Progress Report for ANZHNCS Research Foundation

Project titled: Development and validation of new breath analysis tools for the non-invasive detection of head and neck cancers

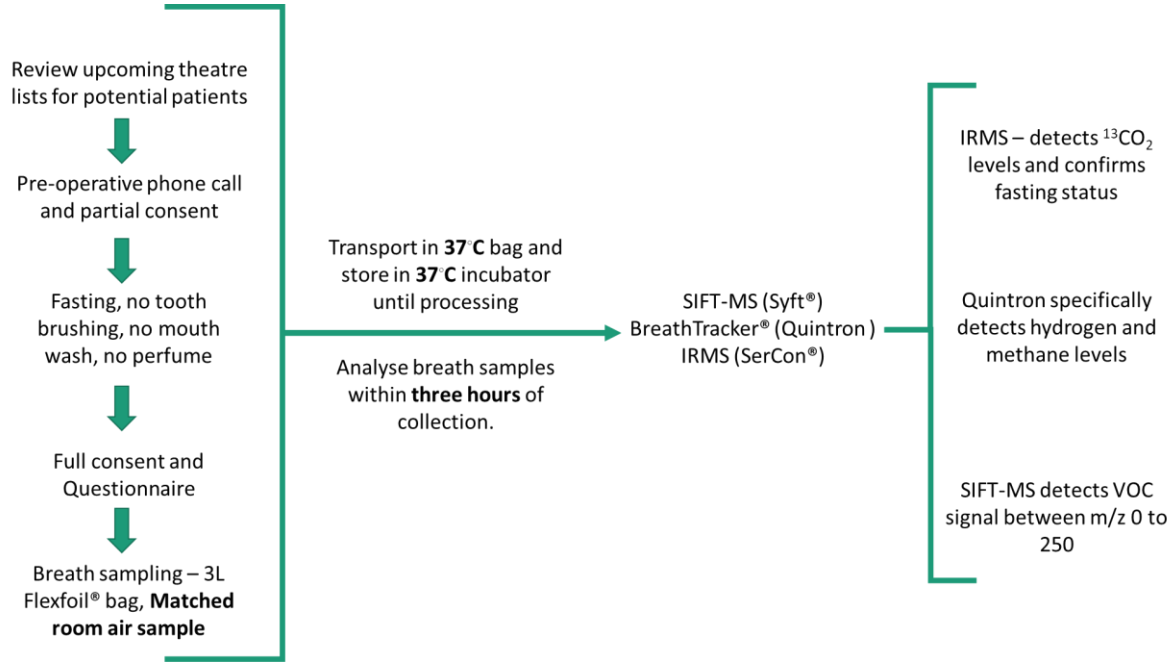
The project has progressed well with Dr Nuwan Dharmawardana actively recruiting patients, obtaining breath samples for Volatile Organic Compound analysis and analysing hundreds of samples. Nuwan was accepted as both a PhD candidate (Flinders University) and an Otolaryngology Head and Neck Surgeon Scientist trainee in 2019. Nuwan received the prestigious Garnett Passe and Rodney Williams Memorial Foundation research scholarship for his PhD and the ANZHNCS research foundation grant has been invaluable for Nuwan to undertake this project. Dr Dhina Chandran was awarded a Masters of Surgery for her research thesis "The role of Volatile Organic Compounds as diagnostic biomarkers of Head and Neck Squamous Cell Carcinoma". Her work has also been invaluable in the initial stages of this project.

Patients recruitment:

Patients were recruited from the Flinders Medical Centre and Royal Adelaide Hospital in South Australia. So far 141 patients have been recruited for this study. We have collected breath samples from 54 non-cancer patients and 87 head and neck squamous cell carcinomas comprising of the following subsites: 33 Oropharyngeal, 22 laryngeal, 24 oral cavity, 1 hypopharynx, and 3 unknown primaries.

Breath analysis methodology:

We have significantly optimised and standardised collection, transport, storage and analysis of patient breath samples. We have also collected room air samples to compare it to patient's breath samples. The SIFT-MS and Breath Tracker and IRMS was used for breath analysis. See our current research protocol below:



The optimisation and standardisation of our methods to minimise cofounding environmental factors is crucial as other studies have been less rigorous in their methodology.

Preliminary Results: Significant volatile organic compounds identified

Compound	P value	Median Percentage Difference (% Higher in Cancer)
Ethanol	0.009	85%
Pentanoic acid	0.001	50%
3-methylbutanoic acid	0.001	40%
Hydrogen Sulfide	0.025	38%
2-furfuryl mercaptan	0.047	31%
Butanoic acid	0.013	24%
Styrene	0.037	21%
Limonene	0.032	15%

The findings to date have been presented at or accepted for upcoming scientific meetings:

"Scoping review of selected ion flow mass spectrometry methodologies for human breath analysis" – Australian Society for Medical Research – South Australia – Annual Scientific Meeting - 2017

"Biomarkers in head and neck caner" – United by Research – Flinders Health Research Week – South Australia - 2017

Accepted for Oral presentation – upcoming meetings

"Characterisation of breath volatile organic compounds in head and neck squamous cell carcinoma"

- Australian Society for Medical Research – ASM – Adelaide, Australia – June 2018
- International Breath Summit – Maastricht, Netherlands - June 2018
- Australia and New Zealand Head and Neck Cancer Society - ASM – Melbourne, Australia – July 2018

Publications: *3 papers from this project are being written up for journal submission*

"Characterisation of breath hydrogen and methane in head and neck squamous cell carcinoma" – Journal of Breath Research

"Characterisation of room air volatile organic compounds in clinical settings" – Journal of Breath Research

Volatile Organic Compounds as diagnostic biomarkers of Head and Neck Squamous Cell Carcinoma – Journal of Breath Research

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