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Performance of a cfDNA methylation–based MCED test in patients with clinicoradiologic suspicion of cancer

Harbinger Health

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INTRODUCTION

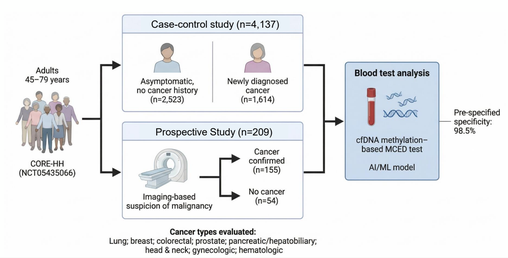
- Radiographic findings resulting in a concern for malignancy frequently initiate prolonged evaluations that may remain nondiagnostic, delay definitive management, and expose patients to uncertainty and potentially avoidable invasive procedures and resource utilization.
- Multi-cancer Early detection (MCED) assays based on circulating cell-free DNA (cfDNA) methylation profiling have demonstrated high specificity in asymptomatic screening populations; however, their performance characteristics in patients undergoing diagnostic evaluation for imaging-based suspicion of malignancy is not fully understood.
- Because pretest probability, disease spectrum, and competing benign etiologies differ substantially between screening and diagnostic settings, test performance observed in asymptomatic cohorts may not generalize to patients with radiographic findings concerning for malignancy.

OBJECTIVES

- To compare the performance of a cfDNA methylation–based multi-cancer detection test between asymptomatic case-control participants and patients undergoing diagnostic evaluation for imaging-based suspicion of malignancy.
- To inform clinical positioning of cfDNA methylation–based multi-cancer detection beyond asymptomatic population screening.

METHODS

- In CORE-HH (NCT05435066), adults aged 45–79 years were evaluated in two distinct study designs: a case-control study (n = 4,137) comprised of asymptomatic participants without self-reported prior cancer (n = 2,523) and participants with newly diagnosed cancer (n = 1,614), and a prospective study (n = 209) of individuals undergoing diagnostic evaluation for imaging findings concerning for malignancy, whereby 155 participants were identified with pathology-confirmed malignancy and 54 had no malignancy identified.
- A standardized pre-pathology clinical suspicion phenotype was assigned to each subject using presentation-level clinical diagnosis, medical history, specimen/procedure data, and indication text through a prespecified rule-based hierarchy informed by NCIt and ICD-10-CM terminology, independent of the test result.[1]
- Samples were analyzed using a cfDNA methylation-based MCED assay using an AI/ML classification model using an AI/ML binary classification model with prespecified thresholds selected to target a specificity of 98.5% in the case-control study.[2]
- Performance was compared between cohorts in non-cancer participants and in early-stage (I–II) and late-stage (III–IV) cancer cases using two-sided Fisher's exact tests.



RESULTS

Characteristic	Case-Control Study			Prospective Study		
	All (N=4,137)	No Cancer (N=2,523)	Cancer (N=1,614)	All (N=209)	Cancer (N=155)	No Ca (N=54)
SEX						
Female	2,225 (53.8%)	1,416 (56.1%)	809 (50.1%)	101 (48.3%)	67 (43.2%)	34 (63.0%)
Male	1,912 (46.2%)	1,107 (43.9%)	805 (49.9%)	108 (51.7%)	88 (56.8%)	20 (37.0%)
AGE						
Mean (SD)	63.5 (8.2) 45–79	62.3 (8.4) 45–79	65.4 (7.5) 45–79	65.8 (7.8) 45–79	66.3 (7.6) 46–79	64.3 (8.4) 45–78
RACE						
White	2,988 (72.2%)	1,705 (67.6%)	1,283 (79.5%)	163 (78.0%)	129 (83.2%)	34 (63.0%)
Black/African American	554 (13.4%)	423 (16.8%)	131 (8.1%)	16 (7.7%)	9 (5.8%)	7 (13.0%)
Asian	55 (1.3%)	37 (1.5%)	18 (1.1%)	3 (1.4%)	2 (1.3%)	1 (1.9%)
Other/Multiple†	105 (2.5%)	46 (1.8%)	59 (3.7%)	8 (3.8%)	4 (2.6%)	4 (7.4%)
Unknown/Not reported	*435 (10.5%)*	*312 (12.4%)*	*123 (7.6%)*	*19 (9.1%)*	*11 (7.1%)*	*8 (14.8%)*
ETHNICITY						
Not Hispanic or Latino	3,396 (82.1%)	2,063 (81.8%)	1,333 (82.6%)	165 (78.9%)	129 (83.2%)	36 (66.7%)
Hispanic or Latino	288 (7.0%)	155 (6.1%)	133 (8.2%)	23 (11.0%)	17 (11.0%)	6 (11.1%)
Unknown/Not reported	*453 (10.9%)*	*305 (12.1%)*	*148 (9.2%)*	*21 (10.0%)*	*9 (5.8%)*	*12 (22.2%)*

Table 1. Patient demographics and baseline characteristics. *†Other/Multiple includes American Indian/Alaska Native, Native Hawaiian/Pacific Islander, two or more races, and other. No Ca, no cancer.*

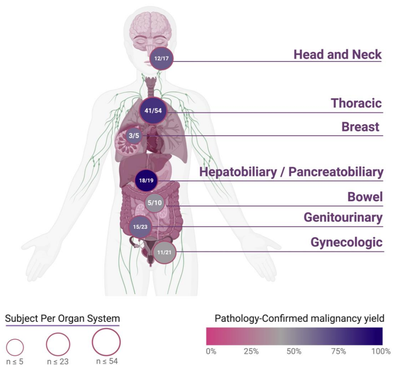


Figure 3. Anatomic schematic of pathology-confirmed malignancy yield by organ system in the prospective study.

Performance Metric	Prospective Clinical Suspicion group	Case-Control group	P
Specificity (non-cancer)	**53/54; 98.1%** (95% CI 90.1–100.0)	**2485/2523; 98.5%** (95% CI 97.9–98.9)	0.56
Overall sensitivity	**85/155; 54.8%** (95% CI 46.7–62.8)	**870/1614; 53.9%** (95% CI 51.4–56.4)	0.87
Stage I–II sensitivity	**18/65; 27.7%** (95% CI 17.3–40.2)	**204/711; 28.7%** (95% CI 25.4–32.2)	1.00
Stage III–IV sensitivity	**58/70; 82.9%** (95% CI 72.0–90.8)	**573/703; 81.5%** (95% CI 78.4–84.3)	0.87

Table 2. Comparison of test performance between the clinical suspicion and case-control cohorts. *Abbreviations: CI, confidence interval; P, Fisher's exact test p-value.*

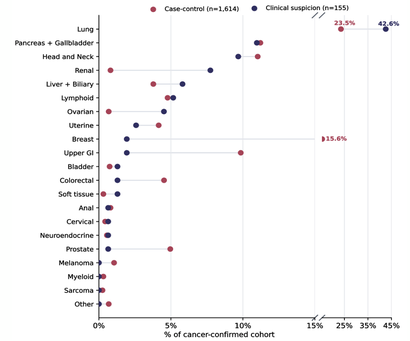


Figure 1. Cancer type distribution between case-control and prospective clinical-suspicion studies.

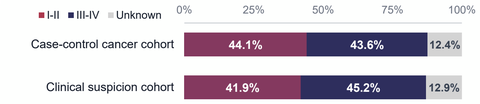


Figure 2. Cancer stage distribution across case-control and prospective clinical-suspicion cohorts.

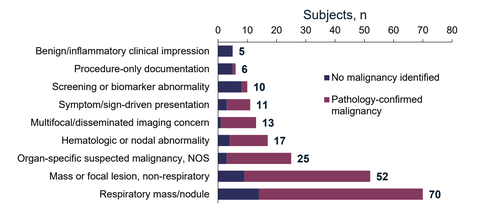


Figure 4. Distribution of prospectively enrolled participants by clinical presentation category, stratified by pathology outcome.

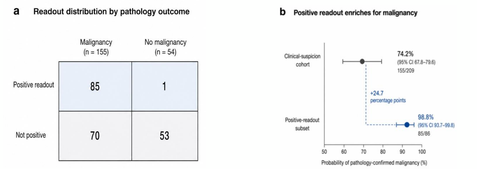


Figure 5. Panel a. Readout distribution by pathology-confirmed outcome. Positive readouts occurred in 85/155 malignant subjects, with a positive likelihood ratio of 29.6 and 1/54 false positives among non-malignant subjects. **Panel b.** Probability of pathology-confirmed malignancy by readout status. Malignancy probability increased from 74.2% in the clinical-suspicion cohort to 98.8% in the positive-readout subset, a +24.7 percentage-point difference. Points show observed proportions; whiskers show 95% confidence intervals. The positive-readout threshold was selected at 98.5% specificity.

DISCUSSION

- The principal methodologic finding was concordance of specificity, overall sensitivity, and stage-stratified sensitivity between the case-control and suspicion studies. Although retrospective case-control designs can overestimate performance because of spectrum bias and differences in stage distribution compared to the intended use population, the prospectively enrolled clinical-suspicion group showed similar performance estimates to the case-control benchmark.
- A specificity of 98.1% in a population with a 74.2% pretest probability of malignancy corresponds to a positive likelihood ratio of 29.6 and a post-test probability of 98.8% following a positive readout (+24.7 percentage-point increment). This profile supports a rule-in role complementary to—rather than substitutive of—standard diagnostic workup. Conversely, stage I–II sensitivity of 27.7% reaffirms that a negative readout has limited capacity to exclude early-stage disease and should not defer definitive evaluation when clinical suspicion is high.
- These findings support a potential adjunctive role for cfDNA methylation–based multi-cancer detection in the diagnostic evaluation of patients with concerning imaging findings. Its value lies in shifting posterior probability across action-relevant thresholds for indeterminate findings—subsolid pulmonary nodules, indeterminate hepatic or adrenal lesions, lymphadenopathy of uncertain etiology—where conventional workup is often protracted, invasive, or non-diagnostic. The performance observed here supports integration as a complement to standard imaging and tissue-based evaluation, with potential to accelerate diagnostic resolution between initial radiographic concern and definitive diagnosis.

CONCLUSIONS

- At the prespecified 98.5% specificity threshold, the cfDNA methylation-based assay showed comparable specificity and sensitivity in prospectively enrolled participants undergoing workup for imaging-based suspicion of malignancy and in a case-control study of newly diagnosed cancer cases versus asymptomatic controls. Because case-control designs may give rise to inflated test performance compared to the intended use population, these results are notable in demonstrating consistent test performance in a diagnostically enriched, clinically relevant population, thereby extending characterization of MCED testing beyond asymptomatic screening use cases. These findings support further characterization of cfDNA methylation–based multi-cancer detection in diagnostically enriched, clinically relevant populations beyond asymptomatic screening.

REFERENCES

1. Sioutos N, de Coronado S, Haber MW, Hartel FW, Shaiu WL, Wright LW. NCI Thesaurus: a semantic model integrating cancer-related clinical and molecular information. J Biomed Inform. 2007;40(1):30-43. doi:10.1016/j.jbi.2006.02.013
2. A. Gregory DiRienzo, Elie Massaad, Hutan Ashrafian. Classification-specific predictive performance: A unified estimation and inference framework for multi-category tests, Statistics in Medicine, 2026; 45:e70431

DISCLOSURES

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