Volatile Organic Compounds Arising from Tracheobronchial Stent-related Biofilm Formation Detected in Patients’ Breath by Ion Mobility Spectrometry

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Abstract

Background: Tracheobronchial stent-related biofilm formation encountered in interventional pulmonology that can result in pneumonia and in tissue granulation. Breath analysis by means of ion mobility spectrometry (IMS) has been reported for detection of volatile organic compounds (VOCs) indicative of bacteria biofilm. We hypothesized that IMS can detect specific VOCs in patients with bacterial biofilm formation resulting from tracheobronchial stent placement, and we tested our hypothesis in a prospective study conducted between October 2011 and October 2012.

Methods: During the study period, stent placement, removal, or replacement was performed in 21 patients with tracheobronchial stenosis. A silicone stent was used in 11 of these patients. In 11 cases, 5 cases were placement procedures and 6 cases were replacement or removal procedures. Breath samples were obtained from these 6 patients before and after stent removal or replacement, and the samples were analyzed by IMS. Peaks were characterized with the use of Visual Now 2.2 software (B&S Analytik, Dortmund, Germany).

Results: Bacteriologic culture from the removed silicone stents showed Pseudomonas aeruginosa biofilm formation in 4 of the 6 patients. Wilcoxon-Rank tests was applied to 36 peaks. Box-and-whisker plots were drawn, and 12 peaks were identified. Five of the 12 peaks differed significantly in signal intensity after removal of the initial stent. Comparison against an IMS database identified the following: peak no. 22 (P22), unknown ($p < 0.05$); P23, limonene ($p < 0.05$); P24: 2,2,4,6,6-pentaheptylmethane ($p < 0.05$); P31, 1-octanol ($p < 0.05$); and P35: phenylacetaldehyde ($p < 0.05$).

Conclusions: Using IMS, we were able to assess the degree of Pseudomonas aeruginosa biofilm formation resulting from stent placement. IMS breath analysis in conjunction with clinical symptoms will provide for non-invasive assessment of the need for stent replacement or removal.

Key words
breath analysis, silicone stent, Pseudomonas aeruginosa

Introduction

In patients with a tracheobronchial stent, the biofilm that forms around the stent often becomes problematic. The formation of such biofilm can result in pneumonia, mucus obstruction inside the stent, and granulation tissue formation. Previously, we reported stent-related complications in 35 patients treated for central airway stenosis. The specific complications listed in the order of prevalence were as follows: mucus obstruction inside the stent (31%), granulation tissue formation at the edge of the stent (49%), tumor overgrowth (9%), and stent migration (11%)\(^1\). In previous patient series that we have reported\(^2\), we found that it was extremely important to control the development of Pseudomonas aerugi-
nosa (P. aeruginosa) biofilm. Moreover, it has been reported that stent-associated respiratory tract infection (SARTI) can develop in as many as 20% of patients who have undergone airway stenting, with P. aeruginosa as the main causative bacteria. For patients who have received an airway stent, it is important that any biofilm be evaluated thoroughly and noninvasively.

Ion mobility spectrometry (IMS) can detect traces of volatile organic compounds (VOCs) in patients’ exhaled breath and is used to diagnose lung cancer, interstitial pneumonia, asthma, and various other lung diseases, including infectious diseases. To our knowledge, there have been no studies of VOCs emitted from bacteria on previously placed stents. We hypothesized that IMS can be used to detect specific VOCs in the exhaled breath of patients in whom P. aeruginosa biofilm has developed as a result of silicone stent placement, and we conducted a prospective single-center study to investigate whether IMS detection of these specific VOCs can be used to prevent the development of bacteria-induced complications in patients with a silicone stent.

Materials and Methods

Ethics committee approval

This study was approved by the Research Ethics Committee of St. Marianna University (approval number 1820) and registered with the Medical Information Network (UMIN 000006696). Written informed consent was obtained from all study patients for their participation.

Patients

The study was conducted between October 2011 and October 2012, and we enrolled patients with various lung diseases who were over 20 years of age and judged to be good candidates for necessary stent placement, replacement, or removal. The study protocol involved the use of IMS to analyze breath samples obtained before and after silicone stent removal or replacement.

Breath sampling and IMS

Exhaled breath was obtained from each patient with a spirometer that has a CO2-controlled sample inlet (Ganhorn Medizin Electronic, Niederlauer, Germany), and 10 mL samples were analyzed by means of a multi-capillary column coupled with an ion mobility spectrometer (MCC-IMS, B&S Analytik, Dortmund, Germany). IMS for analysis of exhaled breath has been described previously. Briefly, within the spectrometer, a 95 MBq 63Ni β-radiation source was applied for ionization of the carrier gas (Nippon Megacare, Tokyo, Japan). The spectrometer was connected to a polar multi-capillary column (MCC, type OV-5, Multichrom Ltd, Novosibirsk, Russia), which was used as the pre-separation unit. The exhaled breath sample was sent through the MCC, which consists of 1000 parallel capillaries, each with an inner diameter of 40 µm and a film thickness of 200 nm. The total diameter of the separation column is 3 mm.

Exhaled breath was taken directly into the spirometer, and the 500 mL dead volume was excluded from analysis. The 10 mL content of the sample loop was transferred to the inlet of the MCC and after preseparation was transferred directly into the ionization region of the ion mobility spectrometer. The MCC and the drift tube of the spectrometer were held isothermally at 40°C.

IMS breath analysis was performed immediately before stenting or removal of a stent. In addition, we cultured the cells present on the stents that were removed to determine the types of bacteria that were prevalent. Breath analysis was performed again 1 week after the stent procedure for comparison of the pre- and post-operative results.

Statistical analysis

IMS analysis was performed before and 1 week after the stent procedure. Peaks on the IMS-chromatograms were characterized with the use of VisualNow 2.2 software (B&S Analytik). All peaks were characterized in relation to drift time (corresponding 1/K0 value) and retention time and their concentration related to the peak height. For the different groups (pre-operative IMS and post-operative IMS) and each of the peaks, a box-and-whisker plot was drawn. The rank sum provided by Wilcoxon-Mann-Whitney test was obtained by VisualNow 2.2, and the peaks showing the greatest differences between groups were ranked.

The observed peaks were compared against those recorded in the IMS 110801 database (B&S Analytik), which contains reference measurements, as described elsewhere. The peaks observed in the present study were compared to those in the reference database.
Results

Study patients and stent procedures

Stenting, with either a metallic or silicone stent, was performed during the study period in 21 patients with tracheobronchial stenosis resulting from lung cancer, tuberculosis, relapsing polychondritis, mediastinal seminoma, or esophageal cancer. Eleven in the 21 cases involved a silicone stent that required initial placement (n = 5) or removal and replacement (n = 6). *P. aeruginosa* was detected in 4 cases of the 6 cases: in 1 case of stent removal after successful chemotherapy and in 3 cases of stent replacement (Fig 1). Of the 4 patients (3 men, 1 woman), 2 were being treated for tracheobronchial malacia due to tuberculosis, 1 was being treated for tracheobronchial malacia due to relapsing polychondritis, and 1 was being treated for tracheal obstruction due to mediastinal seminoma.

![Diagram: Tracheobronchial stenosis with stent placement and procedures](image)

**Figure 1.** Study flow chart.

Bacteriologic inspection

Pre- and post-extraction bronchoscopic images and an electron micrograph of an extracted stent are shown in Fig 2. In 4 patients, *P. aeruginosa* was isolated by bacteriologic culture of cells attached to the removed silicone stents. The pre-bronchoscopic image in Fig 2 shows *P. aeruginosa* biofilm formation on the inside surface of the silicone stent lumen.

Results of IMS

An example IMS-chromatogram is shown in Fig 3. Wilcoxon’s rank-sum test was applied to 36 peaks in total. Box-and-whisker plots were drawn, and 12 peaks were shown to have signal differences. Five of the 12 peaks differed significantly in signal intensity from those observed before the stent procedures (Fig 4). From the database provided, a significant difference was observed for peak no. 22 (P22) (1/K$_0$ (drift time) = 0.7865, RT (retention time) = 26.2): unknown ($p < 0.05$); P23 (1/K$_0$ = 0.6351, RT = 25.4):
limonene ($p < 0.05$), P24 ($1/K_0 = 0.6663$, RT = 25.4); 2,2,4,6,6-Pentaheptylmethane ($p < 0.05$); P31 ($1/K_0 = 0.6985$, RT = 45.3); 1-octanole ($p < 0.05$); and P35 ($1/K_0 = 0.7643$, RT = 26.9); phenylacetaldehyde ($p < 0.05$). Furthermore, although not significant, a decrease was seen in P2 ($1/K_0 = 0.5839$, RT = 39.2); acetophenone; P13 ($1/K_0 = 0.7551$, RT = 10.4); cyclohexanol; P20 ($1/K_0 = 0.7338$, RT = 57.5); nonanal; and P30 ($1/K_0 = 0.5746$, RT = 27.3); 4-Isopropyltoluene. In contrast, an increase was seen in P6 ($1/K_0 = 0.4922$, RT = 5.3); propanol; P15 ($1/K_0 = 0.5487$, RT = 7.3); 3-pentanone; and P32 ($1/K_0 = 0.5487$, RT = 7.5): phenylacetylene (Table 1). Peaks were compared by their actual position to the nearest peak in the reference database.

**Discussion**

To our knowledge, this is the first prospective clinical study to investigate the usefulness of exhaled breath analysis before and after stenting. We found that IMS can detect specific VOCs in patients with *P. aeruginosa* biofilm resulting from silicone stent placement. Furthermore, using IMS in conjunction with patients’ clinical symptoms, we were able to consider whether to replace or remove the silicone stents.

As noted above, bacteria-laden biofilms can cause SARTI or formation of friable granulation tissue. Several bacterial species have been found in stent biofilms, and these are all thought to produce various VOCs. *P. aeruginosa*, *Staphylococcus* species, and fungi are frequently identified in cases of SARTI. The main causative agent of SARTI is *P. aeruginosa*. Bacterial analysis of the stents in our study patients confirmed the presence of *P. aeruginosa* in several biofilms.

Recently, Bos et al. found that propanol and 2-pentanone (VOCs) are related to pathogens includ-
VOCs arising from stent biofilm

Figure 4. Box-and-whisker plots of peaks on IMS chromatograms obtained before and after removal of a silicon stent. After the infectious stent was removed from the respiratory tract, the signal intensity of the 5 peaks decreased significantly. The peak (P) numbers are shown.

Pre: before removal of the silicon stent (n = 4).
Post: after removal of the silicon stent (n = 4).

Table 1. Peaks and the Volatile Organic Compounds They Represent

<table>
<thead>
<tr>
<th>Peak no.</th>
<th>IMS Database</th>
</tr>
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<tbody>
<tr>
<td>P2</td>
<td>Acetophenone</td>
</tr>
<tr>
<td>P6</td>
<td>Propanol</td>
</tr>
<tr>
<td>P13</td>
<td>Cyclohexanol</td>
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<tr>
<td>P15</td>
<td>3-Pentanone</td>
</tr>
<tr>
<td>P20</td>
<td>Nonanal</td>
</tr>
<tr>
<td>P22</td>
<td>Unknown</td>
</tr>
<tr>
<td>P23</td>
<td>Limonene</td>
</tr>
<tr>
<td>P24</td>
<td>2,2,4,6,6-Pentaheptylmethane</td>
</tr>
<tr>
<td>P30</td>
<td>4-Isopropyltoluene</td>
</tr>
<tr>
<td>P31</td>
<td>1-Octanol</td>
</tr>
<tr>
<td>P32</td>
<td>Phenylacetylene</td>
</tr>
<tr>
<td>P35</td>
<td>Phenylacetaldehyde</td>
</tr>
</tbody>
</table>

Peaks identified according to the IMS Visual Now 110801 database.

ing *P. aeruginosa*\(^{33}\). Furthermore, Thomas et al. found 2-aminoacetophenone, which is excreted by *P. aeruginosa*, to be a potential breath biomarker for cystic fibrosis\(^{32}\). We found that a single bacterium may not necessarily produce only a single type of compound; even *P. aeruginosa* was identified on the basis of a combination of VOC peaks. We also found that particular bacteria produced particular VOCs. However, considering that bacteria in biofilms produce several compounds, the combined VOC profile constructed from our total biofilm data may explain the signal differences we observed between some peaks. In addition, the number of cases in which stents were removed and microbiologically analyzed was small. Future investigations should include the assessment of VOCs in patients with newly placed stents in addition to the 5 VOC peaks obtained in this study that were considered biofilm markers useful for
evaluating the presence and degree of biofilm forma-
tion. The increased strength of combined multiple peak signals rather than the increased strength of a single peak signal could aid in biofilm assessment. Other studies on assessment of bacterial infection by means of IMS have shown the importance of correlation when combining peaks\(^1\text{3}^{1}\text{4}\).

Thus, even though assessment can be performed on the basis of a single peak, we believe that the presence and degree of a biofilm can be accurately inferred through the use of a combined peak analysis.

In addition to the breath analysis, we tested the phlegm of all four patients 1 week after the stent procedure; however, no new data were obtained. This is because the absolute numbers of bacteria after stent removal had decreased markedly in comparison to the numbers before stent removal, making our study of phlegm for bacterial composition and number uninformative. We believe that highly accurate results can be obtained when the type of bacteria on the stent is examined microbiologically and the numbers of bacteria are determined on the basis of IMS peak signal strength. Furthermore, our study showed that the presence and degree of biofilm formation can be assessed accurately by means of IMS. By tracking the peaks identified in this study, the removal or exchange of silicone stents can be considered before the harmful effects of bacteria-laden stent biofilms (i.e. SARTI) set in.

The results of our study may be especially important to the future of stent-insertion. Until now, highly invasive methods such as bronchoscopy or highly inaccurate methods such as sputum assessment have been relied upon for assessing the risk associated with stent placement. However, IMS can be used to assess the presence of infectious biofilm with minimal invasion and high accuracy. This will in turn allow for preventive measures to be taken before the onset of SARTI. Our long-term objective is to prevent the onset of SARTI by using the present study results, to perform periodic breath analysis of patients with stents, and to continuously monitor changes in the 5 biofilm markers we identified. The ultimate goal of our research is to use IMS to evaluate biofilm development and control its progress. Studies in larger patient groups are needed to confirm the findings reported herein.

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