspired concentration 0.5 ppb, exhaled 1.1 ppb) and inspired HCN concentrations were comparable to those seen in previous work[150,151]. Group median exhaled HCN concentrations in this study seem to be lower than in studies of nasally exhaled breath in spontaneously breathing healthy volunteers (0.76 ppb versus around 2 ppb)[150,151] but similar to nasally exhaled breath of subjects with CF who were not colonised by P. aeruginosa[239]. The higher concentrations in spontaneously breathing subjects may be a result of oral contamination of nasal breath samples, for example if an oral inspired breath was taken before a nasal exhalation[151] or if swallowing occurred before sample collection.

Lower concentrations of exhaled H_2S and HCN were seen in this study than in orally exhaled breath in other studies[149-151,223,318] because the ETT bypassed the upper airways, therefore preventing contamination of breath samples with H_2S and HCN produced in this area. Both compounds are highly soluble and it is likely that at least some gas exchange occurs in the airways rather than just the

alveoli[157], therefore the presence of the ETT may also have resulted in a reduction in gas exchange in the upper airways.

6.4.2. Bacterial production of H_2S and HCN in breath

As briefly mentioned above, H_2S and HCN may not be produced in the lungs at all, or not in high enough quantities to be detected by this breath collection and analysis method. No patients had evidence of colonisation or infection with Pseudomonas species, either in the chest, urine or skin, so it is not possible to draw any conclusions about whether HCN may be elevated in breath or blood due to the presence of bacteria known to produce HCN in vitro[311,316]. The lack of P. aeruginosa detected may be due to several factors; none of the patients enrolled had hospital acquired or ventilator associated pneumonia, where infection with P. aeruginosa is more common than in community acquired pneumonia[37,38], or COPD or bronchiectasis, specifically no patients with CF, with known P. aeruginosa colonisation, as none were present on the ICU during the data collection period.

H_2S is produced by several bacterial species in vitro, including Escherichia coli, Streptococcus pneumoniae, Staphlococcus aureus, Neisseria meningitidis and Proteus species[352,354]. The production of H_2S by Legionella species and Haemophilus influenzae has not been investigated. These species were predominant in the pneumonia patients investigated, thus it is unclear whether breath H_2S concentration would be expected to be elevated. It is possible that H_2S was produced in the lungs by pathogenic bacteria, but did not reach high enough quantities to be detected in single breath samples without pre-concentration[355].

6.4.3. Systemic and airways production of H_2S and HCN

Hydrogen sulphide

Endogenous H_2S is produced from cysteine, cystathionine and/or homocysteine by the action of the enyzymes CSE and CBS[253], both expressed in human airway smooth muscle[282] where H_2S appears to inhibit airway smooth muscle proliferation and production of the pro-inflammatory cytokine IL-8[282]. Despite

studies showing lower serum H_2S levels in patients with acute exacerbations of COPD compared to stable COPD[287,289], and in pneumonia compared to healthy controls[289], no studies have been performed to examine breath H_2S levels in humans during episodes of pulmonary infection. In a small study of patients with septic shock, plasma H_2S concentration was significantly higher than in healthy controls[261], but breath concentrations were not measured, and unfortunately the authors give little clinical information about these patients. It is therefore not possible to determine whether breath concentrations reflect airway or lung tissue H_2S production or systemic production due to systemic inflammation or cardiovascular instability.

In contrast to the studies above, there was no significant difference in breath or plasma H_2S concentration between patients with pneumonia and those with pulmonary infiltrates caused by other conditions. There was also no significant difference in breath or serum H_2S concentration when patients with SIRS and sepsis were compared to those without SIRS or sepsis, respectively. This may be due to the low numbers in the study, or because breath levels were too low to be quantified in single breath samples without pre-concentration. Some of the less unwell patients in the cohort still had higher levels of inflammation than healthy subjects so there may not have been enough difference in disease states to see a difference in H_2S concentration. Many patients with pulmonary infection, where plasma H_2S may have been expected to be lower[285,287,289], also had septic shock, which has been seen to increase plasma H_2S concentrations in humans[261] and animal models[261,264], thereby cancelling out the effects. It is also unclear whether the use of inotropic and vasopressor support administered to some patients may have affected the concentration of plasma H_2S due to modulation of the cardiovascular system.

Due to the high levels of inflammation in the patients from whom samples were collected in the ICU, it would be expected that there would be a difference between these patients and healthy pre-operative controls. There was no difference in breath H_2S concentrations between these two groups; serum H_2S concentrations were lower in ICU patients than healthy patients, but this difference did not reach statistical significance. Again, this could have been

due to small numbers in the study, or as a result of issues surrounding H_2S analysis in blood, which will be discussed later. Despite low serum concentrations in anaesthetised controls, blood samples were taken prior to anaesthetic delivery so any drugs given or changes in cardiovascular parameters would not have affected blood results.

In contrast to the study by Chen et al.[289], where a weak inverse correlation was seen between plasma H_2S and CRP in patients with pneumonia and COPD, there was no relationship between CRP and plasma H_2S in this study.

There was no correlation between breath and arterial H_2S concentrations in this study. This may be because plasma concentrations were low and any systemic H_2S produced was rapidly scavenged and bound to methaemoglobin (metHb)[282], therefore it could not be excreted in breath. Another possible explanation is that, like NO, any H_2S in breath is produced in the airways themselves, in which case a relationship between breath and plasma concentrations would not be expected. There were a few exhaled breath samples containing an H_2S concentration above the inspiratory concentration and without a corresponding high plasma H_2S concentration. These were likely to have been true high readings, rather than being influenced by high corresponding breath acetone concentrations; all but one breath sample contained an acetone concentration of less than 2000 ppb, which is unlikely to have been high enough to significantly reduce the number of precursor ions available and affect the concentration calculations of the SIFT-MS instrument (precursor ion stripping is discussed in Chapter 3.4.5.).

Hydrogen cyanide

A significant difference in breath HCN concentration was observed when patients who fulfilled the criteria for a diagnosis of SIRS were compared to those who did not; the absolute difference in median breath concentrations was, however, very small and there was overlap between the two groups. The difference may not have been clinically significant and it was also noted that fluctuations in breath HCN concentration over time did not appear to be related to changes in an individual patient's clinical condition.

There was no difference between breath concentrations in ICU patients and healthy anaesthetised subjects; however the range of concentrations was higher in the ICU cohort. This may be a consequence of a greater number of smokers in the group of ICU patients compared to healthy volunteers, although smokers were not excluded. Patients with high breath HCN concentrations, or serum HCN concentrations exceeding physiological levels, were all smokers, which is almost certainly the cause and will be discussed in Chapter 6.4.4.

The production of HCN by neutrophils has been demonstrated in vitro[308,309], but not in vivo. In studies of sodium nitroprusside administration during general anaesthesia, HCN could be detected in breath when the plasma concentration was high and there was a relationship between the two. Only a small proportion of HCN remains unbound in plasma, allowing it to cause toxic effects, with 98% being strongly bound to metHb[299,301]. That remaining in the plasma is detoxified when it travels into the tissues and is converted to thiocyanate[191,301]. This means that, if much lower levels of HCN are produced than seen in poisoning, for example in this study, it is possible that very little HCN will be present in plasma due to a combination of binding to metHb and conversion to thyocyanate, thus no HCN will be excreted in breath. A weakly positive correlation was seen between breath and arterial HCN concentrations in this study, however this may be due to the low concentrations in each type of sample.

The quantification of HCN in blood was only available during the latter half of the study so a limited number of patients had samples analysed. This may have affected the ability of this study to draw any conclusions regarding the analysis of blood HCN concentrations. Unfortunately, HCN concentrations were not analysed in the blood of the healthy anaesthetised volunteers, so comparison with patients in the ICU was not possible.

6.4.4. Effect of smoking on H_2S and HCN concentrations

Hydrogen sulphide

No significant difference was seen between breath or arterial H_2S concentrations in smokers versus non-smokers. This is in contrast to two

studies of subjects with COPD[287] and asthma (reviewed[254]), in which smokers in the disease group or healthy controls displayed lower serum H_2S concentrations. In both of these studies, there was a negative correlation between serum H_2S concentration and the percentage of sputum neutrophils, although no breath samples were taken. There is evidence of neutrophilic inflammation in exhaled breath condensate of smokers[356], which may explain why they have lower plasma H_2S concentrations than non-smokers.

Another study by Chen et al.[281] showed conflicting results, with higher serum H_2S concentration and CSE expression in lung tissue of rats exposed to cigarette smoke compared to controls. In this and another study in rats, the administration of H_2S appeared to improve inflammation caused by cigarette smoke[281,284]. It is possible that the response to cigarette smoke in rats is not identical to that in humans, or that the type of exposure caused an acute response that is different to that of chronic cigarette smoking.

H_2S might be expected to be detected in the breath of smokers as it is present in small quantities in cigarette smoke[187], though the concentration may be too low to enable quantification in breath by SIFT-MS if the last cigarette was smoked several hours or more ago.

Hydrogen cyanide

There was no significant difference in day 1 breath HCN concentration between smokers and non-smokers, however one smoker had a breath HCN concentration over ten times higher than the group median concentration for two days. Unfortunately, no information was available about the time interval between the last cigarette smoked and the time of breath sampling. Day 1 samples should give highest chance of detecting differences associated with cigarettes and would be expected to reduce as time from last cigarette increases. Four smokers were responsible for the eight arterial HCN concentrations above physiological levels, gradually decreasing over time. There were no other important differences between these patients and the rest of the cohort, therefore it is likely that smoking was the cause of the elevated concentrations.

Not only is there evidence for the presence of HCN in cigarette smoke itself[123,186,188], but higher exhaled HCN concentrations have also been see in oral breath samples[123,192,301] and blood samples of smokers compared to non-smokers[301], supporting the results of this study. Higher plasma thiocyanate concentrations have been seen in smokers than non-smokers[191,301], presumably due to detoxification of HCN inhaled from cigarette smoke[191]. Plasma thiocyanate concentrations have been seen to decrease slowly, taking up to 14 days to return to normal after smoking cessation, though the duration of reduction in breath HCN concentrations is unknown[301].

6.4.5. Possible errors in breath sampling and analysis

Minor variation in H_2S and HCN concentrations seen in this study may be due to errors in the sampling and analysis process, thus not clinically significant. The concentrations of H_2S and HCN were very low, nearing the lower limit of detection of the SIFT-MS instrument, so any small errors in absolute concentration would have resulted in large percentage changes. Significant differences and relationships produced by the analysis of both volatiles sometimes reflected very small differences in median concentrations. The results may, therefore, not be clinically significant and may reflect the fact that numerous analyses were conducted.

Another possible explanation for the low concentrations of exhaled volatile detected is loss due to the sampling apparatus or breathing circuit. Owing to the high solubility of H_2S and HCN, if water droplets form due to condensation of humid breath the volatiles will be dissolved in the droplets and cannot be measured in the gas phase, falsely lowering the concentration observed. Condensation could have occurred in the breathing circuit itself (not all circuits contained a humidifier) or in the collection apparatus, which was not heated prior to sample collection. Tedlar bags containing breath samples were immediately incubated to try and prevent condensation of samples and analysed as soon as possible to reduce the chance of volatile loss (see Chapter 2 for further details). Some sample was probably lost due to sticking to the inside of the ETT and ventilator circuit tubing, breath collection tubing, pump, and Tedlar bags, as well as being removed by adhering to the biological filter

in the collection process. Unfortunately it is not easy to quantify all of this loss and it is not routinely reported on in studies of breath volatiles. Any reduction in volatile concentration would have occurred in inspiratory as well as exhaled samples, therefore the relationship between the two sets of samples is not likely to have been significantly affected.

6.4.6. Exhaled H_2S and HCN concentrations following surgery

A significant difference was identified between breath H_2S and HCN concentrations in ICU patients with pulmonary infiltrates compared to patients admitted to the ICU following cardiac surgery. Post-operative breath volatile concentrations were higher, but overlap in concentration range between the two groups prevented their separation on the basis of breath volatile levels. There was also a patient with pneumonia who had an orthopaedic procedure whilst in the study, where the exhaled H_2S and HCN concentrations were elevated post-operatively.

There seems to be an increase in inflammation caused by undergoing a surgical procedure, which may be reflected in an elevation in breath H_2S and HCN concentrations. Any changes seen in this study were, however, confounded by changes in breath acetone concentration, thus interpretation of the difference between ICU patients and post-operative patients is difficult. As has been discussed in Chapter 3, corresponding breath acetone concentrations were very high in the post-operative group and were likely to have caused problems in the calculation of volatiles at much lower concentrations by the SIFT-MS instrument. Precursor ion stripping is unlikely to have occurred in patients with pulmonary infiltrates as breath acetone concentrations were sometimes 100 times lower than in the post-operative group.

6.4.7. Analysis of H_2S in blood samples

Samples for H_2S sampling were partially processed in Christchurch Hospital, then frozen and sent in batches to Canterbury University on the other side of the city for further processing using the protocol most widely used in the literature[334]. This method may measure 'free' H_2S and also H_2S-derived species at physiological pH (HS^- and S^{2-}). It is comparable to other techniques

used to measure H_2S concentration; the main one used in human studies involves a sulphide-selective electrode[287,357](reviewed[255]).

Plasma H_2S concentrations were low in all parts of this thesis, lower than in other studies[285,287,289,357]. Healthy control subjects in these studies had plasma H_2S concentrations greater than 40 μmol/L, compared to 12.8 μmol/L in healthy anaesthetised patients (Chapter 4) and 10.5 μmol/L in ICU patients with pulmonary infiltrates in this study. Due to a lack of data, the normal range for plasma H_2S concentrations in humans is unknown. Five patients with sepsis in a study by Li et al.[261] had a mean plasma H_2S concentration of 150 μmol/L, children with postural orthostatic tachycardia syndrome 100.9 μmol/L[357], but patients in two studies by Whiteman et al. involving patients with knee effusions[358] and lean healthy men[280] had plasma concentrations 36-39 μmol/L. The cause for the discrepancy in plasma H_2S concentrations is unclear. Low plasma H_2S concentrations in patients with all causes of pulmonary infiltrates may be due to high levels of pulmonary inflammation, however concentrations did not seem to change over time with clinical condition or treatment, and this does not explain the low concentrations seen in anaesthetised controls. The measurement of plasma H_2S concentration in ICU patients does not seem to be useful in the diagnosis of pneumonia, sepsis or degree of pulmonary or systemic inflammation, and cannot be used to track changes in clinical condition.

There may have been errors in blood sample processing. Firstly, samples were centrifuged as soon after collection as possible, within a few minutes, although due to the volatile nature of H_2S this delay may have allowed time for reduction in H_2S concentration due to volatilisation or aerial oxidation[255]. Secondly, samples were spun down for 15 min, during which time the centrifuge was not cooled, therefore H_2S contained in the sample may have degraded, with a possible further delay of around 10 min between centrifuging and plasma pipetting. Plasma samples were initially frozen at -25°C for several days, before being transferred and frozen at -80°C. Samples were then transported across the city in an insulated box with ice, which may have permitted a degree of thawing in transit; again, this may have led to sample

degradation. Interestingly, when a group of samples were processed as above and identical samples had the first processing step (the addition of zinc acetate to enable the formation of stable zinc sulphide) performed at Christchurch Hospital prior to freezing, there was no significant difference in H_2S concentration between the matched samples (data not presented). This implies that sample concentration was not significantly affected by being transported to Canterbury University on ice.

6.4.8. Central venous and arterial blood samples

Arterial blood sampling was used in this study because it was the usual method for blood sampling in Christchurch Hospital ICU. It was non-invasive as every patient had an arterial line inserted on admission to the ICU for continuous blood pressure monitoring. By taking all of the patient's daily blood samples at the time of breath sampling as well as study specific samples, the volume of blood aspirated was minimised.

Most patients also had a central line inserted for the administration of medication. It is possible to take blood samples from this line, however it is necessary to stop infusions first so that blood results are not diluted, which affects a patient's treatment. When taking samples from either line, the first 5-10 ml blood aspirated must be discarded due to possible contamination, therefore the collection of blood from both the central venous and arterial lines involved the removal of up to 40 ml blood per day.

For the reasons above, central venous blood samples were not routinely drawn and comparisons between matched central venous and arterial samples were made in only a small number of patients. Despite this, there did not appear to be a significant difference between the concentrations of H_2S and HCN from each site. There was no particular pattern of one site having higher measured volatile concentrations than the other, thus arterial blood sampling was used in preference as it was more convenient than central venous sampling. The lack of difference between central venous and arterial concentrations means there is unlikely to have been a significant difference between peripheral venous and

arterial concentrations, thus blood samples taken from healthy anaesthetised controls and ICU patients should be comparable.

6.4.9. Summary

Exhaled H_2S and HCN were detected at very low concentrations in the group of intubated and ventilated patients in the ICU with new pulmonary infiltrates on CXR. The relationship between inspired and exhaled concentrations makes any further analyses difficult to interpret. There was no relationship between breath and arterial H_2S concentrations, and only a weak relationship between breath and arterial HCN concentrations, therefore the concentrations of these volatiles in exhaled breath do not reflect concentrations in the systemic circulation.

Higher blood HCN, but not breath, concentrations were seen in smokers than non-smokers, however concentrations could not be used to distinguish between the two groups. H_2S and HCN concentrations in breath could not be used to distinguish between patients with pneumonia and other causes for pulmonary infiltrates on CXR, between patients with differing levels of systemic inflammation or organ failure, or between patients in the ICU or healthy anaesthetised controls.

7. Discussion

7.1. Summary of findings

The analysis of breath biomarkers to detect or monitor pulmonary inflammation or infection and metabolic dysfunction is attractive due to its non-invasive nature and the ability to provide results rapidly, repeatably, accurately and in some cases in real time[4]. The challenge rests in identifying useful compounds to act as biomarkers.

Many factors confound the results of breath testing, including those involved in the collection and analysis of samples, patient and environmental factors, particularly the effects of inspired VOCs. Apart from the analysis of exhaled NO in asthma[94], no agreed method of breath sampling or standardisation of results exists[2,147,169,206]. Unfortunately, it is not possible to control all of the elements above, which affect different VOCs in different ways, therefore it may be necessary to develop several standard techniques for individual, or groups of, trace gases. A "Task Force" has recently been set up to explore these areas and hopefully, with the support of the breath research community, will find some solutions[206].

In this thesis, a technique was developed to allow the collection of end-exhaled breath samples repeatedly, reliably and safely from mechanically ventilated patients in the ICU and from anaesthetised patients in the operating theatre. As with other breath studies involving patients in the ICU, due to the size of the SIFT-MS instrument it was necessary to perform analysis off-line[5-8]. In the first part of the study, two different breathing manoeuvres at two breath sampling locations were investigated, and the most appropriate technique chosen for use throughout the rest of the thesis.

Breath sampling directly from the airways is appealing, as it should reduce contamination and dilution due to dead space in the endotracheal tube (ETT) and ventilator tubing. However in practice, with the equipment available in the Christchurch Hospital setting, there were significant difficulties with this

method due to air leakage and loss of tidal volume, preventing its use in some patients. Sampling from a T-piece was, therefore, preferred.

Delivering a recruitment-style breath to a patient, mimicking a vital capacity breath, was possible in most cases, but required knowledge and skill when adjusting ventilator settings. Pressure-controlled rather than volume-controlled breaths were delivered to reduce the risk of barotrauma[327], however that resulted in higher inter-individual variability in breath volume when compared to tidal breath volume. The increased variability makes the method less suitable for use in further studies. Due to its high solubility[154], end-tidal acetone concentration was slightly higher than end-exhaled concentration after a recruitment-style breath. No significant benefit was observed in the repeatability of breath acetone measurement with the delivery of recruitment-style breaths, so end-tidal T-piece sampling was chosen.

As in other studies of breath acetone, including in healthy individuals[5,105,115,167,168,196], there was high inter-subject variation in breath acetone concentration, greater than intra-subject variation due to changing the location of breath sampling or breathing manoeuvre. This was not felt to be important in this part of the study as patients acted as their own controls.

Very high breath acetone concentrations appeared to affect concentration calculations of H_2S and HCN by reacting with most of the precursor ions. This problem may not apply to analytical methods other than SIFT-MS and PTR-MS and may have affected analytes present at higher concentrations to a lesser extent; if the absolute error was the same it would have represented a smaller relative error. Salbutamol administration during breath collection caused similar effects. It is therefore advisable not to collect breath samples for 10 min following inhaled medication[330,331].

Due to the potential effects of recent surgery, other causes of inflammation or drugs on breath VOC concentrations, there was no suitable control group of patients in the ICU. A group of healthy volunteers awaiting surgery was selected so that breath samples could still be collected from intubated individuals. It was therefore necessary to validate an off-line breath collection

method in the operating theatre, comparable to that used in the ICU. By using total intravenous anaesthesia (TIVA) and a relatively high inspiratory gas flow rate, fixing the anaesthesia machine settings and not altering them between patients, and breath sampling as close to the patient as possible, reproducible and reliable results were obtained. The same drugs, or classes of drugs, were used for intubation and TIVA as for sedation and paralysis in the ICU cohort, therefore reducing the number of confounding factors. The control group contained a larger proportion of women than the ICU group, due to the types of surgery for which TIVA is appropriate, and a slightly lower proportion of smokers. A few control patients had obstructive spirometry, which is likely to have been similar in the ICU group, although it was not possible to confirm this by performing spirometry in patients with critical illness.

This is believed to be the first study to investigate the changes in breath VOCs over time in patients with critical illness. Breath acetone concentration tended to decrease over time, being significantly higher in ICU patients on the first day of sampling than in fasted controls, however there was significant overlap in acetone concentrations between the groups. The concentration appeared to rise in association with surgery[105,106], deteriorating clinical condition and low feed intake, as a consequence of reduced input or poor absorption as determined by high volume gastric aspirates. This would be expected due to the known relationship between acetone concentration and fasting[194,227,228,230,232]. Initial breath acetone concentration was not related to outcome measures, such as mortality and length of stay, or other markers of inflammation and organ dysfunction. As in previous studies, there was no difference in breath acetone concentration between patients with, compared to without, pneumonia[5] and in those with sepsis compared to those without[8]. In the study by Schubert et al.[8] a lower blood acetone concentration was reported in patients with sepsis, which was not seen in this study. A slightly higher acetone elimination rate was previously observed in patients with ARDS compared to those without the diagnosis[5], although the difference did not reach statistical significance and was not replicated here.

Irrespective of the type of blood sample, there was a relationship between breath acetone and blood acetone and beta-hydroxybutyrate concentrations in both ICU patients and anaesthetised controls[8,115,195,230,232]. The relationship was not lost in patients in the ICU who would have had higher degrees of pulmonary dead space and shunt[1,174]. Unlike the study of O'Hara et al.[115], there was no difference in the correlations of breath acetone with peripheral venous versus arterial blood concentrations. Identical acetone concentrations were observed when a small number of matched peripheral venous and arterial blood samples were compared. These results confirm those of other studies suggesting breath acetone could be useful as a non-invasive marker of ketosis[8,115,195].

Breath H_2S and HCN were detected at very low ppb concentrations in ICU patients and controls, comparable to previous studies reporting nasally exhaled breath concentrations in patients with bronchiectasis and healthy controls[150,151,223,262]. Breath concentrations of these two volatiles were related to inspired concentrations; in fact exhaled and inspiratory concentrations were similar, which makes interpretation of breath results difficult[2,8]. Breath concentrations could have been truly low, or reduced due to adsorption onto the inner surface of the pump and tubing during sampling. No patients had confirmed infection with P. aeruginosa, so HCN concentrations would not be predicted to be elevated for that reason[311,316]. Systemic H_2S and HCN may have been at low enough concentrations to be completely scavenged by metHb[249,298], thus not easily detectable in breath. As the first study to look at breath H_2S and HCN concentrations in mechanically ventilated patients, the hypothesis was that breath concentrations would be higher in patients with pulmonary infection if they behaved in the same way as NO[117,118]. Unfortunately, this was not observed and neither of the trace gases could be used to distinguish patients with pneumonia, or sepsis, from those without the conditions.

There was no strong correlation between breath and blood H_2S and HCN concentrations or relationships with conventional markers of infection and inflammation. Unlike studies of plasma H_2S concentration in individuals with

COPD and pulmonary infection, in which infection was associated with lower plasma concentration[287,289], there was no difference in plasma H_2S concentration in patients with pneumonia compared to those with an alternative pathology. There was also no difference in plasma H_2S concentration in patients with sepsis compared to other diagnoses, in contrast to a small study of patients with septic shock in which plasma concentrations were high[261]. Little information is reported on the nature of the infections or the respiratory and cardiovascular status of the patients in that study, therefore concentrations may have been high due to vascular dysfunction, which was not the case in patients in this thesis. Of note, plasma H_2S concentrations in ICU patients and controls in this thesis were low compared to previously reported concentrations in other human studies[280,285,287,289,357,358]. The cause of this discrepancy is unknown and may relate to patient factors, or the way in which samples were processed (discussed in more detail in Chapter 6.4.7).

As in other studies, smokers appeared to have higher plasma HCN concentrations than non-smokers. Higher initial breath HCN concentrations were also seen in smokers, although this difference did not reach statistical significance[123,192,301]. Unfortunately, HCN concentration was not measured in blood samples from healthy controls, so the relationship may have been due to other illness factors. Previous human studies have shown lower plasma H_2S concentrations in smokers than non-smokers[254,287], however the relationship was not seen in this study. The differences may relate to the timing of sample collection after the last cigarette.

7.2. Limitations of the study

7.2.1. Study population size

One of the limitations of this study was low patient numbers, particularly in the first ICU investigation and in the subsections of the control group study. Inter-subject variation in acetone concentration was greater than expected and higher than intra-subject variation due to changing the location of breath sampling or breathing manoeuvre. Patients acted as their own controls, enabling conclusions to be drawn about the effects of altering the sampling location or

breathing manoeuvre, however a larger study population may have permitted more specific results of the absolute or percentage change in acetone concentration with different sampling techniques.

Only small numbers of patients were recruited to each of the subsections when validating the breath collection method in the operating theatre. This was because each of the tests was time consuming and needed to be completed before the start of the surgical procedure, thus it was not possible to perform all of them in every patient. It was necessary for the anaesthetist and surgeon to be happy for these tests to be carried out without delaying the flow of patients through the operating theatre. It was not possible to quantify the effects of altering the APL valve, changing the inspiratory gas flow rate and sampling from each side of the biological filter, nevertheless it was possible to conclude that making alterations did affect inspired or exhaled acetone concentrations, highlighting the need to keep settings the same in order to compare results.

7.2.2. Inability to collect breath samples from spontaneously breathing patients

A problem with the method of breath collection developed was that samples could not be obtained from spontaneously breathing patients. It did allow the delivery of recruitment-style breaths, which would not have been possible if the patients' breathing had not been controlled, however it resulted in the curtailment of breath sampling in the ICU cohort during the final study. This was partly a consequence of the flow rate of the hand-held pump, which was measured at 1.8 L/min. In order to collect a large enough breath sample for analysis using this pump flow rate, it had to run for 4 sec, longer than the duration of an exhalation. It should be possible to use a pump with a faster flow rate, as long as it did not induce coughing, to obtain samples from patients with faster respiratory rates (discussed in more detail below).

7.2.3. Timing of breath sample collection

End-expiratory breath samples were collected by turning off the anaesthesia machine or pressing the respiratory pause button on the ICU ventilator and then collecting samples. A patient's residual volume could not be calculated, and

may have differed between patients, possibly affecting breath VOC concentrations. It should be noted, however, that in mechanically ventilated patients breath acetone concentration was not significantly different when mixed breath and CO_2-controlled alveolar breath samples were compared[7]. The timing of breath sample collection using the capnography trace would not have been possible as it did not change quickly enough to allow sampling at CO_2 plateau and there would not have been sufficient time between breaths for adequate breath volumes to be collected via the pump. A CO_2-controlled sampling device was not available, so the technique was developed to collect end-expiratory breath using readily available equipment.

7.2.4. Off-line breath collection

Due to the physical limitations of the SIFT-MS instrument, it was not possible to perform on-line analysis of breath samples, therefore there were inherent problems with collecting and analysing samples off-line[1]. The method developed allowed rapid but not real time breath analysis and did not permit the selection of different parts of the respiratory cycle, which is possible if breath is sampled continuously on-line[106,207,208,210,337]. Losses of around 25% were seen with all analytes due to breath collection and storage in this study. These losses are comparable to other studies using Tedlar bags[134,135,140]. All breath collection methods, on-line and off-line, involve some loss or contamination of samples[7,97], but this is not always investigated or mentioned in research articles. Long sampling lines for on-line analysis will also show a degree of loss of sample due to adsorption onto the inner surface of the tubing and due to reduction in humidity of the sample[106,207], condensation preventing volatile in water droplets being measured. By quantifying the loss in this study it was possible to correct inspiratory and exhaled acetone concentrations, allowing better comparison with other studies.

7.2.5. Arterial versus venous blood analysis

Peripheral venous blood was drawn in control patients and arterial blood in ICU patients, which may have had an impact on the relationship between volatiles in breath and blood and the ability to compare the two groups. The

difference was due to the presence of an arterial line for continuous blood pressure monitoring in ICU patients, which made arterial sampling non-invasive, and was the source of blood for routine tests, therefore little extra blood needed to be drawn for the study itself. Peripheral blood sampling was preferred in control patients due to increased levels of safety and convenience. In the small number of patients where contemporaneous central venous and arterial blood samples were drawn, there was little or no difference in VOC concentrations. This is in contrast to a previous study[115] where a stronger correlation was seen between breath acetone and arterial versus peripheral venous concentrations. It is not clear whether differences in spontaneously breathing compared to mechanically ventilated patients would affect this relationship.

7.3. Future work

There are several ways in which this work could be developed and these are discussed below. Firstly, further study is required to confirm the results of this pilot and secondly, to investigate these trace gases in similar settings to explore their usefulness as biomarkers in clinical practice.

7.3.1. Increased numbers of study participants

It would be important to repeat the breath collection and analysis technique in another ICU to explore its reproducibility by other researchers or clinicians in a similar group of patients. Due to high inter-individual variability, particularly in breath acetone concentration[5,105,115,167,168,196,238], it would also be interesting to study the effects of respiratory pattern and cardiovascular parameters[1,97,169,171] on breath VOCs in a larger group of patients.

7.3.2. Rebreathing around the biological filter

The study by Hornuss et al.[337] was published after the collection of breath samples in this study and reported high isoprene concentrations in inspiratory breath on the patient side of the biological filter in the breathing circuit of an anaesthesia machine. The authors felt that inspiratory gas sampling from that site, rather than from next to the ventilator outlet, more closely reflected the

actual inspired concentration due to rebreathing of VOCs around the filter. Rebreathing of isoprene was not seen in a similar study of ventilated patients in the ICU, however a filter was not used in the circuit[210]. The inspiratory gas flow rate of the anaesthesia machine was not reported, thus this phenomenon may not have occurred in the studies of this thesis due to the use of relatively high gas flow rates in the operating theatre and the use of ICU ventilators that do not use a rebreathing technique.

It would be useful to explore the possibility of rebreathing of acetone, H_2S and HCN around the biological filter, by comparing inspiratory breath from the ventilator outlet and from the same location as the collection of exhaled breath samples in both ICU ventilators and operating theatre anaesthesia machines using the high flow rates used in this thesis.

7.3.3. Breath acetone in stress hyperglycaemia

Stress hyperglycaemia is common in patients in the ICU, particularly those with sepsis[63], and is treated with exogenous insulin. The optimum feed rate for patients in the ICU setting is unclear, although both overfeeding[63,84] and significant underfeeding[85,90,91] appear to be detrimental. The SPRINT method of treating stress hyperglycaemia is unique to the ICU in Christchurch Hospital, New Zealand, and involves the modulation of feeding as well as insulin administration[82,83]. The system was developed by using computer-based modelling of previous insulin handling, including calculations of insulin sensitivity[83]. It has been shown in this and other studies that breath acetone can be used as a surrogate for blood concentrations, which could be an easily repeatable and more reliable measure of ketosis[115].

If breath acetone could be monitored much more frequently than once daily in patients with stress hyperglycaemia, then further investigation into the possible usefulness of acetone in the modulation of feed and insulin would be possible. This may be particularly important in the group of patients reported in this study who remained ketotic despite normal, or near normal, blood glucose concentration. These patients may require increased carbohydrate feeding and insulin to reduce fatty acid metabolism.

7.3.4. The metabolic stress of surgery

Further studies of acetone in post-operative patients, especially comparing blood and breath concentrations to explore the relationship at higher breath concentrations, would be beneficial. It would be useful to enrol larger numbers of patients to compare breath acetone concentrations with disease severity scores and outcome measures to investigate the relationship between metabolic stress and organ dysfunction or length of hospital stay. Further investigation of acetone concentrations may help to explain the metabolic stress of surgery and identify patients who require more intensive monitoring or changes to treatment, including particular types of surgery where patients may benefit from pre-operative carbohydrate rich drinks[342-344].

7.3.5. Breath sampling in spontaneously breathing subjects

Using the method of breath collection described in this study, it was not possible to obtain breath samples from spontaneously breathing subjects, as discussed above. In order to increase the number of days a patient could provide breath samples for, and to allow the inclusion of patients who do not require controlled ventilation at all, a method for sampling in these patients could be developed.

As previously mentioned, it may be possible to use a hand-held pump with a faster flow rate so that the same volume of breath could be collected over a shorter time. Suction units used in the ICU to remove airway secretions by passing a catheter into the ETT had a flow rate of 4.8 L/min when measured by a pneumotachometer (pressure 60 kPa), therefore suction pumps up to this flow rate should be safe to use. It may permit breath sampling in patients with faster respiratory rates than observed in controlled ventilation, as long as the investigator's reaction time for operating the pump was quick enough and the delay before suction was initiated was short. It still may not allow sample collection over a single breath in patients with very rapid respiratory rates, therefore it may be necessary to collect several breath samples into the same bag so that enough gas is available for analysis. This brings up further issues that would have to be investigated, for example how many breaths should be

collected or over what time period[1,97]. Multiple breaths into the same bag may increase dilution by any dead space in the circuit[161], however this should be less of a problem due to the collection of end-expiratory rather than mixed breath samples, needing comparison with single breath concentrations if possible.

If a discontinuous sampling technique was not used, an on-line monitoring system may reduce VOC losses and enable the selection of the phase of breath of interest for analysis[106,207,208,210,337]. Another benefit of an on-line method would be the ability to apply it to the analysis of both spontaneous and controlled breathing patterns, though this would require further specialist equipment and validation, and at present would not be feasible due to the size of instruments. If the size of on-line MS instruments was reduced, this type of analysis would be attractive for monitoring changes in breath VOCs in real time, like the current practice of continuous BP and CO_2 measurement.

7.3.6. Other markers of inflammation and infection

It would be interesting to look at other trace gases or groups of VOCs in patients with pulmonary infiltrates to try to find better markers of pulmonary infection.

Two interesting studies of F_{ENO} in ventilated patients showed promise in the detection of pulmonary infection[117,118]. Unfortunately, NO is not routinely measured by SIFT-MS as it only reacts with the O_2^+ precursor ion[4] and is more commonly measured using a chemiluminescent analyser[116]. There is currently no standardised method for the analysis of F_{ENO} in mechanically ventilated patients[94], as even using an off-line method the breath flow rate cannot be controlled. Further studies that investigate breath NO concentration in this patient group would be useful.

Differences of opinion exist within the breath research community regarding the importance of compound identification in exhaled breath and knowledge of the pathophysiology behind changes in concentration with disease states, versus the appropriateness of simply looking for changes in single or groups of compounds as a fingerprint of disease without actually identifying them[206].

Data trawling by comparing whole mass spectra is quicker than VOC identification but may miss important changes in very low concentration compounds. Identifying individual volatiles for analysis by first looking at their biological plausibility is more time consuming, but understanding their origin in breath may make them more useful.

Due to multiple confounding variables in the analysis of single VOCs, particularly those produced by metabolic processes, it is likely to be better to concentrate on patterns of multiple VOCs and/or explore VOCs produced by microorganisms to identify infection, possibly using qualitative rather than quantitative tests.

7.4. Final remarks

In this study, breath acetone concentration was seen to correlate with blood concentration and, using the technique described, it was possible to sample and analyse breath accurately, allowing the assessment of systemic acetone concentration using breath. The utility of breath acetone to help modify insulin and feed administration in patients with stress hyperglycaemia in the ICU is worthy of further investigation if more frequent sampling is possible. Breath H_2S and HCN in the setting of new pulmonary infiltrates on CXR in mechanically ventilated patients were not useful in the diagnosis of infection and did not allow monitoring of disease progression.

Given the marked number of sources of variability when analysing exhaled volatile gases, particularly in mechanically ventilated patients, it appears to be more important to monitor changes in concentration over time in an individual rather than comparing to a "normal" level. Rapid sequential analysis is also important, where repeated off-line or on-line real time analytical techniques like SIFT-MS will be more useful.

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Appendix A: Patient Information Sheets and Consent Forms

I wrote the application forms as well as the information sheets and consent forms (examples on following pages) for submission to the Upper South Regional Ethics Committee, South Island, New Zealand.

Canterbury DHB
District Health Board
Te Poari Hauora ō Waitaha

Canterbury Respiratory Research Group
Canterbury Respiratory Services
Christchurch Hospital
Private Bag 4710
Christchurch

Telephone: (64) (3) 364-1157
Facsimile: (64) (3) 378-6299

Information Sheet for Elective Surgical Patients

(Version 2, 26 July 2011)

Exhaled Breath in Critical Illness

Principal Investigator

Name: Dr Sharon Sturney, Research Fellow,
Canterbury Respiratory Research Group

Address: Canterbury Respiratory Services
Christchurch Hospital
Private Bag 4710
Christchurch

Phone: (03) 364-1157

Introduction

You are invited to take part in a study designed to develop new techniques for breath testing in sedated, intubated and ventilated patients, looking into markers of inflammation, infection, metabolic and kidney dysfunction and anaesthetic drug levels, by testing breath and blood samples. This study will be written up as part of Dr Sturney's academic thesis project. This leaflet explains the reasons for the study and what is going to happen if you agree to take part. Please read it carefully. You will be given an opportunity to discuss this study with the investigators and ask any questions you may have. You may have a friend, family or whanau support to help you understand the risks and/or benefits of this study and any other explanation you may require. If you decide to take part, you will be asked to sign a consent form.

General

If you need an interpreter, one can be provided.

Participation

Your participation is entirely voluntary (your choice). You do not have to take part in this study, and if you choose not to take part this will not affect any future care or treatment.

If you do agree to take part you are free to withdraw from the study at any time, without having to give a reason and this will in no way affect your health care. Participation in this study will be stopped should any harmful effects appear or if the doctor feels it is not in your best interests to continue.

About The Study

What are the aims of the study?

We are working to identify ways to quickly and easily diagnose inflammation, infection, metabolic and kidney dysfunction in critically ill patients. There are currently blood and sputum tests available to diagnose some of these conditions, however they are not always as accurate as we would like and it can take some time to receive results. The area of critical illness is dynamic, with rapid changes in a patient's condition, therefore it is important to find rapid methods to detect problems in order to allow earlier treatment which might be more effective. For this reason we are trying to develop breath tests and blood tests which might be quicker and more accurate than our current tests.

You have been asked to take part because you will be undergoing an elective surgical procedure requiring sedation, intubation and mechanical ventilation. We are looking at this group of patients to provide us with normal levels of markers in breath and blood. The results will then be compared to those obtained from patients in the Intensive Care Unit, enabling us to look at changes in levels due to critical illness.

We will be measuring several substances in your breath, including acetone, hydrogen sulphide, hydrogen cyanide, 2-Aminoacetophenone, trimethylamine, dimethylamine and ammonia. We believe these substances are higher in the breath of people with critical illness because they have increased metabolic demands due to infection and inflammation and a degree of kidney dysfunction. In addition we are measuring sedative and painkilling drug levels in the breath to look into the properties of the drugs used in general anaesthesia and whether breath samples may help to determine the level of sedation. A blood sample will be taken for substances including glucose, ketones, hydrogen sulphide, renal function, white cell count and C-reactive protein (a marker of inflammation). No samples that are taken will be stored and all samples will be disposed of as per New Zealand standards.

How were participants selected for this study, and who selected them?
We are studying subjects who are undergoing elective surgical procedures, with no evidence of lung disease, infection, inflammation, kidney dysfunction, or diabetes.

We are contacting people via pre-operative assessment clinics. The anaesthetic and surgical teams will be helping to identify people who may wish to take part.

Where will the study be held?
In the pre-operative clinic rooms and operating theatres, Christchurch Hospital.

What is the time span for the study?
Each volunteer will spend 15 minutes participating in the study at the pre-operative assessment clinic. Breath and blood collection will occur whilst you are in the operating theatre. The breath tests will be taken under general anaesthetic.

What will happen during the study?
Firstly, at your pre-operative assessment clinic we will ask some questions about your health. We will then ask you to perform a breath test (spirometry), whereby you will breathe hard and fast into a hand-held machine.

When you come to the hospital for your surgery you will be given a general anaesthetic by the anaesthetist as normal. When you have an intravenous cannula (a little tube) inserted into your vein, as is routine before an operation, we will take some blood samples. Once you are asleep we will take a number of breath samples from your airways via a syringe connected to the breathing circuit. This will not have any impact on your breathing or your surgery.

Benefits Risks And Safety

What are the benefits of the study?
There are no immediate benefits to participating in the study.

What are the risks and/or inconveniences of the study?
The performance of spirometry (breath test) is a simple and safe procedure with no significant risks or side effects. The session will last 15 minutes and will be linked to your preoperative assessment so that you do not need to come up to the hospital for a second appointment.

Collection of exhaled breath when you are intubated is a similar process to suctioning sputum from the airways. There are no identified significant risks or side effects.

Blood tests may cause minor discomfort, which quickly passes. Blood testing may cause minor bruising.

There will be no payment made for participation in this study.

Compensation

The Upper South A Regional Ethics Committee has certified that this clinical trial is being conducted principally for the benefit of the manufacturer or distributor of the medicine or item in respect of which this trial is being carried out. This means that if you suffer injury as a result of your participation, you will not be eligible for cover by ACC. Compensation however, will be provided by Syft Technologies Limited in accordance with the "New Zealand Researched Medicines Industry (RMI) Guidelines on Clinical Trials – Compensation for injury resulting from participation in Industry Sponsored Clinical Trials".

These RMI Guidelines are only guidelines and until your claim is assessed by the insurers of Syft Technologies Limited it cannot be said with any certainty exactly what type or amount of compensation you will receive if you suffer injury as a result of your participation, or exactly what sort of injury will be covered. However the guidelines require that compensation must be provided by Syft Technologies Limited where the injury you suffer is serious and not just temporary and is one caused by the trial medicine or item or where you would not have suffered injury but for your inclusion in this trial. The guidelines also require that the compensation you receive must be appropriate to the nature, severity and persistence of your injury. This means that you will be likely to receive some compensation from Syft Technologies Limited unless your injury is minor or temporary. However, you might not receive compensation from Syft Technologies Limited if your injury was caused solely by you or by the negligence of the research staff of institution.

In the event of a dispute over the cause of the injury or level of compensation, Syft Technologies Limited will accept the decision of a New Zealand medical "referee" as agreed to by yourself and Syft Technologies Limited.

Advocacy

If you have any queries or concerns regarding your rights as a participant in this study you may wish to contact a Health and Disability Advocate. This is a free service provided under the Health and Disability Commissioner Act.

Telephone (NZ Wide):	0800 555-050
Free Fax (NZ Wide):	0800 2787-7678
E-mail:	advocacy@hdc.org.nz

For Maori Health support: Nga Ratonga Hauora Maori Health Service based at Christchurch Hospital campus when available will endeavour to provide awhi/support to turoro/patient and whanau/family while they are involved in study participation. Please contact Tahu Potiki Stirling, Pouarahi Roopu / Team Leader of Nga Ratonga Hauora Maori and Chairperson of Te Komiti Whakarite. Telephone: (03) 364-0640, ext 88797.

Confidentiality

No material which could personally identify you will be used in any reports on this study.

Records and information about the study and the results will be kept at the Canterbury Respiratory Research Group offices, in locked rooms, for 15 years.

Results

This study will be written up as part of Dr Sturney's academic thesis, overseen by the University of Nottingham, UK. We plan to publish the results of this research in international medical journals. No data which might identify you will be published.

Statement Of Approval

This study has received ethical approval from the Upper South A Ethics Committee. The General Manager of Christchurch Hospital has given permission for this study to be carried out.

Who To Contact

Please feel free to ask questions about the study – further information can be obtained at any time from the following researchers:

Dr Sharon Sturney, Principal Investigator
Ph: (03) 364-1157
Dr Michael Epton, Director, Canterbury Respiratory Research Group
Ph: (03) 364-1157
Julie Cook, Research Nurse
Ph: (03) 364-1157

Who to contact in an emergency (24 hours)

Business Hours:	Canterbury Respiratory Research Group Ph: (03) 364-1157
After Hours:	Dr Sharon Sturney Ph: 021 023 20985

Thank you for taking the times to read this information

Canterbury DHB
District Health Board
Te Poari Hauora ō Waitaha

Canterbury Respiratory Research Group
Canterbury Respiratory Services
Christchurch Hospital
Private Bag 4710
Christchurch

Telephone: (64) (3) 364-1157
Facsimile: (64) (3) 378-6299

Participant Study Number: _____

Consent Form for Elective Surgical Patients

Statement by patient

(Version 2, 26 July 2011)

Exhaled Breath in Critical Illness

Principal Investigator

Name: Dr Sharon Sturney, Research Fellow,
Canterbury Respiratory Research Group

Address: Canterbury Respiratory Services
Christchurch Hospital
Private Bag 4710
Christchurch

Phone: (03) 364-1157

Request for an Interpreter

English	I wish to have an interpreter	Yes	No
Deaf	I wish to have a NZ sign language interpreter	Yes	No
Maori	E hiahia ana ahau ki tetahi kaiwhakamaori / kaiwhaka pakeha korero	Ae	Kao
Samoan	Oute mana'o ia iai se fa'amatala upu	Ioe	Leai
Tongan	Oku ou fiema'u ha fakatonulea	Io	Ikai
Cook Island	Ka inangaro au i tetai tangata uri reo	Ae	Kare
Fijian	Au gadreva me dua e vakadewa vosa vei au	Io	Sega
Niuean	Fia manako au ke fakaaoga e taha tagata fakahokohoko kupu	E	Nakai

- I have read and I understand the **Information Sheet** dated **26 July 2011** for participants taking part in a study designed to develop new techniques for breath testing in sedated, intubated and ventilated patients, looking into markers of inflammation, infection, metabolic and renal dysfunction and anaesthetic drug levels, by testing breath and blood samples. I have had the opportunity to discuss this study. I am satisfied with the answers I have been given.

- I have had the opportunity to use whanau support or a friend to help me ask questions and understand the study.

- I understand that taking part in this study is voluntary (my choice) and that I may withdraw from the study at any time and this will in no way affect my future health care, academic progress or employment.

- I understand that my participation in this study is confidential and that no material that could identify me will be used in any reports on this study.
- I understand that the investigation will be stopped if it should appear harmful to me.

- I understand the compensation provisions for this study.

- I know whom to contact if I have questions about the study.

- I consent to the researches collecting samples of my breath and blood and testing it as part of this study. I understand that any blood samples taken during this study cannot be returned to me.

- I consent to the researches doing a breath analysis as part of this research.

- I have had time to consider whether to take part.

- This study has been given ethical approval by the Upper South A Regional Ethics Committee. This means that the Committee may check at any time that the study is following appropriate ethical procedures.

- I wish to receive a copy of the results of this study. YES / NO

- I consent to my GP being informed of the results of this study. YES / NO

I, …… hereby consent to take part in this study.
(Full Name)

_____ _____

Printed Name of Study Participant **Signature of Study Participant**

_____

Date

Using language that is understandable and appropriate, I have discussed this study with the participant. I have offered to answer any questions and have fully answered such questions. I believe that the participant understands my explanations and has freely given informed consent.

Printed Name of Individual Conducting Informed Consent Discussion	Signature of Individual Conducting Informed Consent Discussion

Date

Dr Sharon Sturney, Principal Investigator Ph: (03) 364-1157
Dr Michael Epton, Director, Canterbury Respiratory Research Group Ph: (03) 364-1157
Julie Cook, Research Nurse Ph: (03) 364-1157

Thank you for taking the time to read this Consent Form

Thank you in advance for your help with this study

Canterbury DHB
District Health Board
Te Poari Hauora ō Waitaha

Canterbury Respiratory Research Group
Canterbury Respiratory Services
Christchurch Hospital
Private Bag 4710
Christchurch

Telephone: (64) (3) 364-1157
Facsimile: (64) (3) 378-6299

Information Sheet for Intensive Care Patients

(Version 2, 26 July 2011)

Exhaled Breath in Critical Illness

Principal Investigator

Name: Dr Sharon Sturney, Research Fellow,
Canterbury Respiratory Research Group

Address: Canterbury Respiratory Services
Christchurch Hospital
Private Bag 4710
Christchurch

Phone: (03) 364-1157

Introduction

You are invited to take part in a study designed to develop new techniques for breath testing in sedated, intubated and ventilated patients, looking into markers of inflammation, infection, metabolic and kidney dysfunction and sedative drug levels, by testing breath and blood samples. This study will be written up as part of Dr Sturney's academic thesis project. This leaflet explains the reasons for the study and what is going to happen if you agree to take part. Please read it carefully. You will be given an opportunity to discuss this study with the investigators and ask any questions you may have. You may have a friend, family or whanau support to help you understand the risks and/or benefits of this study and any other explanation you may require. If you decide that you would like to take part, you will be asked to sign a consent form.

General

If you need an interpreter, one can be provided.

Participation

Participation is entirely voluntary (your choice). You do not have to take part in this study, and if you choose not to take part this will not affect any future care or treatment that you receive.

If you do agree to take part you are free to withdraw from the study at any time, without having to give a reason and this will in no way affect your health care. Participation in this study will be stopped should any harmful effects appear or if the doctor feels it is not in the your best interests to continue.

About The Study

What are the aims of the study?

We are working to identify ways to quickly and easily diagnose inflammation, infection, metabolic and kidney dysfunction in critically ill patients. There are currently blood and sputum tests available to diagnose some of these conditions, however they are not always as accurate as we would like and it can take some time to receive results. The area of critical illness is dynamic, with rapid changes in a patient's condition, therefore it is important to find rapid methods to detect problems in order to allow earlier treatment which might be more effective. For this reason we are trying to develop breath tests and blood tests which might be quicker and more accurate than our current tests.

You have been selected to take part because you have a condition requiring sedation, intubation and mechanical ventilation in the Intensive Care Unit. We are comparing people undergoing elective surgery with patients in the Intensive Care Unit to look at differences in levels of breath and blood markers of inflammation, infection, kidney and metabolic dysfunction, and levels of sedative drugs.

We will be measuring several substances in breath, including acetone, hydrogen sulphide, hydrogen cyanide, 2-Aminoacetophenone, trimethyamine, dimethylamine and ammonia. We believe these substances are higher in the breath of people with critical illness because they have increased metabolic demands due to infection and inflammation and a degree of kidney dysfunction. In addition we are measuring levels of sedative and painkilling drugs in the breath to look into the properties of these drugs and whether breath samples may help to determine the level of sedation. A blood sample will be taken for substances including glucose, ketones, hydrogen sulphide, renal function, white cell count and C-reactive protein (a marker of inflammation). Blood samples will be taken to look accurately at the oxygen level, as is routine on the Intensive Care Unit. No samples that are taken will be stored and all samples will be disposed of as per New Zealand standards.

How were participants selected for this study, and who selected them?
We are studying patients in the Intensive Care Unit who are sedated, intubated and ventilated, most with high blood sugar levels and some with evidence of lung changes on chest x-ray or kidney dysfunction.

We are contacting people in the Intensive Care Unit. The Intensive Care specialists and nurses at Christchurch Hospital are helping identify people who may wish to take part.

Where will the study be held?
In the Intensive Care Unit, Christchurch Hospital.

What is the time span for the study?
To help us develop our method of collecting breath samples, some participants will have 4 sets of breath samples taken on a single day. Other participants will have breath and blood samples taken once a day whilst on insulin therapy, or until treatment for lung disease has been stopped, in order to compare these results with the results collected from surgical patients.

What will happen during the study?
Breath samples will be taken from the airways either via a syringe connected to a T-piece within the respiratory circuit, or by passing a suction catheter into the airways and sucking a sample into a syringe. It may be necessary to take samples on a daily basis until your treatment is finished. Arterial blood samples will be taken from an arterial catheter (a small tube that has been inserted into the artery in the wrist) daily in those subjects who also have breath samples taken on a daily basis. The arterial

catheter will already be present for ongoing management by the Intensive Care Unit team. Urine samples will be taken and analysed as per ICU protocol, including for urinary creatinine level.

Benefits Risks And Safety

What are the benefits of the study?
There are no benefits to participating in the study.

What are the risks and/or inconveniences of the study?
Collection of exhaled breath when a patient is intubated is a similar process to suctioning sputum from the airways. There are no identified significant risks or side effects.

Blood tests taken from the arterial line will not cause any extra risk or discomfort.

There will be no payment made for participation in this study.

Compensation

The Upper South A Regional Ethics Committee has certified that this clinical trial is being conducted principally for the benefit of the manufacturer or distributor of the medicine or item in respect of which this trial is being carried out. This means that if you suffer injury as a result of your participation, you will not be eligible for cover by ACC. Compensation however, will be provided by Syft Technologies Limited in accordance with the "New Zealand Researched Medicines Industry (RMI) Guidelines on Clinical Trials – Compensation for injury resulting from participation in Industry Sponsored Clinical Trials".

These RMI Guidelines are only guidelines and until your claim is assessed by the insurers of Syft Technologies Limited it cannot be said with any certainty exactly what type or amount of compensation you will receive if you suffer injury as a result of your participation, or exactly what sort of injury will be covered. However the guidelines require that compensation must be provided by Syft Technologies Limited where the injury you suffer is serious and not just temporary and is one caused by the trial medicine or item or where you would not have suffered injury but for your inclusion in this trial. The guidelines also require that the compensation you receive must be appropriate to the nature, severity and persistence of your injury. This means that you will be likely to receive some compensation from Syft Technologies Limited unless your injury is minor or temporary. However, you might not receive compensation from Syft Technologies Limited if your injury was caused solely by you or by the negligence of the research staff of institution.

In the event of a dispute over the cause of the injury or level of compensation, Syft Technologies Limited will accept the decision of a New Zealand medical "referee" as agreed to by yourself and Syft Technologies Limited.

Advocacy

If you have any queries or concerns regarding your rights as a participant in this study, you may wish to contact a Health and Disability Advocate. This is a free service provided under the Health and Disability Commissioner Act.

Telephone (NZ Wide):	0800 555-050
Free Fax (NZ Wide):	0800 2787-7678
E-mail:	advocacy@hdc.org.nz

For Maori Health support: Nga Ratonga Hauora Maori Health Service based at Christchurch Hospital campus when available will endeavour to provide awhi/support to turoro/patient and whanau/family while they are involved in study participation. Please contact Tahu Potiki Stirling, Pouarahi Roopu / Team Leader of Nga Ratonga Hauora Maori and Chairperson of Te Komiti Whakarite. Telephone: (03) 364-0640, ext 88797.

Confidentiality

No material which could personally identify you will be used in any reports on this study.

Records and information about the study and the results will be kept at the Canterbury Respiratory Research Group offices, in locked rooms, for 15 years.

Results

This study will be written up as part of Dr Sturney's academic thesis, overseen by the University of Nottingham, UK. We plan to publish the results of this research in international medical journals. No data which might identify your relative/friend will be published.

Statement Of Approval

This study has received ethical approval from the Upper South A Ethics Committee. The General Manager of Christchurch Hospital has given permission for this study to be carried out.

Who To Contact

Please feel free to ask questions about the study – further information can be obtained at any time from the following researchers:

Dr Sharon Sturney, Principal Investigator
Ph: (03) 364-1157
Dr Michael Epton, Director, Canterbury Respiratory Research Group
Ph: (03) 364-1157
Dr Geoff Shaw, Intensive Care Specialist
Ph: (03) 364-0640
Julie Cook, Research Nurse
Ph: (03) 364-1157

Who to contact in an emergency (24 hours)

Business Hours:	Canterbury Respiratory Research Group Ph: (03) 364-1157
After Hours:	Dr Sharon Sturney Ph: 021 023 20985

Thank you for taking the times to read this information

Canterbury DHB
District Health Board
Te Poari Hauora ō Waitaha

Canterbury Respiratory Research Group
Canterbury Respiratory Services
Christchurch Hospital
Private Bag 4710
Christchurch

Telephone: (64) (3) 364-1157
Facsimile: (64) (3) 378-6299

Participant Study Number: _____

Consent Form for Intensive Care Patients

Statement by patient

(Version 2, 26 July 2011)

Exhaled Breath in Critical Illness

➢ **Principal Investigator**

Name: Dr Sharon Sturney, Research Fellow,
Canterbury Respiratory Research Group

Address: Canterbury Respiratory Services
Christchurch Hospital
Private Bag 4710
Christchurch

Phone: (03) 364-1157

Request for an Interpreter

English	I wish to have an interpreter	Yes	No
Deaf	I wish to have a NZ sign language interpreter	Yes	No
Maori	E hiahia ana ahau ki tetahi kaiwhakamaori / kaiwhaka pakeha korero	Ae	Kao
Samoan	Oute mana'o ia iai se fa'amatala upu	Ioe	Leai
Tongan	Oku ou fiema'u ha fakatonulea	Io	Ikai
Cook Island	Ka inangaro au i tetai tangata uri reo	Ae	Kare
Fijian	Au gadreva me dua e vakadewa vosa vei au	Io	Sega
Niuean	Fia manako au ke fakaaoga e taha tagata fakahokohoko kupu	E	Nakai

➢ I have read and I understand the **Information Sheet** dated **26 July 2011** for participants taking part in a study designed to develop new techniques for breath testing in sedated, intubated and ventilated patients, looking into markers of inflammation, infection, metabolic and renal dysfunction and anaesthetic drug levels, by testing breath and blood samples. I have had the opportunity to discuss this study. I am satisfied with the answers I have been given.

➢ I have had the opportunity to use whanau support or a friend to help me ask questions and understand the study.

➢ I understand that taking part in this study is voluntary (my choice) and that I may withdraw from the study at any time and this will in no way affect my future health care, academic progress or employment.

- I understand that my participation in this study is confidential and that no material that could identify me will be used in any reports on this study.
- I understand that the investigation will be stopped if it should appear harmful to me.
- I understand the compensation provisions for this study.
- I know whom to contact if I have questions about the study.
- I consent to the researches collecting samples of my breath and blood and testing it as part of this study. I understand that any blood samples taken during this study cannot be returned to me.
- I consent to the researches doing a breath analysis as part of this research.
- I have had time to consider whether to take part.
- This study has been given ethical approval by the Upper South A Regional Ethics Committee. This means that the Committee may check at any time that the study is following appropriate ethical procedures.
- I wish to receive a copy of the results of this study. YES / NO
- I consent to my GP being informed of the results of this study. YES / NO

I, …… hereby consent to take part in this study.
(Full Name)

_____ _____

Printed Name of Study Participant **Signature of Study Participant**

_____
Date

Using language that is understandable and appropriate, I have discussed this study with the participant. I have offered to answer any questions and have fully answered such questions. I believe that the participant understands my explanations and has freely given informed consent.

_____	_____
Printed Name of Individual Conducting Informed Consent Discussion	**Signature of Individual Conducting Informed Consent Discussion**

_____
Date

Dr Sharon Sturney, Principal Investigator
Ph: (03) 364-1157
Dr Michael Epton, Director, Canterbury Respiratory Research Group
Ph: (03) 364-1157
Dr Geoff Shaw, Intensive Care Specialist
Ph: (03) 364-0640
Julie Cook, Research Nurse
Ph: (03) 364-1157

Thank you for taking the time to read this Consent Form
Thank you in advance for your help with this study

Canterbury DHB
District Health Board
Te Poari Hauora ō Waitaha

Canterbury Respiratory Research Group
Canterbury Respiratory Services
Christchurch Hospital
Private Bag 4710
Christchurch

Telephone: (64) (3) 364-1157
Facsimile: (64) (3) 378-6299

Information Sheet for Intensive Care Patients
For use after urgent enrolment
For relatives/friends/family/whanau
(Version 3, 7 October 2011)

Exhaled Breath in Critical Illness

Principal Investigator

Name: Dr Sharon Sturney, Research Fellow,
Canterbury Respiratory Research Group

Address: Canterbury Respiratory Services
Christchurch Hospital
Private Bag 4710
Christchurch

Phone: (03) 364-1157

Introduction

Your relative/friend is invited to continue to take part in a study designed to develop new techniques for breath testing in sedated, intubated and ventilated patients, looking into markers of inflammation, infection, metabolic and kidney dysfunction and sedative drug levels, by testing breath and blood samples. It was important that your relative/friend received treatment for his/her illness swiftly, therefore he/she has already been enrolled in the study and breath and blood samples have been taken. The Upper South A Regional Ethics Committee has approved this process including your relative/friend in the study without prior consent at the time, but with your relative/friend/whanau or yourself being approached about the study as soon as possible afterwards.

This study will be written up as part of Dr Sturney's academic thesis project. This leaflet explains the reasons for the study, what has already happened and what is going to happen if you agree for them to continue to take part. Please read it carefully. You will be given an opportunity to discuss this study with the investigators and ask any questions you may have. You may have a friend, family or whanau support to help you understand the risks and/or benefits of this study and any other explanation you may require. If you decide that your relative/friend would want to continue to take part, you will be asked to sign a consent form.

General

If you need an interpreter, one can be provided.

Participation

Participation is entirely voluntary (your choice). Your relative/friend does not have to continue to take part in this study, and if you choose for them not to continue to take part this will not affect any future care or treatment that they receive.

If you do agree for them to continue to take part they are free to withdraw from the study at any time, without having to give a reason and this will in no way affect their health care. Participation in this study will be stopped should any harmful effects appear or if the doctor feels it is not in the participant's best interests to continue.

About The Study

What are the aims of the study?
We are working to identify ways to quickly and easily diagnose inflammation, infection, metabolic and kidney dysfunction in critically ill patients. There are currently blood and sputum tests available to diagnose some of these conditions, however they are not always as accurate as we would like and it can take some time to receive results. The area of critical illness is dynamic, with rapid changes in a patient's condition, therefore it is important to find rapid methods to detect problems in order to allow earlier treatment which might be more effective. For this reason we are trying to develop breath tests and blood tests which might be quicker and more accurate than our current tests.

Your relative/friend has been selected to take part because they have a condition requiring sedation, intubation and mechanical ventilation in the Intensive Care Unit. We are comparing people undergoing elective surgery with patients in the Intensive Care Unit to look at differences in levels of breath and blood markers of inflammation, infection, kidney and metabolic dysfunction, and levels of sedative drugs.

We will be measuring several substances in breath, including acetone, hydrogen sulphide, hydrogen cyanide, 2-Aminoacetophenone, trimethyamine, dimethylamine and ammonia. We believe these substances are higher in the breath of people with critical illness because they have increased metabolic demands due to infection and inflammation and a degree of kidney dysfunction. In addition we are measuring levels of sedative and painkilling drugs in the breath to look into the properties of these drugs and whether breath samples may help to determine the level of sedation. A blood sample will be taken for substances including glucose, ketones, hydrogen sulphide, renal function, white cell count and C-reactive protein (a marker of inflammation). Blood samples will be taken to look accurately at the oxygen level, as is routine on the Intensive Care Unit. No samples that are taken will be stored and all samples will be disposed of as per New Zealand standards.

How were participants selected for this study, and who selected them?
We are studying patients in the Intensive Care Unit who are sedated, intubated and ventilated, most with high blood sugar levels and some with evidence of lung changes on chest x-ray or kidney dysfunction.

We are contacting people in the Intensive Care Unit. The Intensive Care specialists and nurses at Christchurch Hospital are helping identify people who may wish to take part.

Where will the study be held?
In the Intensive Care Unit, Christchurch Hospital.

What is the time span for the study?

Participants will have breath and blood samples taken prior to and once a day whilst on insulin therapy, or until treatment for lung disease has been stopped, in order to compare these results with the results collected from elective surgical patients.

What will happen during the study?
Breath samples will have been taken from the airways either via a syringe connected to a T-piece within the respiratory circuit, or by passing a suction catheter into the airways and sucking a sample into a syringe. It will be necessary to continue to take samples on a daily basis until treatment is finished. Arterial blood samples will have been taken from an arterial catheter (a small tube that has been inserted into the artery in the wrist) and will be taken daily in those subjects who also have breath samples taken on a daily basis. The arterial catheter will already be present for ongoing management by the Intensive Care Unit team. Urine samples will be taken and analysed as per ICU protocol, including for urinary creatinine level.

Benefits Risks And Safety

What are the benefits of the study?
There are no benefits to participating in the study.
What are the risks and/or inconveniences of the study?
Collection of exhaled breath when a patient is intubated is a similar process to suctioning sputum from the airways. There are no identified significant risks or side effects.
Blood tests taken from the arterial line will not cause any extra risk or discomfort.
There will be no payment made for participation in this study.

Compensation

The Upper South A Regional Ethics Committee has certified that this clinical trial is being conducted principally for the benefit of the manufacturer or distributor of the medicine or item in respect of which this trial is being carried out. This means that if you suffer injury as a result of your participation, you will not be eligible for cover by ACC. Compensation however, will be provided by Syft Technologies Limited in accordance with the "New Zealand Researched Medicines Industry (RMI) Guidelines on Clinical Trials – Compensation for injury resulting from participation in Industry Sponsored Clinical Trials".

These RMI Guidelines are only guidelines and until your claim is assessed by the insurers of Syft Technologies Limited it cannot be said with any certainty exactly what type or amount of compensation you will receive if you suffer injury as a result of your participation, or exactly what sort of injury will be covered. However the guidelines require that compensation must be provided by Syft Technologies Limited where the injury you suffer is serious and not just temporary and is one caused by the trial medicine or item or where you would not have suffered injury but for your inclusion in this trial. The guidelines also require that the compensation you receive must be appropriate to the nature, severity and persistence of your injury. This means that you will be likely to receive some compensation from Syft Technologies Limited unless your injury is minor or temporary. However, you might not receive compensation from Syft Technologies Limited if your injury was caused solely by you or by the negligence of the research staff of institution.

In the event of a dispute over the cause of the injury or level of compensation, Syft Technologies Limited will accept the decision of a New Zealand medical "referee" as agreed to by yourself and Syft Technologies Limited.

Advocacy

If you have any queries or concerns regarding your rights, or those of your relative/friend as a participant in this study, you may wish to contact a Health and Disability Advocate. This is a free service provided under the Health and Disability Commissioner Act.

Telephone (NZ Wide): 0800 555-050
Free Fax (NZ Wide): 0800 2787-7678
E-mail: advocacy@hdc.org.nz

For Maori Health support: Nga Ratonga Hauora Maori Health Service based at Christchurch Hospital campus when available will endeavour to provide awhi/support to turoro/patient and whanau/family while they are involved in study participation. Please contact Tahu Potiki Stirling, Pouarahi Roopu / Team Leader of Nga Ratonga Hauora Maori and Chairperson of Te Komiti Whakarite. Telephone: (03) 364-0640, ext 88797.

Confidentiality

No material which could personally identify your relative/friend will be used in any reports on this study.
Records and information about the study and the results will be kept at the Canterbury Respiratory Research Group offices, in locked rooms, for 15 years.

Results

This study will be written up as part of Dr Sturney's academic thesis, overseen by the University of Nottingham, UK. We plan to publish the results of this research in international medical journals. No data which might identify your relative/friend will be published.

Statement Of Approval

This study has received ethical approval from the Upper South A Ethics Committee. The General Manager of Christchurch Hospital has given permission for this study to be carried out.

Who To Contact

Please feel free to ask questions about the study – further information can be obtained at any time from the following researchers:

Dr Sharon Sturney, Principal Investigator, Ph: (03) 364-1157
Dr Michael Epton, Director, Canterbury Respiratory Research Group, Ph: (03) 364-1157
Dr Geoff Shaw, Intensive Care Specialist, Ph: (03) 364-0640
Julie Cook, Research Nurse, Ph: (03) 364-1157

Who to contact in an emergency (24 hours)

Business Hours: Canterbury Respiratory Research Group, Ph: (03) 364-1157
After Hours: Dr Sharon Sturney, Ph: 021 023 20985

Thank you for taking the times to read this information

Canterbury DHB
District Health Board
Te Poari Hauora ō Waitaha

Canterbury Respiratory Research Group
Canterbury Respiratory Services
Christchurch Hospital
Private Bag 4710
Christchurch

Telephone: (64) (3) 364-1157
Facsimile: (64) (3) 378-6299

Participant Study Number:_____

Consent Form for Intensive Care Patients For use after urgent enrolment

Statement by relative/friend/family/whanau

(Version 3, 7 October 2011)

Exhaled Breath in Critical Illness

Principal Investigator

Name: Dr Sharon Sturney, Research Fellow,
Canterbury Respiratory Research Group

Address: Canterbury Respiratory Services
Christchurch Hospital
Private Bag 4710
Christchurch

Phone: (03) 364-1157

Request for an Interpreter

English	I wish to have an interpreter	Yes	No
Deaf	I wish to have a NZ sign language interpreter	Yes	No
Maori	E hiahia ana ahau ki tetahi kaiwhakamaori / kaiwhaka pakeha korero	Ae	Kao
Samoan	Oute mana'o ia iai se fa'amatala upu	Ioe	Leai
Tongan	Oku ou fiema'u ha fakatonulea	Io	Ikai
Cook Island	Ka inangaro au i tetai tangata uri reo	Ae	Kare
Fijian	Au gadreva me dua e vakadewa vosa vei au	Io	Sega
Niuean	Fia manako au ke fakaaoga e taha tagata fakahokohoko kupu	E	Nakai

- I have read and I understand the **Information Sheet** dated **7 October 2011** for relatives/friends of people taking part in the study designed to develop new techniques for breath testing in intubated and ventilated patients, looking into markers of inflammation, infection, metabolic and renal dysfunction and anaesthetic drug levels, by testing breath and blood samples. I have had the opportunity to discuss this study. I am satisfied with the answers I have been given.

- I have had the opportunity to use family/whanau support or a friend to help me ask questions and understand the study.

- I believe that (participant's name) would have chosen and consented to participate in this study if he/she had been able to understand the information that I have received and understood.

- I understand that taking part in this study is voluntary and that my relative/friend may withdraw from the study at any time if he/she wishes. This will not affect his/her continuing health care.

- I understand that his/her participation in this study is confidential and that no material which could identify him/her will be used in any reports on this study.

- I understand that the investigation will be stopped if it should appear harmful to my relative/friend.

- I understand the compensation provisions for this study.

- I know whom to contact if I have any questions about the study.

- I consent to the researches collecting samples of breath and blood and testing it as part of this study. I understand that any blood samples taken during this study cannot be returned.

- I consent to the researches doing a breath analysis as part of this research.

- I know whom to contact if my relative/friend has any side effects to the study or if anything occurs which I think he/she would consider a reason to withdraw from the study.

- This study has been given ethical approval by the Upper South A Ethics Committee. This means that the Committee may check at any time that the study is following appropriate ethical procedures.

- I believe my relative/friend would agree to an auditor approved by the Upper South A Ethics Committee reviewing my relative's/friend's relevant medical records for the sole purpose of checking the accuracy of the information recorded for the study.

I/my relative/friend would like a copy of the results of the study.	☐	Yes	☐	No
I believe my relative/friend would agree to his/her GP being informed of his/her participation in this study.	☐	Yes	☐	No

Signed:		Date:	
Printed name:			
Relationship to participant:			
Address for results:			

I confirm that if the participant becomes competent to make an informed choice and give an informed consent, full information will be given to him/her as soon as possible, and his/her participation will be explained. If the participant makes an informed choice to continue in the study, written consent will be requested and if the participant does not wish to continue in the study, he/she will be withdrawn.

Using language that is understandable and appropriate, I have discussed this study with the participant's relative/friend. I have offered to answer any questions and have fully answered such questions. I believe that they understands my explanations and have freely given informed consent.

Printed Name of Individual Conducting Informed Consent Discussion	Signature of Individual Conducting Informed Consent Discussion

Date

Dr Sharon Sturney, Principal Investigator, Ph: (03) 364-1157
Dr Michael Epton, Director, Canterbury Respiratory Research Group
Ph: (03) 364-1157
Dr Geoff Shaw, Intensive Care Specialist, Ph: (03) 364-0640
Julie Cook, Research Nurse, Ph: (03) 364-1157

(If applicable at a later stage)

I (participant) having been fully informed about this study agree to continue taking part in it.

Signed:		Date:	

(Participant)

Thank you for taking the time to read this Consent Form and thank you in advance for your help with this study

Appendix B: Ethics Approval

Upper South A Regional Ethics Committee
c/- Ministry of Health
Montgomery Watson Building
6 Hazeldean Road
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Phone: (03) 974 2304
Email: uppersoutha_ethicscommittee@moh.govt.nz

24 August 2011

Dr Sharon Sturney
Canterbury Respiratory Research Group
Canterbury Respiratory Services
Private Bag 4710
Christchurch

Dear Dr Sturney

Ethics ref:	**URA/11/07/032 (please quote in all correspondence)**
Study title:	**Measurement of exhaled volatile organic compounds in critical illness measured via Selected Ion Flow Tube - Mass Spectometry (SIFT-MS) and Gas Chromatography Mass Spectroscopy (GCMS-MS)**
Investigators:	**Dr S Sturney, Dr M Epton, Ms M Storer, Ms J Cook, Ms F McCartin, Dr G Shaw, A/Prof R Kennedy, Prof S Chambers, Dr J Pickering, Prof M Bhatia**

This study was given ethical approval by the Upper South A Regional Ethics Committee.

Approved Documents

- Information sheet and consent form for elective surgical patients, version 2 dated 26/07/11
- Information sheet and consent form for intensive care patients, version 2 dated 26/07/11
- Information sheet and consent form for intensive care patients (relative/friend/whanau) version 2 dated 26/07/11

This approval is valid until **31 December 2013**, provided that Annual Progress Reports are submitted (see below).

Access to ACC

For the purposes of section 32 of the Accident Compensation Act 2001, the Committee is satisfied that this study is not being conducted principally for the benefit of the manufacturer or distributor of the medicine or item in respect of which the trial is being carried out. Participants injured as a result of treatment received in this trial will therefore be eligible to be considered for compensation in respect of those injuries under the ACC scheme.

Amendments and Protocol Deviations

All significant amendments to this proposal must receive prior approval from the Committee. Significant amendments include (but are not limited to) changes to:

- the researcher responsible for the conduct of the study at a study site
- the addition of an extra study site
- the design or duration of the study
- the method of recruitment
- information sheets and informed consent procedures.

Health and Disability Ethics Committees

Upper South A Regional Ethics Committee
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Significant deviations from the approved protocol must be reported to the Committee as soon as possible.

Annual Progress Reports and Final Reports

The first Annual Progress Report for this study is due to the Committee by 31 July 2012. The Annual Report Form that should be used is available at www.ethicscommittees.health.govt.nz. Please note that if you do not provide a progress report by this date, ethical approval may be withdrawn.

A Final Report is also required at the conclusion of the study. The Final Report Form is also available at www.ethicscommittees.health.govt.nz.

Requirements for the Reporting of Serious Adverse Events (SAEs)

SAEs occurring in this study must be individually reported to the Committee within 7-15 days only where they:

- are *unexpected* because they are not outlined in the investigator's brochure, and
- are not defined study end-points (e.g. death or hospitalisation), and
- occur in patients located in New Zealand, and
- if the study involves blinding, result in a decision to break the study code.

There is no requirement for the individual reporting to ethics committees of SAEs that do not meet all of these criteria. However, if your study is overseen by a data monitoring committee, copies of its letters of recommendation to the Principal Investigator should be forwarded to the Committee as soon as possible.

Please see www.ethicscommittees.health.govt.nz for more information on the reporting of SAEs, and to download the SAE Report Form.

Statement of compliance

The committee is constituted in accordance with its Terms of Reference. It complies with the *Operational Standard for Ethics Committees* and the principles of international good clinical practice.

The committee is approved by the Health Research Council's Ethics Committee for the purposes of section 25(1)(c) of the Health Research Council Act 1990.

We wish you all the best with your study.

Yours sincerely

Alieke Dierckx
Administrator
Upper South A Regional Ethics Committee
Uppersoutha_ethicscommittee@moh.govt.nz
25 October 2011

Upper South A Regional Ethics Committee
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Dear Dr Sturney

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Amendments
- To change the consenting process for Phase 3 of the study from prospective to delayed consent
- To allow data obtained from deceased participants to be used without consent from family/whanau

The above amendments were discussed at the meeting of the Upper South A Regional Ethics Committee on 17 October 2011. The committee thanks you for participating in the meeting by teleconference.

The committee's decisions regarding the amendments are as follows:
- The change in consenting process in Phase 3 from prospective to delayed consent is **approved**
- The proposal to use data from deceased participants without delayed consent from family/whanau is **not approved**.

If you have any queries, please do not hesitate to contact me.

Yours sincerely

Alieke Dierckx
Administrator
Upper South A Regional Ethics Committee
Uppersoutha_ethicscommittee@moh.govt.nz

Canterbury
District Health Board
Te Poari Hauora ō Waitaha

2nd August 2011

Dr Sharon Sturney
Canterbury Respiratory Research Group, Respiratory Services
Christchurch Hospital

Re: Measurement of exhaled volatile organic compounds in critical illness measured via Selected Ion Flow Tube – Mass Spectrometry (SIFT-MS) and Gas Chromatography Mass Spectroscopy (GCMS-MS)

Tena koe Dr Sturney,

Ka nui te mihi tenei ki a koe me tou roopu o ka Kairapukorero ki te hapai o te kaupapa whakahirahira mou, moku mo tatou katoa. Ko Rapuka Korero te mea nui. No reira tena koe me te roopu o ka Kairapukorero, tena koutou katoa.

Firstly apologies for lateness due to snow but thank you for submitting your research for assessment by Te Komiti Whakarite. We have made several general comments and commendations for you and the applicable Ethics Committee to consider.

- The researcher acknowledges the importance of ethnicity data collection. Findings from this study may contribute to the development of future research hypotheses or projects and we therefore support the demographic information being collected using the Census 2006 ethnicity question.
- Inclusion of accurate contact details for Māori health support in the study's Participant Information Sheet has been implemented.
- Allowing a support person or whanau member to accompany the participant during research interviews/visits/sessions is a positive inclusion.
- We agree with your acknowledgement that your research will benefit Maori and also see that it opens the door for some exciting developments in the future.
- You have acknowledged the importance of enrolling Māori participants in your research and using appropriate communication. It is worthy to note that you have used a macron on the word Māori which may seem small but certainly a move in the right direction of correctly using the language and particularly when pronouncing a Māori patients name ensuring their engagement.
- Researchers are aware of the importance of explaining to Maori participants and/or their whanau how the study/research samples will be stored, any security measures, the length of time and the process that takes place up to the destruction of the sample.
- Presentation of study/research findings through journals, publications, conferences and local iwi / participants are excellent forms of dissemination.
- We appreciate you sending your findings to us. We are committed to building on-going relationships with researchers in the hope of improving Maori health.

We do not intend to hinder the ethics approval process and therefore we do not require a response as the final decision is with the Ethics Committee. We are confident our concerns will be considered by the applicable Ethics Committee. Thanks you for your application.

Heoi ano

Tahu Potiki Stirling
Chairperson
Te Komiti Whakarite

Appendix C. Inspired and mean exhaled volatile concentrations using the four breath collection methods described in Chapter 3.

Subject	Inspiratory concentration (ppb)			Mean concentration of breath from T-piece after tidal breathing (ppb)			Mean concentration of breath from airways after tidal breathing (ppb)			Mean concentration of breath from T-piece after recruitment-style breath (ppb)			Mean concentration of breath from airways after recruitment-style breath (ppb)		
	Acetone	H_2S	HCN	Acetone	H_2S	HCN	Acetone	H_2S	HCN	Acetone	H_2S	HCN	Acetone	H_2S	HCN
1	38.7	0.760	0.769	6750	2.21	1.71	6813	2.05	1.70	5993	1.86	0.888	6423	1.89	1.49
2	54.6	0.961	0.755	2977	1.27	0.917	4057	1.67	1.10	-	-	-	-	-	-
3	32.5	1.06	0.788	362	0.892	0.679	449	1.26	0.667	322	0.950	0.661	465	0.900	0.741
4	41.1	1.21	0.901	261	0.915	0.868	540	1.07	0.964	697	0.812	0.718	963	1.10	0.686
5	50.7	0.774	1.02	689	1.37	2.12	938	2.12	1.86	619	1.13	1.29	946	1.88	1.38
6	116	0.634	0.807	4060	2.60	1.92	4983	2.71	2.06	4543	1.61	1.27	4887	2.16	1.92
7	22.6	0.420	0.760	3470	1.08	1.02	4350	1.22	1.13	3467	1.02	0.806	4253	0.975	0.936
8	51.5	0.727	1.08	3360	1.45	0.977	-	-	-	3243	1.16	1.05	-	-	-
9	43.4	1.58	0.899	8940	1.63	1.21	12067	1.66	-	-	-	-	-	-	-
10	82.2	0.847	0.893	4637	2.44	2.28	6016	2.66	2.22	4787	1.96	1.45	5613	2.56	2.10
11	97.1	0.868	0.880	1743	0.881	0.787	2233	0.910	0.832	1787	0.68	0.744	2377	0.793	0.845
12	52.8	0.597	0.662	722	0.673	0.821	948	0.657	0.862	515	0.73	0.768	900	0.702	0.954
13	110	1.44	1.13	10157	4.18	2.53	11620	4.69	2.43	8663	2.56	1.56	11300	3.68	2.55
14	107	0.933	0.766	1011	1.08	0.813	-	-	-	931	1.10	0.827	-	-	-
15	291	0.800	0.972	11733	4.04	4.17	12700	4.20	3.23	9053	2.90	2.61	13733	4.04	5.13
16	89.6	0.866	0.830	13167	3.96	6.86	14367	4.49	8.06	11933	3.83	5.64	15500	4.45	9.44
17	150	1.62	1.14	-	1.82	-	722	1.81	6.26	661	1.45	-	734	1.21	5.24
18	265	1.26	1.30	9780	2.87	2.32	7763	2.90	2.25	8470	2.06	1.63	9910	2.34	1.80
19	61.2	0.997	0.861	11133	3.02	2.85	11157	3.44	3.04	7360	1.58	1.19	10633	2.87	2.31
20	175	0.859	0.836	27800	4.05	13.2	35367	3.75	18.2	20500	3.91	9.80	28800	3.60	14.1
Median	71.7	0.867	0.871	4060	1.73	1.71	5500	2.09	2.06	4005	1.52	1.19	5250	2.03	1.86

Appendix D. Comparison of patient demographics and ventilation data from Chapters 4 and 5/6.

	Healthy controls (n=26)	ICU patients		Significance of difference (p value)	
		All (n=32)	Pulmonary infiltrates only (n=28)	HC v A	HC v PI
Subject demographics					
Age years (±SD)	53 (±19)	61 (±16)	61 (±16)	0.079	0.092
Male/Female	9/17	20/12	17/11	-	-
Smoking status n (smoker/ex-smoker/non-smoker)	2/6/18	13/8/11	12/6/10	-	-
Ventilation parameters		Day 1	Day 1		
V_T ml (IQR)	500 (450-500)	455 (390-500)	445 (360-485)	**0.033**	**0.0080**
RR breaths/min (IQR)	10 (10-10)	18 (16-22)	20 (16-22)	**<0.0001**	**<0.0001**
MV L/min (IQR)	5 (4.5-5.1)	8.8 (7.5-9.9)	8.8 (7.4-9.9)	**<0.0001**	**<0.0001**
$P_{et}CO_2$ mmHg (IQR)	38 (35-40)	38 (32-43)	40 (33-43)	0.92	0.78

ICU, Intensive Care Unit; HC, healthy controls; A, all; PI, group of patients with pulmonary infiltrates on chest radiograph; SD, standard deviation; V_T, tidal volume; RR, respiratory rate; MV, minute ventilation; $P_{et}CO_2$, end-tidal partial pressure of carbon dioxide; IQR, interquartile range.