Faecal volatile organic compounds and NEC

Andrew Ewer
Professor of Neonatal Medicine
Birmingham University
Birmingham Women’s Hospital
Smells and human disease

...encouraged his students to smell the breath of patients and to pour sputum on hot coals to produce smells as predictors of disease.

Hippocrates 460-370 BC
Smells and human disease

Wrote the Canon of Medicine.

Diagnosed illnesses by noting changes in the smell of patients urine

Avicenna 980-1037 AD
Smells and human disease

Noted the similarity between odours of infected wounds and those of pathogenic bacteria in culture. Germ theory.

Robert Koch 1843-1910
Dogs trained to detect prostate cancer with more than 90% accuracy

The ability of two German shepherds to identify the most common form of cancer in British men has sparked hopes of finding a practical application.

Surviving prostate cancer: a prostate surgeon’s story
Sniffing out cancer with electronic noses

By William Kremer
BBC World Service

9 March 2014

Lung cancer
Ovarian cancer
Diagnosis of rotavirus gastroenteritis by smell

J Poulton and M J Tarlow

East Birmingham Hospital, Birmingham

852 Archives of Disease in Childhood, 1987, 62

69% of stool specimens recognised by smell alone

We conclude that the smell of the stools of infants with acute gastroenteritis may help to diagnose rotavirus and possibly, other infections.
Diagnosis of NEC

• Clinical suspicion – symptoms often vague and non-specific

• Examination

• X-rays
Specific diagnostic techniques limited to established disease – x-rays, histology

No specific treatment

Few treatment options other than supportive management (medical and surgical) – often unsuccessful in established disease
Prevention and early diagnosis are the key initiatives likely to influence the outcome for NEC

Is there are characteristic smell that might predict NEC?
Volatile organic compounds (VOCs)

- Organic compounds characterised by low vapour pressure
- Mainly, but not exclusively, carbon-based
- Intermediates in metabolic pathways
- Levels in biofluids may reflect changes in body state caused by disease
Volatile organic compounds (VOCs)

- chemical fingerprint of compounds in faeces - may be altered in specific disease states\(^1\)
- Prof Chris Probert - Bristol and Liverpool
- Campylobacter, C Diff, Cholera, Giardia, UC\(^2\)
- Identified using HS - SPME GC-MS

Healthy adults

Compounds

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
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<tbody>
<tr>
<td>A</td>
<td>ethanol</td>
</tr>
<tr>
<td>B</td>
<td>dimethyl disulfide</td>
</tr>
<tr>
<td>C</td>
<td>acetic acid and hexanol</td>
</tr>
<tr>
<td>D</td>
<td>limonene</td>
</tr>
<tr>
<td>E</td>
<td>dimethyl trisulfide</td>
</tr>
<tr>
<td>F</td>
<td>4-methyl-phenol</td>
</tr>
<tr>
<td>G</td>
<td>indole</td>
</tr>
<tr>
<td>H</td>
<td>3-methyl-1H-indole</td>
</tr>
</tbody>
</table>

 Garner et al. FASEB J. 2007
Campylobacter gastroenteritis

Comparison of two different Campylobacter samples in human faeces

Top TIC asymptomatic human faeces, Lower TIC human Campylobacter faeces

1 Garner et al. FASEB J. 2007
Hypothesis

Can the alterations in the microbiome (dysbiosis) and the intestinal mucosa which predispose to clinical NEC be predicted by changes in faecal VOCs
Early diagnosis

The Pooh Study - Birmingham and Bristol
Aims of study

• To identify VOCs emitted from the stools of babies with mass spectrometry

• To compare VOCs from stools of babies who subsequently developed NEC with matched controls
6 babies with NEC
7 healthy controls

HS SPME GC MS

Reduced numbers of VOCs in NEC
Absence of 4 specific esters
Healthy Twins
FIG. 2. Chromatograms from twin pair 2 (discordant for NEC) at day 16. Headspace VOC of faeces were analysed by GC-MS. These chromatograms show the similarities of the VOCs extracted from faeces passed 3 days before the diagnosis of NEC in 1 of a pair of twins (twin pair 2). However, the similarity is less than that found in healthy twin pairs (see Fig 1).

FIG. 3. Chromatograms from twin pair 2 (discordant for NEC) at day 18. Headspace VOC of faeces were analysed by GC-MS. These chromatograms show that 1 day before the diagnosis of NEC in 1 of a pair of twins (twin pair 2), there are significant differences between the VOCs that have been extracted.
FIG. 5. Example of absent VOC, 2-ethylhexyl acetic acid ester in NEC diagnosed infants. Headspace VOC of faeces were analysed by GC-MS. These chromatograms show an enlarged area between 31 and 32.4 minutes in which 2-ethylhexyl acetic acid ester is shown in the VOCs extracted from faeces of 1 non-NEC infant; this ester was absent in those infants with NEC, of which 4 examples are shown.
Conclusions

- Babies who develop NEC fail to demonstrate an increase in VOCs around the time of onset of disease.
- Differences noted 4 days prior to onset of NEC and up to 12 days afterwards.
- Faecal VOC could be utilised as potential biomarkers for NEC prior to acute presentation.

Early Detection of Necrotizing Enterocolitis by Fecal Volatile Organic Compounds Analysis

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3 NNUs in Netherlands. No probiotic use
Infants ≤30 weeks
Congenital intestinal anomalies and intestinal surgery excluded

Analysed stool samples up to 5 days before diagnosis using E-Nose
Stool collection

- Prospective daily stool collection

- 13 NEC (Modified Bell’s ≥ stage II)
- 31 Sepsis (signs and Sx and +ve BC)
- 14 Controls

Independent review of diagnoses

- 3 ‘time windows’ (day 4, 5; 2, 3 and 0, 1 before diagnosis)
Electronic nose (eNose)
Electronic Nose (eNose)

Biological olfactory system
- Olfactory receptors
- Olfactory bulb
- Brain (memory)

Artificial electronic nose
- Sensor array
- Pre-processing
- Pattern recognition (database)

Interaction
Signal generation
Processing
Identification
ODOUR

Volatile odorant

Trends in Food Science & Technology 2011;22:165-174
VOC analysis

- Handheld eNose (Cyranose 320)
- 32 polymer sensors
- Interaction with VOCs $\rightarrow$ change in resistance
- Multiple interactions
- Final resistance $\rightarrow$ ‘smell print’
- Pattern recognition analysis
Statistical analysis

• Principle component analysis (PCA)
• Highest variability in lowest n of variables
• Identify differentiating components and group into factors
• Discriminant analysis to identify best predictor of disease using ROC curves
Table V. Performance characteristics of fecal VOC analysis for the discrimination of NEC, sepsis, and controls

<table>
<thead>
<tr>
<th>Time window</th>
<th>AUC ± 95% CI</th>
<th>P value</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>+LR</th>
<th>−LR</th>
</tr>
</thead>
<tbody>
<tr>
<td>NEC vs control</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T₀₋₁</td>
<td>0.99 ± 0.04</td>
<td>&gt;.001</td>
<td>88.9</td>
<td>88.9</td>
<td>8.1</td>
<td>0.1</td>
</tr>
<tr>
<td>T₋₂₋₃</td>
<td>0.77 ± 0.21</td>
<td>.024</td>
<td>83.3</td>
<td>75.0</td>
<td>3.3</td>
<td>0.2</td>
</tr>
<tr>
<td>T₋₄₋₅</td>
<td>0.65 ± 0.25</td>
<td>.257</td>
<td>60.0</td>
<td>60.0</td>
<td>1.5</td>
<td>0.7</td>
</tr>
<tr>
<td>NEC vs sepsis</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T₀₋₁</td>
<td>0.64 ± 0.18</td>
<td>.216</td>
<td>88.9</td>
<td>56.5</td>
<td>2.1</td>
<td>0.20</td>
</tr>
<tr>
<td>T₋₂₋₃</td>
<td>0.80 ± 0.17</td>
<td>.004</td>
<td>83.3</td>
<td>75.0</td>
<td>3.3</td>
<td>0.2</td>
</tr>
<tr>
<td>T₋₄₋₅</td>
<td>0.52 ± 0.23</td>
<td>.886</td>
<td>50.0</td>
<td>40.0</td>
<td>0.8</td>
<td>1.3</td>
</tr>
</tbody>
</table>

+LR, positive likelihood ratio; −LR, negative likelihood ratio.
Sensitivities, specificities, and positive and negative likelihood ratios are reported for the optimum cut-points.
Figure 2. Receiver operator characteristic curve with 95% CI for diagnosis of NEC compared with controls and NEC vs sepsis, at time windows A, T_{-1,0}, B, T_{-3,-2}, and C, T_{-5,-4}.
No significant differences in VOCs between sepsis patients and controls

- More patients with sepsis
- Younger age at presentation
The DOVE study
Diagnostic testing of Organic Volatiles in necrotising Enterocolitis
Methods

- Preterm babies 23-34 weeks gestation
  - excluded if unlikely to survive or GI malformation
- 8 UK NICUs
  - no probiotic use
- Clinical data collected
  - demographics, feeds antibiotic use
- Diagnosis of definite NEC (Modified Bell’s 2 or 3)
  - Independent review of all clinical data
Methods

• Prospective stool collection in babies <34 weeks from 8 UK NICUs

VOCs analysed using HS –SPME–GC–MS

VOCs identified using AMDIS* software, NIST** library and METAB

*AMDIS - Automated Mass spectral Deconflation and Identification System
**NIST – National Institute of Standards and Technology
Sample gas analysis: SPME GC-MS

Statistical analysis

• Principle component analysis

• Factors identified

• Logistic regression of factors to predict NEC

• Longitudinal discriminant analysis of individual VOCs
Results

• 51 cases of NEC Bell’s ≥ 2 (SIPs excluded)
  34 with adequate samples

• Matched with 70 controls
  gestation, BWt, Unit, delivery mode, feed and Ab use

• Samples selected 1-6 days prior to diagnosis
  and age-equivalent samples from controls
**Principle component analysis**

9 factors – 3 were associated with NEC

<table>
<thead>
<tr>
<th>Factor</th>
<th>1</th>
<th>2</th>
<th>3</th>
</tr>
</thead>
<tbody>
<tr>
<td>3-methylsulfanylpropanal</td>
<td></td>
<td>heptan-4-one</td>
<td>3-methylbutanoic acid*</td>
</tr>
<tr>
<td>2-phenylacetaldehyde</td>
<td></td>
<td></td>
<td>methanedithione*</td>
</tr>
<tr>
<td>Benzaldehyde</td>
<td></td>
<td></td>
<td>2-methylbutanoic acid*</td>
</tr>
<tr>
<td>2-methylpropanal</td>
<td></td>
<td>2-butanone**</td>
<td></td>
</tr>
<tr>
<td>3-methylbutanal</td>
<td></td>
<td></td>
<td>butanoic acid**</td>
</tr>
<tr>
<td>2-methylbutanal</td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

1.61 [1.10, 2.35] 0.51 [0.32, 0.82] 0.70 [0.52, 0.96]

(Odds ratio (95% CI) of NEC risk)

* Produced by bifidobacteria
** Produced by bifidobacteria and lactobacilli
# Principal Component Analysis

<table>
<thead>
<tr>
<th>AUC</th>
<th>Day -6</th>
<th>Day -5</th>
<th>Day -4</th>
<th>Day -3</th>
<th>Day -2</th>
<th>Day -1</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.68</td>
<td>0.75</td>
<td>0.52</td>
<td>0.67</td>
<td>0.74</td>
<td>0.71</td>
</tr>
<tr>
<td></td>
<td>[0.45, 0.92]</td>
<td>[0.60, 0.90]</td>
<td>[0.31, 0.73]</td>
<td>[0.50, 0.83]</td>
<td>[0.55, 0.92]</td>
<td>[0.54, 0.87]</td>
</tr>
</tbody>
</table>

Mean AUCs
Longitudinal Discriminant Analysis (LoDA)

27 individual VOCs could predict NEC with AUC >0.7 between 3 and 4 days prior to diagnosis
LoDa of best predictor VOCs
Summary

• Small numbers of patients

• Both GCMS and eNose show ability to discriminate NEC and healthy patients to some degree

• eNose has greater sensitivity albeit in a smaller group of patients
Conclusions

More studies needed in larger populations to corroborate initial findings

Cotside eNose has potential for identifying patients at higher risk before onset of disease

Early diagnosis with eNose may enable treatment and prevent disease progression

GCMS may shed more light on mechanisms of pathogenesis
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