Breath Biopsy® Discovery of Biomarkers for Lung Cancer in Former Smokers

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1. Background and Objectives

Early detection of lung cancer is critical for improving treatment options and survival. 1-year survival can be 85% at early stages but falls to just 15% in Stages III and IV [1]. Two large randomized controlled trials (NLST, NELSON) have demonstrated >20% mortality reduction in lung cancer with low dose CT screening, however important questions remain prior to widespread implementation, including high risk patient selection criteria and indeterminate nodule follow up.

Former smokers remain a high-risk group for lung cancer. Around 50% of diagnoses in the US and Canada are in former smokers and 40% of these occur more than 15 years after quitting.

Metabolic changes associated with lung cancer are thought to produce unique volatile organic compounds (VOCs) detectable on breath that could be applied as diagnostic biomarkers [2,3,4].

Aims:

- Identify VOCs in the breath of former smokers to differentiate those with and without lung cancer.
- Investigate the behaviour of these VOCs in subjects with intermediate risk lung nodules detected through CT scanning.
- Propose candidate biomarkers for early detection of lung cancer in former smokers.

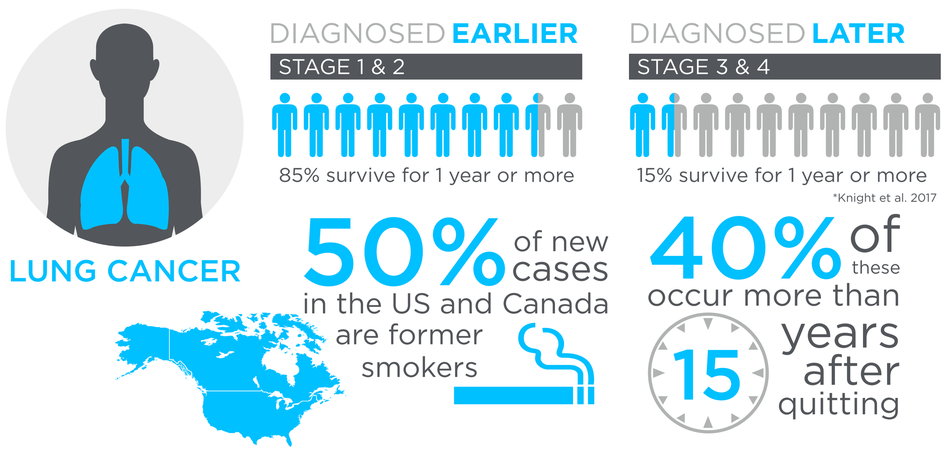


Figure 1: Key statistics relating to the need for improved lung cancer screening for former smokers.

2. Methods

Breath Biopy samples were collected using the ReCIVA® Breath Sampler (**Figure 2**), developed by Owlstone Medical, and analysed with thermal desorption gas chromatography mass spectrometry (TD-GC-MS)* using the Breath Biopsy Platform.

Samples were all collected at a single site with control subjects recruited from the PanCan Lung Cancer Screening trial and intermediate risk subjects (one or more non-calcified nodules, malignancy risk score 1.5%-30%) from the BC Lung Screen Trial.

	Controls	Cases	Intermediate Risk	Full Cohort
N° of Subjects	20	19	25	64
Female	11	13	13	37
Age	74.5±3.5	71.0±6.0	69.0±5.2	71±5.21
BMI	26.6±4.0	26.7±4.8	26.4±3.6	26.4±4.07
Node Risk Score	0.36±1.46	46.9±20.3	3.20±4.66	N/A

Table 1: Test subject groups and demographic overview.

*All subjects were adult former smokers aged 55 to 80. All stopped smoking at least one year previous and have a minimum smoking exposure of 20 pack-years (years smoked * packs per day).*

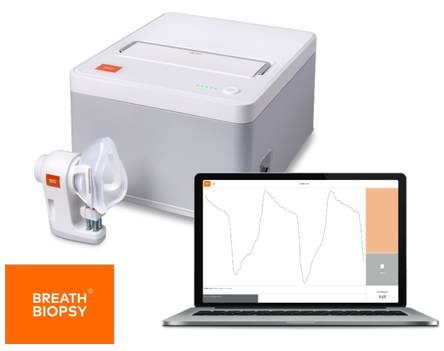


Figure 2: The Breath Biopsy® Collection Station, consisting of ReCIVA® Breath Sampler (left), CASPER™ Portable Air Supply (top) and Breath Biopsy Collect Software (lower right).

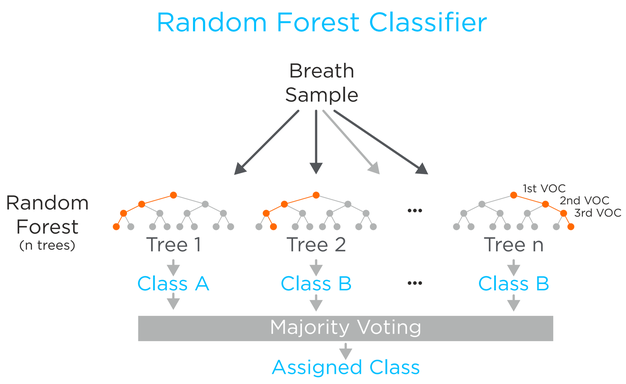


Figure 3: An illustrative schematic of a Random Forest classifier used to create case-control discrimination models. Data from each sample is presented to numerous decision trees and the majority outcome of these trials is used to assign a classification to each sample.

3. Results

22 of 67 molecular features were shown to differ (unadjusted P<0.05) between cases and controls (Mann-Whitney U-test). 8 of which persist following correction for multiple sampling (**Figure 4**).

Features prospectively identified as hydrocarbons and aldehydes (**Figure 5**) show progressive changes in abundance from control to intermediate and cases.

Both groups of VOCs can be produced as a result of lipid peroxidation resulting from oxidative stress (**Figure 6A**) and associated with a range of factors and diseases including cancer and smoking (**Figure 6B**).

A random forest classifier was found to have the highest accuracy in discriminating cases from controls based on these data. The resulting model, based on three VOCs, gives 80% accuracy, 75% sensitivity and 80% specificity (**Figure 7**).

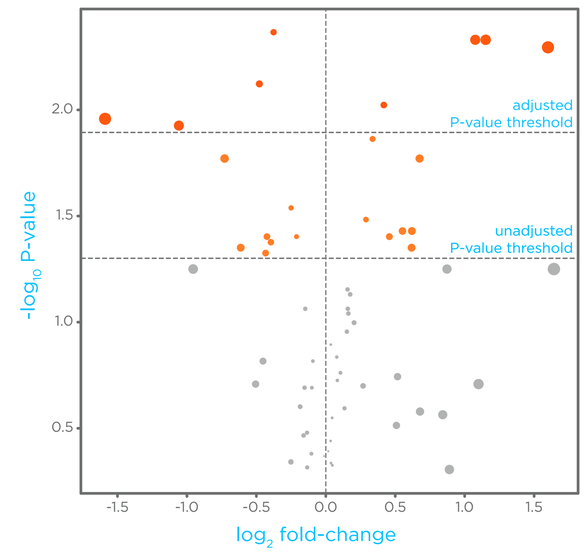


Figure 4: Volcano plot of molecular features plotted by Mann-Whitney P-value and fold-change between cases and controls.

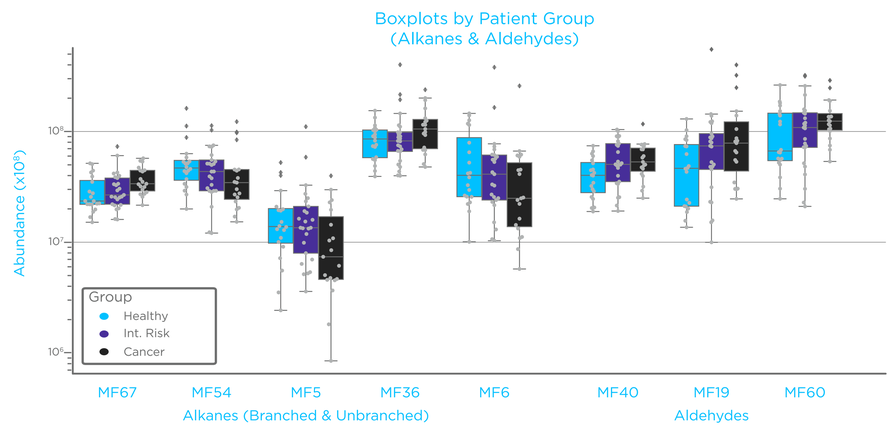
Features are colored by significance thresholds and sized by fold-change.

22 features attain unadjusted P-values <0.05 and 8 attain an adjusted P-value <0.05 following correction for multiple sampling.

Figure 5: Boxplots of VOC abundance.

Plots are colored by study group and grouped by molecular feature.

Eight VOCs, five putatively identified as alkanes and three as aldehydes. All show trends of increasing or decreasing abundance across the three sample groups and significant changes in abundance between cases and controls.



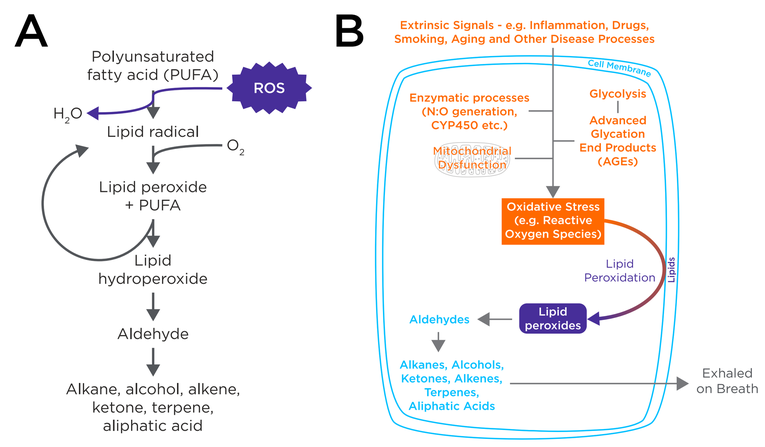


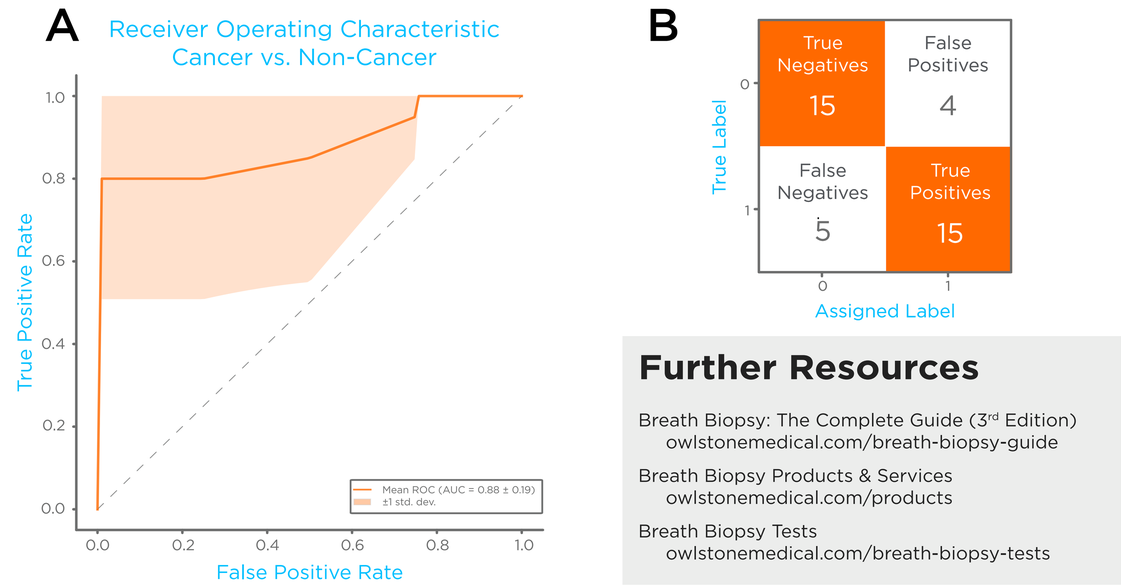
Figure 6: Lipid peroxidation and VOC production.

(A) The chemical chain reaction between reactive oxygen species (ROCs) and polyunsaturated fatty acids that produces various VOC species.

(B) A schematic illustration of reported relationships between various diseases and lifestyle factors (orange text), oxidative stress (orange box), lipid peroxidation (purple) and VOC production by cells (cyan text)

*Aldehydes and alkanes (as seen in **Figure 5**) both result from lipid peroxidation processes.*

*Figure 7: Random Forest predictive model (see **Figure 3**) for lung cancer in former smokers.*
Receiver operating characteristic (ROC) curve (A) and confusion matrix based on average data performance (B).
Model is based on three VOCs (MF67, MF44, MF60) and gives 80% accuracy, 75% sensitivity and 80% specificity.
*Boxplots for MF67 and MF60 are included in **Figure 5**.*



4. Conclusions

Identified eight VOCs with significant differences between cases and controls. Abundance for intermediate risk subjects between cases and controls for most significant and borderline VOCs.

Random forest model based on three VOCs resulted in 80% accuracy in discriminating cases from controls but does not agree strongly with image-based algorithms.

Increased abundance of VOC tentatively identified as aldehydes has previously been reported in lung cancer [5,6] and may be a result of oxidative stress and lipid peroxidation associated with altered metabolic flux in cancer. This may also explain some of the hydrocarbons prospectively identified.

This study demonstrates the feasibility of using breath biomarkers for lung cancer screening. There are several future directions for this work. First, tentative VOC assignments could be verified by comparison to standards analyzed with the same method.

Several candidate biomarkers have been proposed and, if validated, could be applied for cancer screening. This would require a follow-up study. Power calculations based on the current data suggest 30 to 40 subjects per study group would be needed to statistically validate our results.

The intermediate risk group has only been qualitatively assessed relative to cases and controls, these could be investigated further with a longitudinal study to understand which subjects go on to develop cancer.

Further Resources

Breath Biopsy: The Complete Guide (3rd Edition)
owlstonemedical.com/breath-biopsy-guide

Breath Biopsy Products & Services
owlstonemedical.com/products

Breath Biopsy Tests
owlstonemedical.com/breath-biopsy-tests

5. References

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