Multi-centre cross-validation study in the search for volatile colorectal cancer biomarkers in breath and faeces

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Introduction

Colorectal cancer (CRC) is a highly prevalent malignancy that results in almost a quarter of a million deaths annually in the EU. Current screening methods have limitations in relation to sensitivity and specificity, late-stage identification, and discomfort. An alternative screening procedure that offers earlier and more accurate prediction via non-invasive means is highly desirable. Exhaled breath or stool headspace offer a potential screening medium for prospective biomarkers[1,2,3,4].

The exploitation of volatile biomarkers to support disease diagnosis has found limited success, to date. This can be attributed to a lack of reproducibility from one study to the next, amongst other factors. Efforts towards establishing standardised practices and protocols will help in generating reproducible and validated data.

Study concept

Our multi-centre European study in Munich (Germany), Maastricht (Netherlands) and Torun (Poland) will explore the presence of volatile biomarkers in exhaled breath and stool headspace of CRC sufferers in comparison to other bowel disorders and healthy controls.

The study design uses a multi-cohort, cross-validation constellation, comprising the following aspects (see fig. 1):

- **triplicate sample collection** of breath/stool headspace per patient of each cohort
- each sample is **analysed in three independent laboratories**
- **data pooling and cross-validation** for biomarker discovery

The project will recruit ca. 300 participants for each cohort (CRC, irritable bowel syndrome, inflammatory bowel disease - including Crohn's disease and ulcerative colitis - and healthy controls) across the three centres.

Sampling and analytical methods

Standard operating procedures (SOPs) for sampling and analysis will be established prior to commencing the clinical trials for implementation across the three centres. Proficiency training of clinical personnel prior to the start of the clinical phase will reduce discrepancies in procedures across the centres.

- all samples will be collected in triplicate on Tenax/Carbograph adsorption tubes
- samples will be analysed as soon as possible after collection; within four weeks
- breath will be sampled using a ReCIVA device (Owlstone Medical)
- faecal headspace will be sampled using a micro-chamber/thermal extractor (μ-CTE; Markes International)
- replicate sample tubes will be shipped for analyses at the partnering centres
- analyses will be made using thermal desorption-gas chromatography-mass spectrometry (TD-GC-MS; different manufacturers) (see fig. 2).
- machine learning will be applied on the complex datasets using unsupervised Random Forest, PCA, clustering and robust-PCA

Aim of the study

Our aim is to successfully identify and validate volatile biomarkers to ultimately enable the future development of non-invasive, early diagnostic tests for CRC.

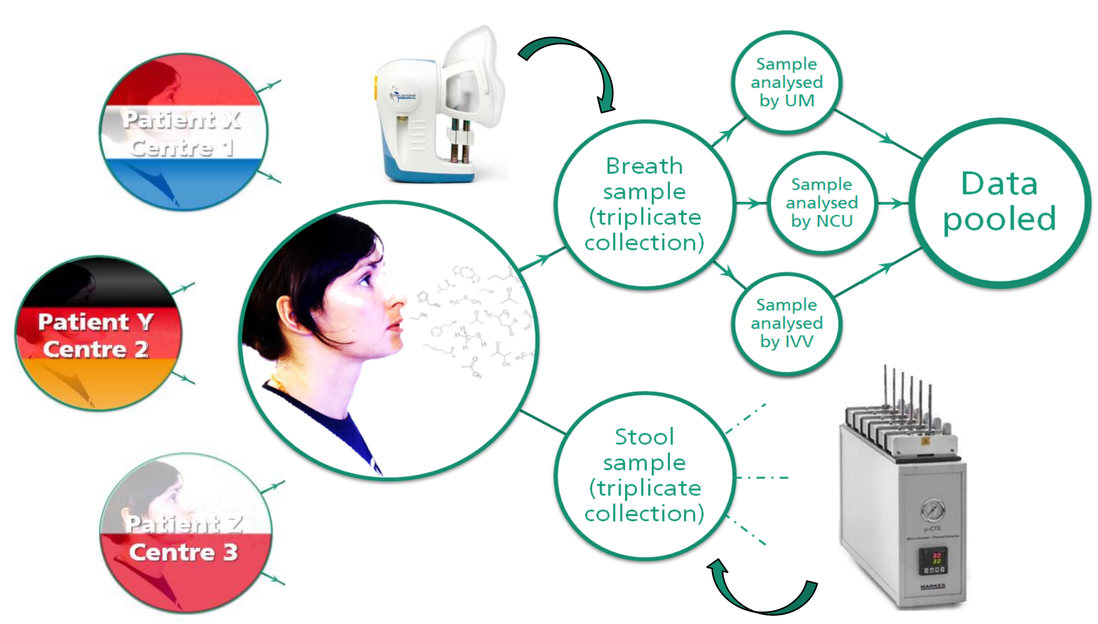

Figure 1: Cross-validation concept for identifying volatile biomarkers for CRC. Exhaled breath or stool headspace is sampled on Tenax/Carbograph adsorbent tubes using a ReCIVA or micro-chamber/thermal extractor, respectively. A replicate of each sample will be comparatively analysed at each of the three centres.

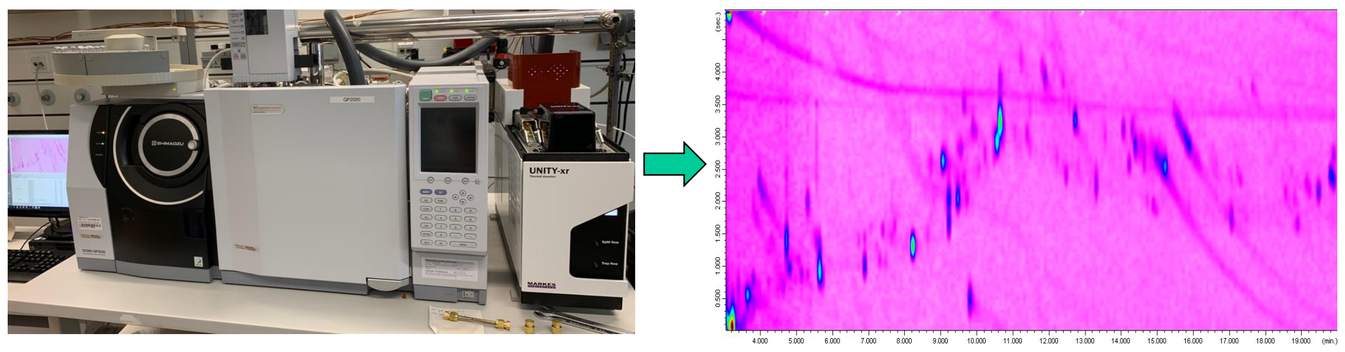

Figure 2: Exploring CRC-specific volatiles using TD-GCxGC-MS, with the instrument (left) and an example of a GC×GC chromatogram of a breath sample (right).

Conclusions

Identifying unique volatile fingerprints for CRC sufferers is of little value if these cannot be reproducibly found in different cohorts at different locations. Our project concept addresses this challenge with its multi-centre, cross-validation approach.

This poster outlines its concept, the methods being employed, and intends to spark debate and seek additional advice on searching for CRC-specific volatile biomarkers.

References

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