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Variation in the levels of volatile trace gases within three hospital environments: implications for clinical breath testing

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Abstract
Selected ion flow tube mass spectrometry, SIFT-MS, has been used to determine the levels of volatile trace gases in the room air of three clinical environments within a busy teaching hospital. The main aims of this study were to establish background levels of trace gases and to compare them to levels typically found within exhaled breath. Over a period of one month, room air samples were collected daily from an outpatient clinic, a hospital ward and an operating theatre, in either the morning or the evening. The concentrations of seven volatile compounds were compared between different locations and different times of the day. Having established the baseline levels for these compounds, breath was collected from healthy volunteers working within each of the three clinical locations. The concentrations of isoprene, acetone, hydrogen cyanide and ammonia within room air samples collected from at least one of the three clinical environments were less than 25% of levels typically found in human breath. Based on the recommendation of previous authors these compounds may therefore be suitable for use as exhaled markers of disease. In comparison high levels of ethanol, propanol and acetic acid within room air samples collected from each location may diminish confidence in their use as breath biomarkers.

1. Introduction

The concept that breath contains molecules useful for the detection of abnormal physiology has been recognized for centuries. Growing concern about the delivery of healthcare at both local and national levels, reflecting an ailing population and declining resources, has reinvigorated international efforts to seek out future breath tests that will be fast, non-invasive and cost effective. Crucially, these endeavours have been supported by recent advances in analytical techniques which now possess the ability to detect both on-line, and in real time, multiple metabolites within a single exhalation [1, 2].

In addition to the anticipated influence of both normal and abnormal physiology, the levels of volatile trace gases in the breath may be influenced by several other factors, including environmental exposure through inhaled air. Without reference to room air levels, it is, therefore, difficult to determine whether metabolites within the breath are significantly altered as a result of a disease process or as a function of the local environment. Moreover, if not monitored, individuals may be exposed to unacceptably high levels of volatile gases which, in turn, may have a detrimental effect on health.
Herein we report the findings of a longitudinal study investigating the levels of selected trace gases, commonly found in exhaled breath, in different clinical environments within a busy teaching hospital. Specific aims of this work were to determine: (i) variation in the levels of trace gases within different clinical environments; (ii) intra- and inter-day variability in the levels of volatile trace gases in these locations; and (iii) potential implications for collection and analysis of breath within clinical environments.

2. Methods

2.1. Experimental design

Gas samples were analysed using the multiple ion-monitoring (MIM) mode of a Profile-3 SIFT-MS instrument (Instrument Science, Crewe, UK), a full description of which is provided elsewhere [2]. Room air samples were collected in double thickness (2 × 25 μm) Nalophan® (Kalle UK Ltd, Witham, UK) sample bags (~2 L) using a room air sampler (developed at Innsbruck Medical University, Innsbruck, Austria), which relies on the conduction of air samples along heated sample lines into a Nalophan® bag held under sub-atmospheric pressure within a plastic chamber. Nalophan® has previously been reported as a suitable material for the construction of gas sample bags [3, 4]. Our own studies have confirmed that breath samples remain stable within double thickness Nalophan® sample bags for a minimum of 1 h following collection (unpublished data). Over a period of 1 month, ambient room air samples were collected in the afternoon (after 17:00) from three hospital environments. The locations were: the waiting room of an outpatient clinic, a surgical ward, and an operating theatre used for general surgical procedures. Over the course of the second month, further room air samples were collected from the same three locations in the morning (between 08:30 and 10:00). On selected days, room air samples were collected both in the morning and afternoon. Breath samples were also collected from ten hospital staff working in one or more of the three clinical environments. At the time of breath sample collection the staff had been within each hospital environment for a minimum of 2 h. ‘Mixed’ breath samples were collected by asking subjects to exhale directly into Nalophan® sample bags (the same as described above).

To reduce condensation, the bag samples were heated within an incubator set at 37 °C 5 min prior to, and for the duration of, sample analysis. All room air and breath samples were analysed within 1 h of collection. Trace gases investigated in both room air and breath samples during
Table 3. Median levels of selected trace gases in ambient air collected in the morning and evening on the same day from three clinical environments.

<table>
<thead>
<tr>
<th></th>
<th>Hospital ward (n = 5)</th>
<th>Outpatient clinic (n = 5)</th>
<th>Operating theatre (n = 5)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>AM&lt;sup&gt;b&lt;/sup&gt;</td>
<td>PM&lt;sup&gt;c&lt;/sup&gt;</td>
<td>AM</td>
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<tr>
<td></td>
<td>Median&lt;sup&gt;a&lt;/sup&gt;</td>
<td>IQR</td>
<td>Median</td>
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</tbody>
</table>

<sup>a</sup> Number of days upon which samples were analysed.

<sup>b</sup> AM, morning.

<sup>c</sup> PM, evening.

<sup>d</sup> All values are given as parts-per-billion.

<sup>e</sup> IQR, interquartile range. Values that are given the prefix, <, were considered to be below the limit of detection of the SIFT-MS instrument.
the current study were as follows: acetone, ethanol, propanol, ammonia, hydrogen cyanide, isoprene and acetic acid. The quantification of these trace gases by SIFT-MS has been described previously [5, 6]. The calculation of trace gas levels, in parts-per-billion (ppb), was performed by determining their levels over a 60 s MIM mode scan.

2.2. Statistical analysis

Data analysis was conducted using Microsoft Office Excel 2007 (Microsoft, Redmond, WA, USA) and SPSS version 17 (SPSS Inc., Chicago, IL, USA). Trace gas concentrations are presented as median values and their associated interquartile range (IQR). Statistical differences between the trace gas concentrations measured in each of the three hospital locations were assessed using the Kruskal–Wallis test. All other pairwise comparisons were made using the Mann–Whitney U test. The level of statistical significance was assigned to \( P \) values \( \leq 0.05 \).

3. Results

The levels of selected trace gases within ambient air samples collected in the evening from the three clinical environments were determined (table 1). One-way analysis of variance determined that levels of acetone, ethanol and propanol were significantly different between the three hospital environments \( (P < 0.05) \). A pair-wise comparison identified higher acetone levels in the clinic room air compared to the operating theatre \( (P = 0.015) \). In contrast, ethanol was found in higher concentrations within the ward compared to the clinic \( (P = 0.003) \) and operating theatre \( (P < 0.001) \). Ambient propanol levels were significantly higher in both the ward and clinic environments compared to the operating theatre \( (P < 0.001) \). The levels of hydrogen cyanide, isoprene, acetic acid and ammonia were equivalent in room air samples collected from each of the three locations.

Variations in the morning levels of the selected trace gases among the three clinical environments were also assessed (table 2). One-way analysis of variance determined that the levels of acetone, ethanol, propanol and ammonia were significantly different in room air samples collected from different environments during the mornings \( (P < 0.05) \). In the morning samples, the concentration of acetone and ethanol was higher in the ward compared to the clinic \( (P \leq 0.001) \). Both ward and clinic levels of acetone, ethanol, propanol and ammonia were greater than levels found in theatre air \( (P \leq 0.005) \). The levels of hydrogen cyanide, isoprene and acetic acid were equivalent in room air samples collected from each of the three locations during the morning.

A comparison of the morning and evening samples (collected on different days) indicated the presence of higher ethanol levels in ward air at the beginning of the day \( (P < 0.001) \). In contrast, ammonia levels tended to be higher in all three locations towards the end of the day \( (P < 0.001) \). On selected days room air samples were collected from all three clinical environments both in the morning and in the afternoon (table 3). The findings of these limited studies confirmed the presence of higher ethanol levels within the ward in the morning \( (P > 0.05) \). A rise in ammonia levels was, however, not observed when both morning and afternoon sampling were performed on the same day.

Breath samples from hospital staff working in each of the three clinical environments were collected and analysed by SIFT-MS to allow a comparison with ambient levels (table 4). At all locations, the levels of acetone, hydrogen cyanide, isoprene and ammonia within exhaled breath were higher than in ambient air (table 5). It was only in the theatre environment that breath propanol levels were found to be higher than those in ambient air; whilst acetic acid was higher in the breath samples collected from the staff in both the ward and clinic, but not in the theatre. At all locations ethanol was higher in the ambient air compared to breath.

4. Discussion

The principal findings of this study were: (i) that the levels of several volatile trace gases were markedly different in the room air of the three distinct clinical environments; (ii) to ascertain a clearer understanding of variability in the room air levels of prominent breath metabolites; and (iii) that the consistently high levels of certain trace gases within the room air of clinical environments may diminish confidence in their use as breath biomarkers. In particular, the observation of a trend towards lower ambient trace gas levels in the operating theatre may be an indication of the efficiency of the ventilation systems used within this environment.

As suggested by Schubert et al, high inspiratory concentrations of a trace gas may disproportionately affect the alveolar concentration gradient of that compound and hence, the rate of elimination in the exhaled breath [7]. The same authors further suggested that, based on their own experimental evidence, only compounds whose inspired concentrations are <5% of exhaled concentrations can be confidently analysed in exhaled breath. This recommendation could, however, have major implications for breath testing, especially in the clinical setting, where there is expected to be an abundance of volatile compounds within the ambient air. Furthermore, background concentrations of many compounds may vary significantly depending on the time and locality of breath testing.

Despite these acknowledged factors, there currently exists no consensus agreement as to the most appropriate method to correct for the potential effects of inspired trace gas levels. Two suggested methods supported by different sets of authors for inspired air correction are: (i) background subtraction; and (ii) breathing air ‘scrubbed’ of volatiles prior to breath sampling. Both these correction methods suffer from important limitations. Background subtraction, although relatively easy to implement, is associated with increased uncertainties when interpreting recalculated breath analyte concentrations, especially when room air levels are comparable, or greater, than levels in exhaled breath [8, 9]. Furthermore, it has been demonstrated that the correlation between compound concentrations in blood and breath, as influenced by inspired concentrations, is not purely linear [10], and hence cannot be accounted for simply by background.
subtraction [7]. Alternatively, whilst breathing scrubbed air
prior to breath sampling is arguably a more scrupulous method
of background correction, it is laborious and impractical to
implement routinely within the clinical setting. In addition,
there remains considerable uncertainty as to the necessary
washout period that is required prior to breath sampling. Some
authors suggested that successful washout of the lungs can be
achieved in as little as 4 min if a subject breathes scrubbed
air [11]. However, depending on the compound, to achieve
washout of the entire body, significantly longer periods may
be required on account of latent storage of volatile compounds
within lipid-rich body compartments [10].

In order that the appropriate guidelines can be created, it
is, therefore, of the utmost importance that researchers produce
accurate toxicokinetic models to predict the disposition of
molecules present in inspiratory air. Until such guidelines
exist it is, however, recommended that samples of inspiratory
air are collected and analysed in parallel to breath sampling.
According to Risby and Solga, if it is subsequently found that
the analyte concentration of inspiratory air is greater
than 25% of concentrations in exhaled breath, then the data
should be treated with caution [12]. It is noted that this
figure of 25% is higher than the 5% quoted by Schubert
et al [7], but perhaps represents a more practicable guideline,
especially for breath sampling within the clinical setting.
By this recommendation, the analysis of isoprene, acetone,
hydrogen cyanide and ammonia are possible in at least one
of the environments described in this study. In comparison,
the current study, 'mixed' mouth-exhaled breath
samples were collected from all subjects. The site of release of
breath metabolites within the respiratory tract and its relation
to the chosen sampling methodology is likely to contribute to
observed variability. Several recent publications comparing
metabolite levels within the mouth- and nose-exhaled breath
and in the oral cavity contribute new evidence relevant
to this hypothesis [5]. In particular, levels of ammonia,
hydrogen cyanide and ethanol are reported to be lower in nose-
compared to mouth-exhaled breath, and as a consequence,
current experiments may overpredict their levels. Whilst
sampling methodology has been shown to influence the level
of repeatability and variability related to breath sampling,
metabolite concentration and physiologic variability appear
to remain the principal determinants of these parameters [13].
Accordingly, the small number of breath samples collected
from the hospital staff working in each clinical environment
limits the strength of conclusions that can be drawn. The
recorded breath metabolite levels were, however, similar to
those reported by previous SIFT-MS studies [5, 14–16].

It is intended that this work should serve as a starting
point for future studies aimed at accurately interpreting the
influence of inspiratory trace gas levels on related expiratory
concentrations. Such information will help to inform
those attempting to develop clinical breath testing, thereby

| Table 4. Median levels of selected trace gases within the exhaled breath of hospital staff. |
|-----------------------------------------------|-----------------|-----------------|-----------------|
| Hospital ward (n = 6)a                         | Outpatient clinic (n = 8) | Operating theatre (n = 6) |
| Acetone                                      | Medianb IQRc       | Median IQR       | Median IQR       |

| Table 5. Ratios of the levels of selected trace gases measured within three clinical environments and the breath of hospital staff working within those environments. |
|-----------------------------------------------|-----------------|-----------------|-----------------|
| Hospital ward | Outpatient clinic | Operating theatre | All |
| Acetone          | 0.07a           | 0.04           | 0.04           | 0.05           |
| Ethanol          | 2.07           | 1.38           | 1.75           | 1.87           |
| Propanol         | 1.86           | 3.33           | 0.32           | 2.37           |
| Hydrogen cyanide | 0.16           | 0.13           | 0.31           | 0.18           |
| Isoprene         | <0.01          | <0.01          | <0.01          | <0.01          |
| Acetic acid      | 0.79           | 0.62           | 1.07           | 0.77           |
| Ammonia          | 0.35           | 0.12           | 0.38           | 0.24           |

a Ratio calculated as [median level within room air]/[median level within breath].

b All values are given as parts-per-billion.
c IQR, interquartile range.
a Number of breath samples analysed.
making possible the establishment of appropriate consensus guidelines.

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