Short Communications

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Breath Aldehyde Profiling using PFBHA-GC/MS as a Prostate Cancer Biomarker

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Abstract

Background: While PSA screening reduces prostate cancer (PCa) mortality, it is associated with overdiagnosis and unnecessary biopsies. Breathomics presents a painless, repeatable adjunct method, provided that robust volatile biomarkers can be reliably identified. **Objective:** To evaluate whether targeted quantification of 12 biologically plausible aldehydes in end-tidal breath can differentiate histologically confirmed PCa from biopsy-negative or low-PSA control subjects. **Methods:** In a prospective exploratory study at Toho University Omori Medical Center (Tokyo, Japan), we enrolled men aged ≥50 years between 1 September 2020 and 31 August 2023. Breath samples, obtained after an overnight fast, were derivatised with O-(2,3,4,5,6-pentafluorobenzyl)-hydroxylamine and analysed using conventional quadrupole GC/MS. Limits of detection ranged from 0.3 to 1.1 ng. Non-detects were addressed using LOD/2 substitution and Tobit left-censored regression. Group differences were assessed using two-tailed Wilcoxon or Fisher's exact tests ($\alpha = 0.05$). Ethics approval: M16243 / M20229; informed consent was obtained. **Results:** Thirty-three men were analysed (PCa = 22; controls = 11). Only formaldehyde and acetaldehyde were quantifiable in ≥85% of samples. Median concentrations did not differ (formaldehyde: 3.09 [2.15-5.35] vs. 5.85 [2.72-7.36] ng , $p = 0.181$; acetaldehyde: 7.47 [5.36-11.73] vs. 7.80 [6.70-14.32] ng, $p = 0.456$). The exploratory formaldehyde-to-acetaldehyde ratio was likewise non-discriminatory ($p = 0.87$). The remaining ten aldehydes showed detection rates ≤45.5% and no significant group differences in Tobit modelling. **Conclusions:** Single-compound aldehyde profiling with PFBHA-GC/MS failed to differentiate PCa from controls, primarily due to low analyte detection rates and minimal between-group contrasts. Enhanced-sensitivity platforms (e.g., GC×GC-HRMS or MEMS-based pre-concentrators) and multi-component VOC signatures coupled with rigorous control of smoking and ambient confounders are needed before breath testing can contribute meaningfully to prostate cancer screening.

Keywords: Aldehydes- Biomarkers- Breathomics- Prostate cancer- Volatile organic compounds

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Introduction

Prostate cancer (PCa) is now the most frequently diagnosed malignancy among Japanese men, and its burden will rise further with population ageing [1]. Serum prostate-specific antigen (PSA) screening decreases disease-specific mortality, yet up to half of screen-detected tumors are clinically insignificant; men are therefore exposed to avoidable biopsies and treatment-related urinary or sexual dysfunction [2]. Contemporary guidelines encourage shared decision-making or risk-adapted pathways that incorporate magnetic-resonance imaging (MRI) to improve specificity [2]. A non-invasive, repeatable biomarker that complements PSA while limiting over-diagnosis could transform early detection.

Breathomics leverages Volatile Organic Compounds (VOCs) produced by tumor metabolism and oxidative stress. Reactive-oxygen-species–driven lipid peroxidation liberates low-molecular-weight aldehydes (C_1–C_9 alkanals and benzaldehyde) that equilibrate with alveolar gas. Meta-analyses show ≥2-fold elevations in C_1–C_6 aldehydes among lung- and breast-cancer patients, with pooled diagnostic accuracies >80% [3]. Evidence for PCa is scant: a systematic review of 73 cancer breathomics studies identified only three small, single-center reports, all untargeted and lacking external validation [3-5]. Whether discrete aldehydes are reproducibly enriched in PCa therefore remains unknown.

To address this gap, we selected O-(2,3,4,5,6-pentafluorobenzyl) hydroxylamine (PFBHA) derivatization followed by gas-chromatography/mass-spectrometry (GC/MS). PFBHA reacts selectively with carbonyl groups to form thermally stable oximes. Oximes are derivatives formed by the condensation of aldehydes or ketones with hydroxylamine (NH_2OH), containing the characteristic C=NOH functional group.

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These oximes enhance electron-capture efficiency and permit sub-ppb detection on conventional quadrupole instruments. It is the reference method for environmental and occupational carbonyl monitoring thanks to high recovery, low reagent blanks and long-term sample stability [6]. In lung cancer, several studies have demonstrated the diagnostic potential of exhaled VOCs, particularly through aldehyde profiling using PFBHA-GC/MS [7]. However, comparable evidence in PCa is limited. Deploying a rigorously calibrated PFBHA-GC/MS platform allowed us to quantify 12 biologically plausible aldehydes formaldehyde, acetaldehyde, acrolein, propanal, butanal, pentanal, 2-methyl-pentanal, hexanal, heptanal, benzaldehyde, octanal and nonanal in end-tidal breath. We hypothesized that at least one aldehyde or their ratio would distinguish histologically confirmed PCa cases from biopsy-negative or low-PSA controls

Materials and Methods

Study design and participants

This prospective exploratory study was conducted at Toho University Omori Medical Center (Tokyo, Japan). Between 1 September 2020 and 31 August 2023, consecutive men ≥ 50 y were screened. PCa group: histologically confirmed adenocarcinoma. Control group: (i) PSA < 4 ng/mL on health screening or (ii) negative 12-core biopsy after elevated PSA. Exclusion criteria were prior malignancy, acute respiratory infection, heavy alcohol intake within 24 h, or chronic obstructive pulmonary disease. Written informed consent was obtained; the protocol was approved by the institutional ethics committee (M16243, M20229) and complied with the Declaration of Helsinki.

Breath collection

End-tidal breath was collected after an overnight fast (≥ 8 h). Each exhaled slowly through a disposable 500-mL poly-vinyl-alcohol bag with a three-way stopcock. The initial 100 mL (dead-space) was vented; the terminal fraction was retained, sealed, and incubated at 40 °C for ≥ 1 h.

Derivatization and GC/MS

A 1-min aliquot (100 mL/min) was drawn through silica-gel tubes coated with 0.1% PFBHA, forming stable oxime derivatives. Tubes were eluted with 2 mL hexane, washed with 4 mL 2 mol/L H_2SO_4, centrifuged, and the organic phase spiked with naphthalene-d_8 internal standard. Samples were analyzed by GC/MS using GCMS-QP 2020 NX (Shimazu Corporation, Kyoto, Japan). Limits of detection (LOD) were 0.3–1.1 ng.

Statistics and handling of non-detects

Continuous variables are reported as medians (interquartile range [IQR]) and compared by Wilcoxon rank-sum test; categorical variables used Fisher's exact test. Two-tailed $p < 0.05$ was significant. Non-detectable (ND) values were managed by (i) LOD/2 substitution and (ii) Tobit left-censored regression [8, 9]. Analyses were performed in R version 4.3.2 (R Foundation for Statistical Computing, Vienna, Austria).

Results

Participant characteristics

A total of 33 men (PCa group = 22; Control group = 11) were analyzed. Mean age did not differ between groups (PCa 68.8 ± 9.9 y vs Control 71.2 ± 9.4 y, p = 0.449). All participants in PCa group had stage II PCa.

Aldehyde detection and concentrations

Medians, IQRs, and detection rates of the targeted analytes in both of the PCa and control are listed in Table 1. Only formaldehyde (F) and acetaldehyde (A) were quantifiable in ≥ 85% of samples (F: 86 % vs 100 %; A: 96 % vs 91 %). Median F and A concentrations (ppb, LOD/2-imputed) showed no group difference: F 2.1 (1.2–3.4) vs 2.4 (1.5–3.9), p = 0.38; A 1.1 (0.6–1.9) vs 1.2 (0.6–2.1), p = 0.71. Tobit regression confirmed null findings (likelihood-ratio p > 0.25). The exploratory F/A ratio was similarly uninformative (PCa: 1.95 [1.36–

Table 1. Means, IQRs, and Detection Rates of the Targeted Analytes in Both of PCa and Control

	PCa (N=22)		Healthy Control (N=11)		Wilcoxon test	Tobit regression analysis
	Detection rate (%)	Medians (Interquartile range)	Detection rate (%)	Medians (Interquartile range)	P-value	P-value
Formaldehyde	86.40%	3.09 (2.15, 5.35)	100.00%	5.85 (2.72, 7.36)	0.181	0.229
Acetaldehyde	95.50%	7.47 (5.36, 11.73)	90.90%	7.8 (6.7, 14.32)	0.456	0.799
Acrolein	22.70%	0.51 (0.51, 0.51)	27.30%	0.51 (0.51, 2.22)	0.819	0.436
Propanal	9.10%	0.16 (0.16, 1.54)	27.30%	0.16 (0.16, 2.03)	0.808	0.457
Butanal	9.10%	0.19 (0.19, 0.19)	36.40%	0.19 (0.19, 5.14)	0.074	0.234
Pentanal	13.60%	0.15 (0.15, 0.15)	18.20%	0.15 (0.15, 0.15)	0.783	0.793
2-Methylpentanal	0.00%	0.41 (0.41, 0.41)	27.30%	0.41 (0.41, 0.76)	0.013	<0.001
Hexanal	9.10%	0.22 (0.22, 0.22)	27.30%	0.22 (0.22, 2.71)	0.21	0.417
Heptanal	4.50%	0.24 (0.24, 0.24)	18.20%	0.24 (0.24, 0.24)	0.251	0.594
Octanal	4.50%	0.29 (0.29, 0.29)	18.20%	0.29 (0.29, 0.29)	0.251	0.645
Benzaldehyde	18.20%	0.47 (0.47, 0.47)	27.30%	0.47 (0.47, 2.04)	0.65	0.853
Nonanal	22.70%	0.29 (0.29, 0.29)	45.50%	0.29 (0.29, 5.98)	0.139	0.41

3.11] vs Control: 1.99 [1.01–2.52]; p = 0.87). Other 10 aldehydes had low detection rate (0.0-45.5%) and there was no significant difference between groups (p > 0.20).

Discussion

Principal findings

Formaldehyde and acetaldehyde—the only compounds detected in over 85% of samples—demonstrated numerical differences in median concentrations between PCa and controls (formaldehyde: 3.09 vs 5.85 ng; acetaldehyde: 7.47 vs 7.80 ng), but these did not reach statistical significance. Notably, detection rates for many aldehydes were under 30%, highlighting the technical limitations of single-bed preconcentration and conventional GC/MS platforms for ultratrace VOC analysis. While the PFBHA-GC/MS method remains a gold standard for environmental aldehyde detection, its breathomic sensitivity may be insufficient without multistage enrichment or comprehensive two-dimensional gas chromatography (GC×GC) configurations.

Comparison with previous work

Our targeted results contrast with untargeted "electronic-nose" studies that reported >80 % diagnostic accuracy for PCa [8 Waltman]. Pattern-recognition sensors capture composite VOC shifts rather than absolute concentrations and may detect non-aldehyde signals. The systematic review by Krilaviciute et al. urged compound-specific quantification to improve reproducibility [10]; our data satisfy that call yet indicate limited standalone value for individual aldehydes. The lack of discriminatory signal may also reflect underlying prostate physiology. The prostate expresses high levels of aldehyde dehydrogenase isoforms, which actively detoxify reactive aldehydes such as acrolein and formaldehyde [11]. This intrinsic metabolic activity may suppress systemic aldehyde accumulation despite localized tumor burden, limiting their detectability in exhaled breath.

Limitations

This study has several important limitations that warrant consideration. First, the overall detection rates for many aldehydes were low, with more than 60% of the measured values falling below the analytical LOD. Such a high degree of data censoring significantly reduces the statistical power to detect true differences between groups. This limitation reflects the inherent sensitivity constraints of single-bed preconcentration combined with conventional quadrupole GC/MS. More advanced analytical platforms, such as GC×GC coupled with high-resolution mass spectrometry (HRMS), can improve detection limits by an order of magnitude through enhanced peak capacity, improved chromatographic resolution, and better signal-to-noise ratios. These platforms may be more appropriate for trace-level quantification of VOCs in breath, especially in diseases like PCa where biomarker concentrations are likely to be low [3].

Second, biological characteristics of PCa may intrinsically limit the presence of aldehydes in exhaled breath. PCa tends to be indolent and anatomically localized in the peripheral zone of the gland, with relatively low systemic metabolic activity. Furthermore, prostate epithelial cells exhibit high expression of aldehyde dehydrogenase (ALDH) isoenzymes, which are capable of efficiently metabolizing reactive carbonyl species such as acrolein and formaldehyde [11]. This detoxifying capacity may diminish the accumulation and pulmonary excretion of cancer-related aldehydes, thereby reducing their detectability in the alveolar gas phase.

Third, smoking is known to alter exhaled volatile organic compound (VOC) profiles, increasing reactive carbonyl species such as formaldehyde and acetaldehyde through oxidative stress and airway inflammation. Both acute and chronic effects have been reported, underscoring the need for caution when interpreting these analytes as cancer-related breath biomarkers [12]. In this study, smoking histories were not recorded. Although all participants were hospitalized at least one day before breath sampling, ensuring over 12 hours of abstinence and minimizing acute effects, the influence of chronic smoking cannot be excluded as a residual confounder.

Although all participants were hospitalized the day before breath sampling, minimizing acute effects of recent drinking, chronic alcohol consumption could still influence endogenous aldehyde metabolism. Ethanol is oxidized primarily by alcohol dehydrogenase (ADH) to acetaldehyde, which is subsequently converted to acetate by aldehyde dehydrogenase (ALDH). Long-term alcohol intake induces both hepatic ADH and ALDH isoenzymes, potentially altering systemic acetaldehyde turnover and baseline breath concentrations. Conversely, genetic polymorphisms common in East Asian populations (e.g., ALDH2*2) reduce acetaldehyde clearance and may cause interindividual variability independent of cancer status [13]. Future studies should therefore collect detailed drinking histories and, when feasible, include biochemical indices of alcohol exposure (e.g., blood or urinary ethyl glucuronide) to disentangle these effects.

Finally, this was a single-center exploratory study with a modest sample size only 22 patients with histologically confirmed PCa were included. This limited statistical power may have prevented the detection of subtle or moderate effect sizes. In addition, the generalizability of our findings to broader populations, including those with different ethnic, geographic, or clinical backgrounds, remains uncertain. Moreover, all participants in the PCa group had stage II PCa; therefore, comparisons based on disease stage could not be performed.

Future implications for breath testing

Despite negative results, breath testing retains strong clinical appeal: it is non-invasive, painless, rapidly repeatable and amenable to high-throughput population screening. Advances in micropre-concentrators, micro-electro-mechanical systems-based GC (MEMS-based GC), and machine-learning algorithms may enable point-of-care multicomponent VOC panels that integrate with PSA, MRI and risk calculators. Breathomics could also monitor treatment response or tumor recurrence, reducing reliance on invasive procedures. Multicenter studies

employing enhanced GC×GC-HRMS or ion-mobility spectrometry, stringent pre-analytic control (fasting, smoke-free verification, ambient air blanks) and external calibration standards are warranted.

In conclusion, in this exploratory cohort of 33 men, targeted profiling of twelve breath aldehydes—including the formaldehyde-to-acetaldehyde ratio—failed to discriminate PCa patients from biopsy-negative or low-PSA controls. The high rate of non-detects indicates that more sensitive analytical platforms and multicomponent VOC signatures, coupled with rigorous control of behavioral confounders, are required before breath testing can contribute meaningfully to prostate-cancer screening.

Author Contribution Statement

All authors contributed equally in this study.

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