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# Evaluation of a multimodal ctDNA-based assay for detection of aggressive cancers lacking standard screening tests

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## Addressing the Unmet Need for Cancer Screening in Absence of Standard Methods

**70%** of cancer deaths result from late-stage diagnoses in cancers that lack standard-of-care screening (LSS) [1].

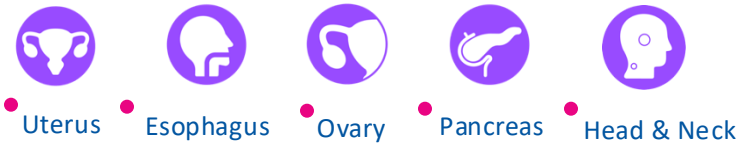
**Limitations** in current screening for LSS cancers arise from their, necessitating numerous tests to detect a single case, which increases the likelihood of erroneous positive results. Techniques such as imaging diagnostics and protein markers often exhibit insufficient sensitivity and specificity, resulting in late-stage diagnoses and poor outcomes. For instance, CT scans for pancreatic cancer and CA125 tests for ovarian cancer demonstrate limited early detection capabilities and high rates of unnecessary recalls, leading to patient distress or late diagnosis [2]. These factors significantly reduce the effectiveness of screening and early detection programs based on single-cancer-type methods for LSS cancers on a broad scale.

## Complementing new LSS cancers into Multi-Cancer Early Detection Test

**05 Common Cancers**




**05 Lack-standard-of-care (LSS) cancers**




We previously developed a multimodal ctDNA assay, Screening for the Presence of Tumor by Methylation And Size (SPOT-MAS), designed to profile methylation and fragmentomic signatures for multi-cancer early detection. SPOT-MAS has demonstrated its capability to capture ctDNA signatures from 05 common cancer types—breast, lung, liver, colorectal, and gastric cancer—and successfully facilitated early-stage detection in both retrospective [4] and prospective validation [5] studies.

This study extends the analysis to the 05 lethal LSS cancers: endometrial, esophageal, head and neck, ovarian and pancreatic.

We identified numerous shared ctDNA signals across five common cancers and LSS cancers, demonstrating that SPOT-MAS could serve as a non-invasive method for detecting low-prevalence cancers with 96.2% specificity and 74.8% overall sensitivity, including a sensitivity of 60.7% for early-stage detection.

## Clinical characteristics of cancer & healthy participants

In this study, 739 healthy individuals and 135 patients with one of five LSS cancer types (endometrial, esophageal, head and neck, ovarian, and pancreatic) were recruited from 15 hospitals between May 2023 and July 2024. Healthy participants were monitored for 12 months to confirm their cancer-free status, while cancer diagnoses were confirmed histologically for all patients. The cancer group (median age 60) was significantly older than the control group (median age 47), with similar gender distribution overall, though ovarian and endometrial cancers occurred exclusively in females, and esophageal and head and neck cancers were mostly in males. Most cancer patients (79.3%) were diagnosed at advanced stages (III and IV).

## Distinct methylation & fragmentomic alterations in cell-free DNA of LSS cancers

Methylation: In this study, the SPOT-MAS multimodal analysis was used to examine unique ctDNA signatures in plasma from patients with five LSS cancers. This approach integrated deep target and shallow genome-wide sequencing, identifying 347 differentially methylated regions (DMRs) across LSS cancers. Notably, genome-wide hypomethylation was observed in all cancer groups compared to healthy controls, indicating widespread epigenetic alterations.

Fragment length: Fragment length patterns of cfDNA varied by cancer type, with esophageal and head and neck cancers showing more short fragments (<150 bp), while endometrial, ovarian, and pancreatic cancers had fewer short fragments compared to healthy controls.

End motifs: Additionally, specific 4-mer end motifs were identified as significant for distinguishing ctDNA from different cancer types, with 11 shared motifs across all five cancers.

These methylation, fragment length, and motif end changes in cfDNA could serve as potential biomarkers for detecting LSS cancers that currently lack standard screening options.

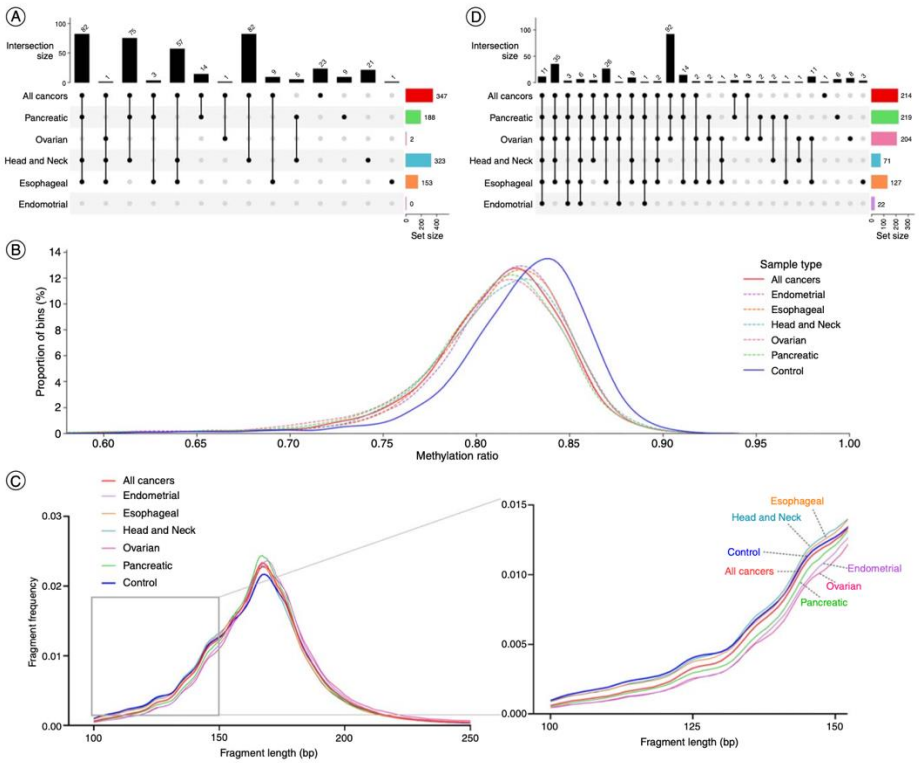
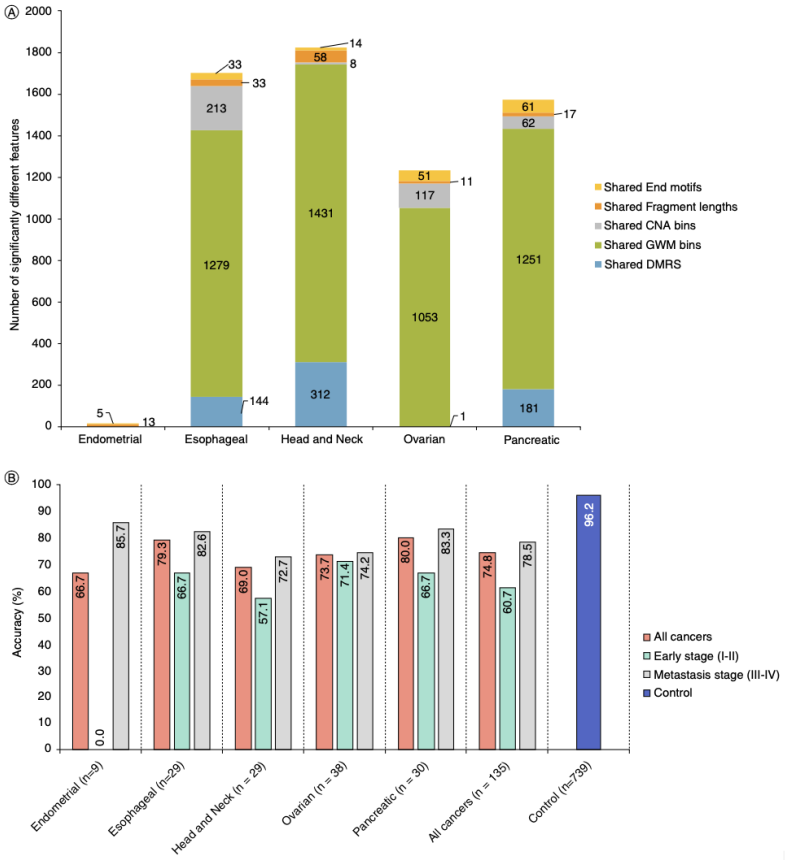



Figure 1. Methylation and fragmentomic profiles of cancers lacking standard screening (LSS) tests. (A) The number of differentially methylated regions (DMRs) profiled in each cancer type and their overlapping. (B) LSS cancers exhibited a consistent pattern of genome-wide hypomethylation. (C) Cell-free DNA fragment length distribution in each LSS cancer type compared with healthy samples. (D) The number of end motifs (EMs) significantly different between LSS cancers and control samples, along with their overlap.

# Classification of LSS cancers using a multimodal ctDNA machine learning model




The study investigated whether ctDNA signatures from LSS cancers overlap with those from the five most common cancers (breast, colorectal, lung, liver, and prostate). Significant shared methylation patterns were found in esophageal, head and neck, and pancreatic cancers, with over 1,000 overlapping methylation bins identified across four LSS cancers (excluding endometrial cancer). Additionally, shared fragment length changes and end motifs were observed across all five LSS cancers. Chromosomal copy number alterations (CNA) common to LSS and common cancers were detected, with notable differences in endometrial cancer.

Figure 2. Overlapping significant ctDNA signatures between the five LSS cancers and the top five common cancers, and detection accuracy by a multimodal machine learning algorithm. (A) Number of features overlapping with the top five common cancer types. (B) Accuracy of the multimodal machine learning algorithm in distinguishing between these LSS cancers and healthy individuals.

**96.2%**

Specificity

were achieved by a machine learning model trained on shared signatures from five common cancers and applied to LSS cancers

**74.8%**

Overall Sensitivity

Sensitivities ranged from 66.7% to 80.0% across the five LSS cancers, with higher sensitivity in metastatic stages (78.5%) compared to early-stage cancers (60.7%). These findings suggest that shared ctDNA signatures could serve as universal biomarkers for detecting multiple cancer types.

The study revealed shared methylation and fragmentomic signatures in plasma samples from patients with cancers lacking standard-of-care screening. This proof-of-concept study demonstrates that SPOT-MAS, a non-invasive test trained on five common cancer types, could detect a number of LSS cancer cases, potentially complementing existing screening programs.


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