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Prospective validation study: a non-invasive circulating tumor DNA-based assay for simultaneous early detection of multiple cancers in asymptomatic adults

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Abstract

Background Non-invasive multi-cancer early detection (MCED) tests have shown promise in enhancing early cancer detection. However, their clinical utility across diverse populations remains underexplored, limiting their routine implementation. This study aims to validate the clinical utility of a multimodal non-invasive circulating tumor DNA (ctDNA)-based MCED test, SPOT-MAS (Screening for the Presence Of Tumor by DNA Methylation And Size).

Methods We conducted a multicenter prospective study, K-DETEK (ClinicalTrials.gov identifier: NCT05227261), involving 9057 asymptomatic individuals aged 40 years or older across 75 major hospitals and one research institute in Vietnam. Participants were followed for 12 months.

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Results Of the 9024 eligible participants, 43 (0.48%) tested positive for ctDNA. Among these, 17 were confirmed with malignant lesions in various primary organs through standard-of-care (SOC) imaging and biopsy, with 9 cases matching our tissue of origin (TOO) predictions. This resulted in a positive predictive value of 39.53% (95%CI 26.37–54.42) and a TOO accuracy of 52.94% (95%CI 30.96–73.83). Among the 8981 participants (99.52%) who tested negative, 8974 were confirmed cancer-free during a 12-month period after testing, yielding a negative predictive value of 99.92% (95% CI 99.84–99.96). The test demonstrated an overall sensitivity of 70.83% (95%CI 50.83–85.09) and a specificity of 99.71% (95% CI 99.58–99.80) for detecting various cancer types, including those without SOC screening options.

Conclusions This study presents a prospective validation of a multi-cancer early detection (MCED) test conducted in a lower middle-income country, demonstrating the potential of SPOT-MAS for early cancer detection. Our findings indicate that MCED tests could be valuable additions to national cancer screening programs, particularly in regions where such initiatives are currently limited.

Trial registration ClinicalTrials.gov ID: NCT05227261. Date of registration: 07/02/2022.

Keywords Liquid biopsy, Multimodal analysis, Multicancer early detection, Tissue of origin, Clinical validation

Background

Cancer is the second leading cause of death globally, significantly increasing mortality rates and placing immense pressure on healthcare systems worldwide [1]. This burden is further exacerbated by the commonly late detection of the disease [2]. In an effort to combat cancer, screening methods such as those recommended by the United States Preventive Services Task Force (USPSTF) have shown promise in increasing overall survival rates, improving treatment efficiency, and reducing long-term medical costs [3–5]. However, certain screening methods, particularly colonoscopy or cervical cytology tests, are invasive and have low accessibility [6, 7]. Moreover, they can only detect a single cancer type, leading to a high cumulative false positive rate when performed sequentially [8].

One of the most promising advancements in cancer detection in recent years is the development of non-invasive multi-cancer early detection (MCED) tests [9]. MCED tests, using blood-based liquid biopsy (LB) approaches, have the potential to revolutionize cancer screening by enabling early detection of multiple types of cancer through a single blood draw [10]. LB assays can detect specific cancer-related biomolecules including circulating tumor cells (CTC), cell-free DNA (cfDNA), circulating tumor DNA (ctDNA), circulating cell-free RNA (cfRNA), and exosomes [11]. Of these, ctDNA released into the circulation when tumor cells undergo apoptotic and necrotic cell death processes has been extensively studied due to its tissue- and cancer-type specificity [12–14]. The OverC test (Burning Rock) or Galleri test (Grail) can detect multiple cancer types simultaneously with high performance by analyzing methylation changes in cfDNA [15, 16]. The Galleri test has undergone clinical validation in both asymptomatic cohort (PATHFINDER study) and symptomatic cohort (SYMPLIFY study). In asymptomatic individuals, the Galleri test demonstrated a PPV of 38% for cancer detection and achieved a tissue of origin (TOO) prediction accuracy of 97% [15].

Despite promising results, MCED methods face significant challenges in detecting certain cancers, such as breast cancer, and early-stage tumors. These limitations arise from the low quantity and high heterogeneity of ctDNA [10, 17, 18]. While high-depth sequencing can enhance ctDNA detection sensitivity, its economic infeasibility for large-scale screening necessitates alternative approaches [10, 19]. To address these constraints, we developed a multimodal method known as Screening for the Presence Of Tumor by DNA Methylation And Size (SPOT-MAS). The SPOT-MAS test utilizes a multimodal approach, combining both targeted and genome-wide bisulfite sequencing to enable the simultaneous analysis of multiple circulating tumor DNA (ctDNA) signatures. These include methylation patterns, fragment length variations, DNA copy number aberrations, and end motifs. By leveraging advanced machine learning algorithms and an integrated, all-in-one protocol, this approach not only enhances the sensitivity of ctDNA detection but also optimizes cost efficiency. In addition to detecting ctDNA, SPOT-MAS identifies the tissue of origin (TOO), providing greater precision to guide subsequent diagnostic steps and treatment decisions. The SPOT-MAS test was previously validated in a case–control retrospective study involving 738 cancer patients with common cancers such as breast, liver, colorectal, lung, and gastric cancers, as well as 1550 cancer-free individuals [20]. The method achieved a sensitivity of 72.4 at 97.0% specificity across five common cancer types (liver, breast, colorectal, gastric, and lung), with 70.0% accuracy in TOO prediction [17, 20, 21]. Subsequently, we evaluated the clinical performance of SPOT-MAS in a prospective cohort of 2795 participants within the K-DETEK study [22]. However,

our interim report on this evaluation, with incomplete 12-month follow-ups, hindered our ability to achieve diagnostic resolution for all participants, particularly those with positive test results, and to accurately determine the performance of our test. Hence, in this study, we present a thorough evaluation of SPOT-MAS performance on a large prospective cohort of 9057 asymptomatic participants across 75 major hospitals and one research institute in Vietnam (Fig. 1).

Methods

Study design

The study recruited 9057 participants having follow-up visits for chronic conditions or undergoing annual health check-ups across 75 major hospitals and one research institute in Vietnam from April 2022 to April 2023. The study was registered with the U.S. National Institutes of Health (ClinicalTrials.gov identifier: NCT05227261). The institutional ethics and scientific committee of the University of Medicine and Pharmacy, Ho Chi Minh City, Vietnam, reviewed and approved this study (approval number: 192/HĐĐĐ-ĐHYD). All participants provided written informed consent prior to participating in this study. Participants enrolled in the K-DETEK study were required to meet the age criterion of ≥ 40 years. Those who met this criterion and were not in the high-risk group were classified as moderate risk, as being over 40 is recognized as a significant risk factor for cancer [23]. Participants were eligible for K-DETEK study if they were aged 40 years or older, willing to return for required follow-up visits at 6 months and 12 months, had neither clinical suspicion of cancer nor history of confirmed cancer, had no history of blood transfusion or bone marrow transplantation in 3 months prior to recruitment time and had no clinical manifestations of pregnancy. The exclusion of participants with a prior cancer diagnosis is a fundamental aspect of our study's design, ensuring that the evaluation of the SPOT-MAS test is conducted within a population representative of either healthy individuals, managing chronic conditions, or participating in general healthy check-ups health screenings without a known history of cancer. Pregnant individuals were excluded for two reasons. First, cell-free fetal DNA (cffDNA) released from the placenta shares certain characteristics with circulating tumor DNA, which could confound cancer prediction [24]. Second, any participant with a positive result would need to undergo cancer diagnostic tests, some of which—such as mammography and CT scans—involve X-ray exposure and could be harmful to the developing fetus. All the clinical characteristics of participants are de-identified and listed in Additional file 1: Table S1.

Laboratory workflow

Blood samples of 10 ml were collected in Streck cfDNA tubes and transported to the central laboratory for plasma cfDNA extraction. The median time from blood collection to plasma isolation was 2 days, ranging from 0 to 5 days. Subsequently, the isolated cfDNA underwent the SPOT-MAS assay to detect 5 common cancers (breast, liver, colorectal, lung, and gastric cancers), following previously described protocols [20]. The multiple features of cfDNA, including methylation profiles of 450 target regions, genome-wide methylation profiles (global methylation density of 2734 1-Mb bins on 22 chromosomes), fragment length, DNA copy number of 588 5-Mb bins on 22 chromosomes and end motifs, were simultaneously analyzed by SPOT-MAS workflow [20]. Using machine learning algorithms, the model returned the probability scores of ctDNA signal detection (SPOT-MAS score) and TOO for those with ctDNA signal detected (Fig. 1). Our TOO prediction model consists of five binary classifiers, each identifying one specific

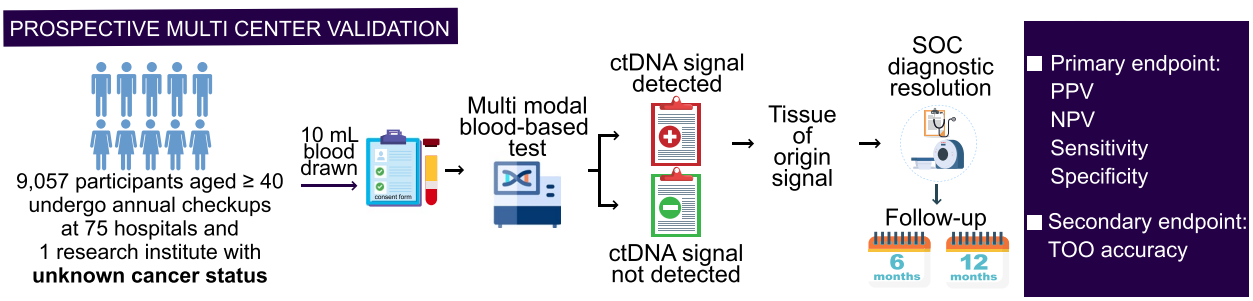

Fig. 1 K-DETEK study design. K-DETEK is a prospective study involving the recruitment of 9057 asymptomatic participants aged 40 or older from 75 hospitals and one research institute in Vietnam. Plasma cell-free DNA (cfDNA) was extracted from 10 ml of blood collected from eligible participants and analyzed using the SPOT-MAS assay. SPOT-MAS assay provides two possible results: "ctDNA signal detected" or "ctDNA signal not detected," along with the predicted TOO. Participants with a "ctDNA signal detected" result were consulted by physicians and underwent further confirmation through diagnostic imaging tests based on the TOO probability values. All participants were followed up at 6 and 12 months to collect information on possible cancer diagnoses

cancer type among all unclassified. For each model, a cutoff value was determined to maximize accurate detection of the corresponding cancer type. When predicting new samples, each is assessed by all five models, with the predicted probabilities compared to the respective cutoffs. If a model's score exceeds its cutoff, the sample is classified as the corresponding cancer type. If none of the scores meet their cutoffs, the sample is assigned to the "Unclassified" category, indicating that it possesses methylation and fragmentomic signatures that do not fully match any of the predefined cancer types.

Informing participants of test results

The SPOT-MAS test results were returned to the study participants within a 30-day period following blood collection. Time to diagnostic resolution for each participant was calculated as the duration in days between the availability of test results to the ordering physicians and the date of diagnostic resolution, as determined by the ordering physicians. SPOT-MAS provides two types of test results: "ctDNA signal not detected" (negative) or "ctDNA signal detected" (positive), with up to two prediction results for TOO.

Participants with a positive ctDNA signal and tissue of origin (TOO) prediction were recommended to undergo "on-site" diagnostic imaging tests, in accordance with the National Comprehensive Cancer Network guidelines, to confirm the presence of a tumor. The diagnostic procedures for specific cancer types included breast ultrasound and mammography for breast cancer, colonoscopy for colorectal cancer, gastroscopy for gastric cancer, chest CT with contrast for lung cancer, and three-phase abdominal CT for liver cancer. For participants who had a mammogram within the past 3 months, a breast MRI was recommended. Those with a TOO prediction of "unclassified cancers" were advised to undergo a whole-body CT scan at the physician's discretion. If imaging tests identified suspected malignant lesions, a diagnostic biopsy was performed to assess histopathological characteristics, with the final cancer diagnosis confirmed through histopathological results. Participants with a negative result were informed about their low risk for the five cancer types covered by SPOT-MAS test. All participants were followed up after 6 months and 12 months to obtain information on possible cancer diagnosis. The machine learning algorithms were trained on data from five common cancer types at early stages. Thus, there is a possibility that SPOT-MAS may not detect asymptomatic metastatic cancer or other cancer types that possess distinct molecular characteristics that are not yet integrated into the SPOT-MAS detection model. Therefore, patients may still be at risk for other cancers, necessitating ongoing vigilance.

The study provided structured post-test counseling sessions for all individuals with positive results to minimize unnecessary psychological harm. Specifically, those who received a positive SPOT-MAS result were scheduled for consultations with oncology and genetics experts. These sessions aimed to offer comprehensive explanations and interpretations of the test results, along with a clear follow-up and diagnostic plan. Patients with a positive SPOT-MAS result were subsequently referred for further diagnostic imaging or biopsy, depending on the predicted TOO [25]. Invasive procedures were only recommended when imaging or clinical findings were suspicious, in accordance with established guidelines for diagnostic workups in screening programs and with consideration for local medical practices and patient-centered communication.

In cases where the histopathological results did not confirm cancer or the imaging tests failed to detect suspicious lesions, the counseling sessions were focused on educating participants about the nature of false positive results and the importance of follow-up diagnostic testing. Previous research indicated that providing patients with comprehensive education can help alleviate the anxiety associated with screening outcomes [25].

For all participants with negative results, we conducted follow-ups by phone at the 6th and 12th months using a survey follow-up form. Additionally, we gathered clinical information for participants enrolled at hospitals where electronic health records are available. The cancer surveillance process is considered complete only when there is either an update in the electronic health record indicating a cancer diagnosis, or no cancer-related findings are recorded in both the electronic health record and the phone interview at the 12th month post-blood collection. The survey questionnaire used in these follow-ups is provided in Additional file 2: Appendix 1. Participants were considered cancer-free if no cancer was diagnosed during the follow-up period. Participants who exhibit symptoms suggestive of cancer were recommended for further screening and comprehensive diagnosis.

The performance of the SPOT-MAS assay was determined, including true positive rate, false positive rate, positive predictive value (PPV), negative predictive value (NPV), sensitivity and specificity for cancer signal detection (%). The overall prediction accuracy (%) of TOO was also assessed. Regarding the classification criteria, test-positive cases with tissue biopsy results confirming malignant lesions were considered true positives. Test-positive cases with precancerous or benign lesions, or no lesions detected, were considered false positives. For test-negative cases, follow-up was conducted at 6 and 12 months using self-reporting and questionnaires, where false negatives were defined as cases diagnosed

with cancer within this 12-month follow-up period. Cases with no cancer detected after 12 months of follow-up were classified as true negatives.

Participant demographic and statistical analysis

The demographic information of all participants is listed in Additional file 1: Table S1. Participants in the high-risk group were identified based on factors such as heavy smoking, alcohol consumption, hepatitis B/C infection, type 2 diabetes, having first-degree relatives (FDR) diagnosed with two types of cancer at an age younger than 45, and carrying inherited cancer mutations. The remaining participants were classified into the moderate-risk group.

All statistical analyses were performed by using R (4.3.2) with standard data analysis packages and the ggplot2 package for visualization. Confidence intervals were estimated by the Wilson method using R (4.3.2) [26].

Results

Clinical characteristics of study participants

Of the 9057 eligible participants enrolled in K-DETEK, 9024 (99.64%) completed the 12-month follow-up and were included in the study analysis, while 33 participants (0.36%) were excluded (Fig. 2). The reasons for exclusion included a cancer diagnosis ($n=1$), pregnancy ($n=1$), high levels of blood hemolysis ($n=31$). The clinical characteristics of the participants are summarized in Table 1, with a higher percentage of females than males (54.67% versus 45.33%) and a median age of 50 years, with a range of 40 to 79 years. A substantial proportion of the study population (42.27%, $n=3814$) was classified as high-risk based on the presence of one or more of the following factors: hepatitis B/C infection (16.38%), alcohol consumption (16.10%), heavy smoking (16.25%), type 2 diabetes (5.73%), FDR (first-degree relatives) diagnosed with two types of cancer at an age younger than 45 (11.58%), and carrying inherited cancer mutations (0.93%). The remaining 57.73% ($n=5210$) of participants were categorized as moderate risk (Table 1). Notably, the distribution of risk factors such as heavy smoking, hepatitis B/C infection, and type 2 diabetes was consistent with previous reports, suggesting that the K-DETEK cohort is representative of the screening population in Vietnam [27–30].

Test performance

We detected 43 cases (0.48%, Table 2 and Fig. 2) with "ctDNA signal detected" results, all of whom were referred for SOC imaging tests to confirm the presence of tumors according to our consultation protocol (Additional file 1: Table S2). All cases agreed to undertake diagnostic tests for the cancer types corresponding to the prediction of TOO provided in the SPOT-MAS test

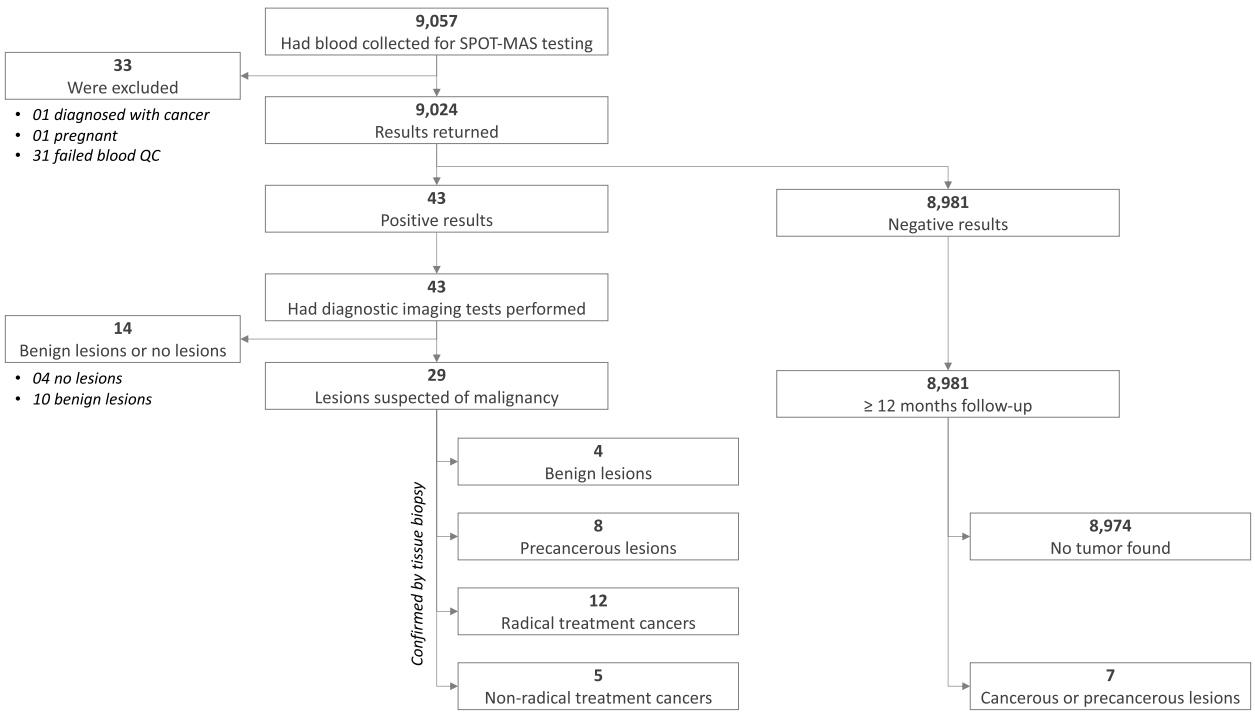

Fig. 2 The flow chart of recruiting and following up participants in the K-DETEK study

Table 1 Clinical characteristics of 9024 eligible participants

Risk classification		*N* = 9024	%
Gender	Female	4933	54.67
	Male	4091	45.33
Age	Median	50	
	Min	40	
	Max	79	
High risk		3814	42.27
Moderate risk		5210	57.73
Liver infection (HBV/HCV)	Yes	1478	16.38
	No	7546	83.62
Alcohol consumptions	Yes	1453	16.10
	No	7571	83.90
Heavy smoking	Yes	1466	16.25
	No	7558	83.75
Diabetes	Yes	517	5.73
	No	8507	94.27
Family history of cancer[a]	Yes	1045	11.58
	No	7979	88.42
Carrying inherited cancer mutations	Yes	84	0.93
	No	8940	99.07

[a] Participants who have first-degree relatives (FDR) diagnosed with two types of cancer at an age younger than 45

reports. Among the 43 participants with positive test results, 29 had imaging results with lesions suspected of malignancy and were advised to perform tissue biopsies (Fig. 2). Of those, 25 cases had precancerous (8 cases) or cancerous lesions (17 cases), while four cases were found to have benign lesions (Fig. 2, Additional file 3: Appendix 2 and Additional file 4: Figure S1). Among the 17 cases with cancerous lesions, 12 were detected at early stages (I to IIIA) and underwent curative interventions, which could significantly improve their survival prognosis. These 12 cases (0.13%, Table 2) were diagnosed with localized (Fig. 3, red squares) or locally advanced cancer (Fig. 3, purple squares), while the remaining five cases (0.06%, Table 2) had metastatic late-stage cancer (Fig. 3, blue squares). We recorded 26 false positive cases, including eight cases (0.09%, Table 2) with precancerous lesions in the colon and 18 cases (0.20%, Table 2) with diagnostic results unable to confirm the presence of malignant or precancerous tumors. Accurate prediction of the tumor origin was observed in 9 out of the 17 cancer cases (52.94%) (Fig. 3, Table 2). The test achieved an overall sensitivity of 70.83% (95%CI 50.83–85.09), a PPV of 39.53% (95%CI 26.37–54.42), and a TOO prediction accuracy of 52.94% (95%CI 30.96–73.83) when considering only malignant lesions as true positives (Table 2).

Among the confirmed cancer cases, four cases (K6348, K6642, K4101, and K9012) exhibited "unclassified" TOO signals, which were later identified as cancer types outside the five tested common cancer types (prostate cancer, endometrial cancer, cervical cancer, thyroid cancer). Additionally, there were four cases with misidentified TOO, involving samples K6583, K0156, K3404, and K9024 (Fig. 3, Additional file 1: Table S3 and Additional file 3: Appendix 2). In case K6583, the TOO was reported

Table 2 SPOT-MAS test performance

Test performance			*N* = 9024[a]	%	95% CI
ctDNA signal detected			43	0.48	
	True positive		17	0.19	
		Early and non-metastatic stage (I, II, IIIA)	12	0.13	
		Late metastatic stage (IIIB, IV)	5	0.06	
	False positive		26	0.29	
		No lesions	18	0.20	
		Precancerous lesions	8	0.09	
ctDNA signal not detected			8981	99.52	
	True negative		8974	99.44	
	False negative		7	0.08	
Sensitivity				70.83 (17/24)	50.83–85.09
Specificity				99.71 (8974/9000)	99.58–99.80
Positive predictive value				39.53 (17/43)	26.37–54.42
Negative predictive value				99.92 (8974/8981)	99.84–99.96
Prediction accuracy of tumor origin				52.94 (9/17)	30.96–73.83

[a] Participants at 12-month follow-up

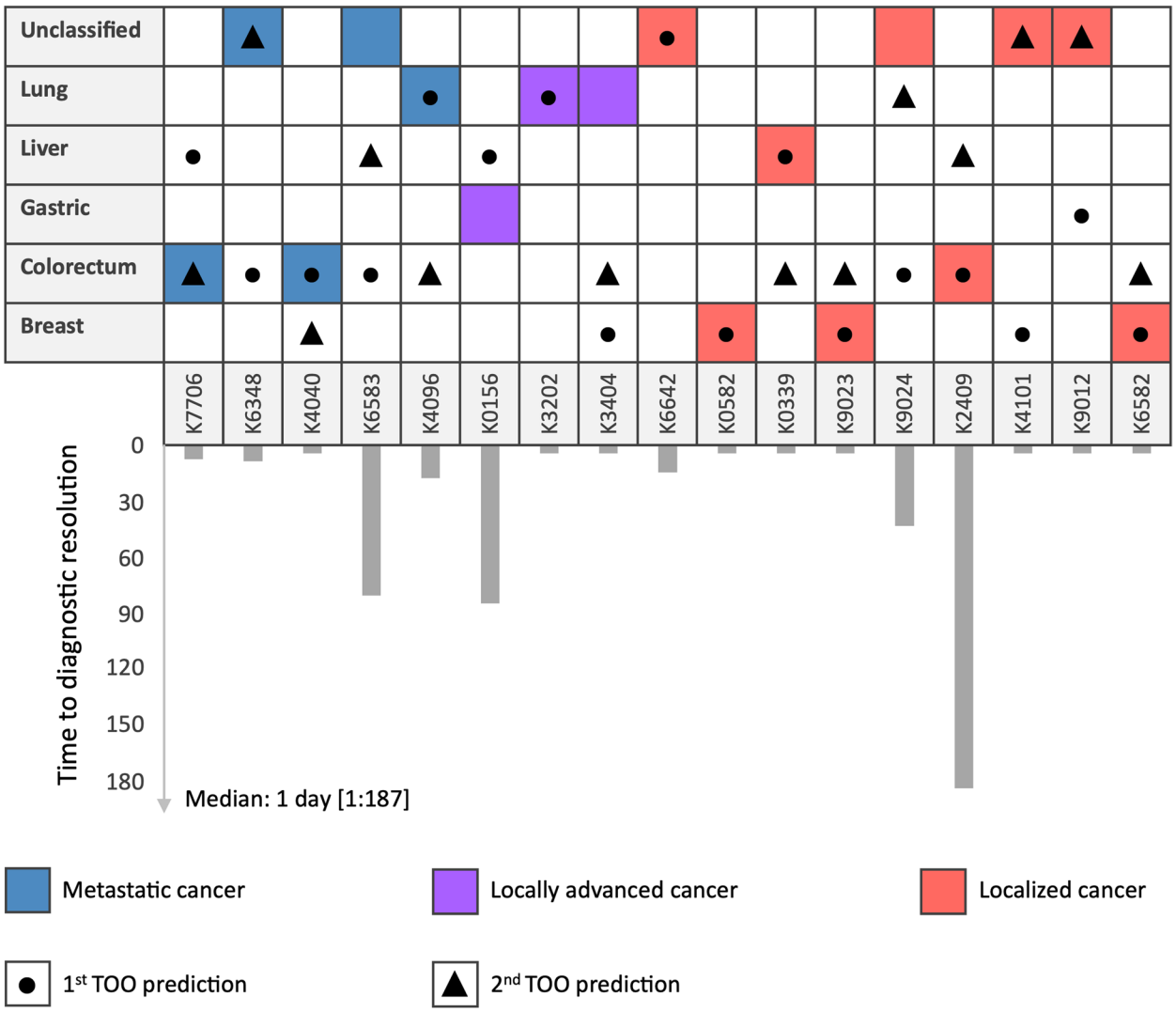

Fig. 3 The analysis of diagnostic results of 17 participants with a true positive result. Colored squares indicate the lesion-specific origin, while circles and triangles denote the first and second predicted TOO by SPOT-MAS assay. Colors represent the cancer diagnostic outcomes: metastatic cancer (blue), locally advanced cancer (purple), localized cancer (red). Intersections between colored squares and circles or triangles signify correct TOO predictions by SPOT-MAS. The bar charts display the observed time from the receipt of positive ctDNA results to final diagnosis confirmation. Unclassified group including cancers outside the scope of SPOT-MAS: Prostate cancer (K6348), Bile duct cancer (K6583), Endometrial cancer (K6642), Lymphoma (K9024), Cervical cancer (K4101), Thyroid cancer (K9012)

as colorectum and liver but no abnormalities were detected during colonoscopy. A subsequent three-phase contrast-enhanced abdominal CT scan identified multiple metastatic lesions in the liver, suggesting the primary tumor was in the biliary tract. Further imaging with magnetic resonance cholangiopancreatography (MRCP) confirmed a malignant lesion within the biliary ducts, which was diagnosed as cancer following biopsy and histopathological analysis (Additional file 3: Appendix 2).

In case K0156, TOO was predicted as liver. A three-phase abdominal CT scan did not reveal hepatic lesions, but did detect a mass in the pyloric antrum with associated perilesional fat infiltration suggestive of malignancy. Gastroscopy, followed by biopsy of the suspicious lesion, confirmed the diagnosis of gastric carcinoma through histopathological evaluation (Additional file 3: Appendix 2).

Patients K3404 (TOO in breast and colorectum) and K9024 (TOO in colorectum and lung) opted for comprehensive whole-body CT scanning in addition to the recommended on-site imaging tests. In case K3404, a suspicious pulmonary lesion was identified, while case

K9024 presented findings indicative of potential lymphoma. Both cases were subsequently confirmed to be malignant through biopsy and detailed histopathological examination. (Additional file 3: Appendix 2).

The majority of participants (8981[99.52%] of 9024) showed "ctDNA signal not detected" results and 8974 (99.92%) of them were confirmed to be cancer-free at 12 months after enrollment, indicating a NPV of 99.92% (95%CI 99.84–99.96) and a specificity of 99.71% (95%CI 99.58–99.80) (Table 2 and Fig. 2). Among these participants, seven (0.08%) cases were found to develop cancer during the 12-month follow-up (Additional file 1: Table S4). Specifically, two cases developed metastatic lung cancer ($n=2$, patients K1452 and K7249), 2 cases had locally advanced colorectal cancer ($n=2$, patients K6250 and K6956), and 3 cases developed localized cancer including colon (patient K3947), lung (patient K4047), and gastric cancer (patient K6690).

We observed that the median time from receipt of positive SPOT-MAS results to final diagnosis confirmation was 12 days, ranging from 1 to 187 days, for all 43 cases with ctDNA signal detected (Additional file 1: Table S3 and Table S5). Interestingly, the true positive group had a shorter median time (1 day) compared with the false positive group (12.5 days) to achieve diagnostic resolution (Fig. 3, Additional file 1: Table S3 and Table S5). Notably, our test not only detects cancers included in the current USPSTF guidelines but also identifies six (35%) cases with cancers that lack SOC screens, including liver cancer, biliary tract cancer, gastric cancer, endometrial cancer, thyroid cancer, and lymphoma (Fig. 4).

Seven false negative cases were identified through phone follow-up and were found to have one of the five cancer types through their routine medical care or regular wellness checkups. Since previous studies have reported that the performance of a MCED could be dependent on the risk of target populations [31], we next examined such association in our cohort. We did not observe any noticeable difference in NPV and specificity across diverse groups of participants (NPV > 99.80%, specificity > 99.50%, Fig. 5, Additional file 1: Table S6). By contrast, the PPV increased from 36.36% (95% CI 22.19–53.38), Fig. 5, Additional file 1: Table S6) in moderate-risk participants to 50.00% (95% CI 23.66–76.34, Fig. 5, Additional file 1: Table S6) in high-risk participants. Moreover, we observed higher PPV in the group over 50 years old as compared to the younger group < 50 years old (44.44%, 95% CI 29.54–60.42 versus 14.29%, 95% CI 2.57–51.31, Additional file 1: Table S6). For sex, the PPV was higher in male participants than female participants (50.00%, 95%CI 25.38–74.62 versus 35.48%, 95%CI 21.12–53.05, Additional file 1: Table S6). The test exhibited a slightly lower sensitivity for early and non-metastatic stage cancer compared to metastatic late-stage cancer (70.59%, 95% CI 46.87–86.72 versus 71.43%, 95% CI 35.89–91.78, Additional file 1: Table S6).

Our findings demonstrate that the SPOT-MAS test effectively identifies various cancer types, including those lacking SOC screens, at early stages. The test exhibits a PPV of 39.53% (95%CI 26.37–54.42), a NPV of 99.92% (95% CI 99.84–99.96), a sensitivity of 70.83% (95%CI 50.83–85.09), and a specificity of 99.71% (95% CI 99.58–99.80). In addition to its cancer detection capabilities, SPOT-MAS accurately localizes tissue-specific cancer signals, achieving an accuracy of 52.94% (95%CI 30.96–73.83).

Discussion

The paradigm of cancer diagnosis is undergoing a significant shift with the development of MCED tests. MCED from a single blood draw is key to successful treatment and improved survival outcomes for cancer patients. To ensure the effectiveness and reliability of MCED tests in clinical practice, thorough validation is crucial. Here, we conducted a multi-center prospective clinical trial, K-DETEK, to determine the clinical performance of SPOT-MAS in a large asymptomatic cohort in Vietnam. The primary endpoints included the report of NPV, PPV, sensitivity, and specificity, while the secondary endpoint involved reporting TOO accuracy (Fig. 1).

Our data observed a NPV of 99.92% (95%CI 99.84–99.96), corresponding to 8974 out of 8981 negative cases that remained cancer-free at 12 months after enrollment (Table 2). Among these cases, 2 out of 7 (patient K1452 and K7249) developed metastatic cancer. Metastatic tumors are known to display methylation signatures that differ from those of primary early-stage tumors [32]. This variation may explain why our algorithms, trained on samples meeting stringent selection criteria for early and nonmetastatic cancer (stage I-II and IIIA), did not identify these two cases.

Among individuals with positive SPOT-MAS results, 58.1% (25/43) were found to have either cancerous ($n=17$) or precancerous lesions ($n=8$) during further diagnostic workup. In this group, 68% (17/25) had lesions in the organs matched with predictions. While precancerous lesions are not categorized as true positives in this analysis, these individuals are clinically high risk. Prestigious medical organizations, including the US Multi-Society Task Force on Colorectal Cancer (MSTF), the American College of Gastroenterology (ACG), the American Gastroenterological Association (AGA), and the American Society for Gastrointestinal Endoscopy (ASGE) [33–36], emphasize the potential for malignant transformation of such lesions, particularly in the gastrointestinal tract. Early detection of these lesions enables

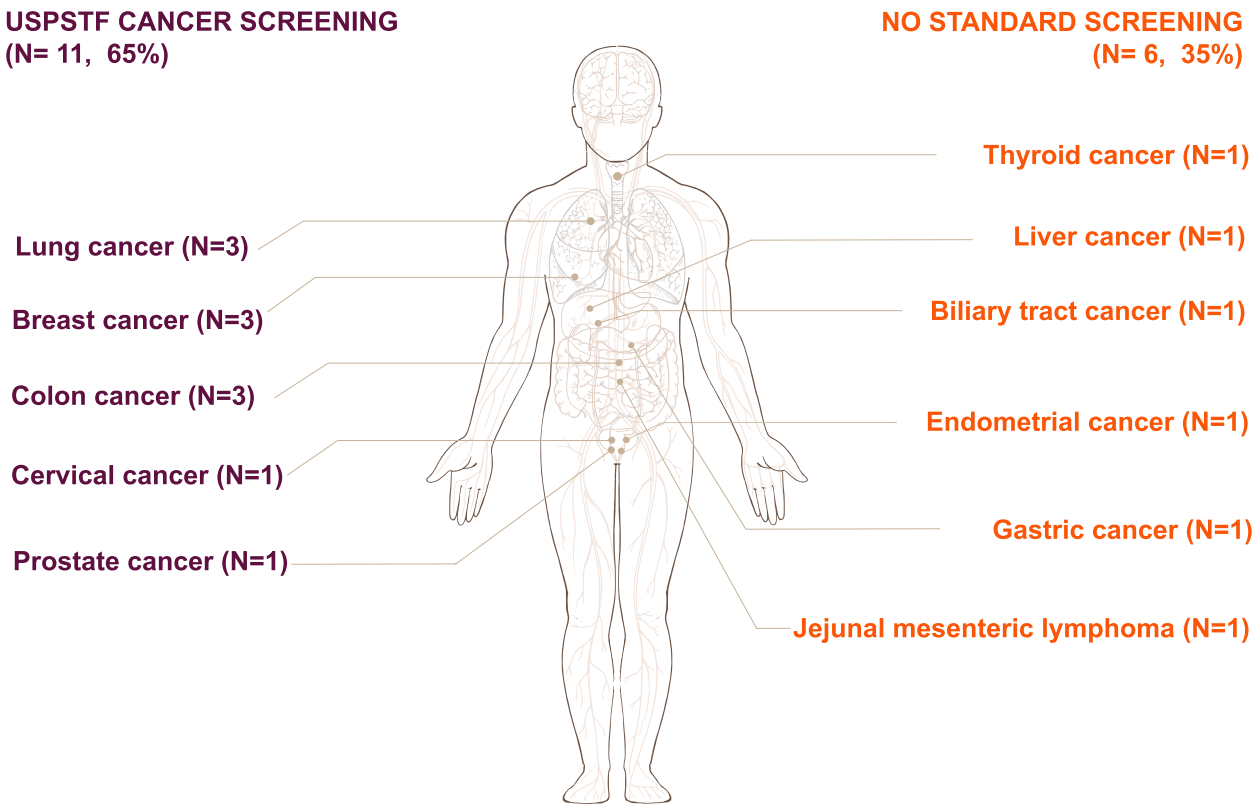

Fig. 4 The distribution of cancer types detected in the 17 true-positive participants. SPOT-MAS test can detect a wide range of cancers, including those with standard-of-care (SOC) screens and those lacking SOC screens

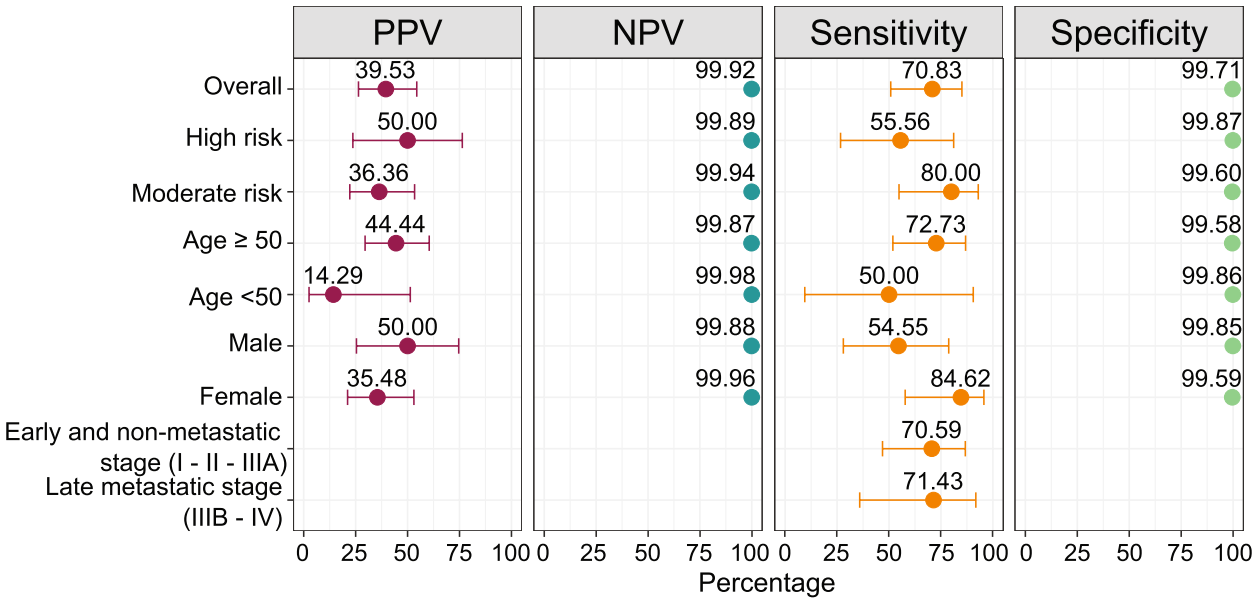

Fig. 5 Positive predictive value (PPV), negative predictive value (NPV), sensitivity and specificity of SPOT-MAS test in different demographic groups

timely intervention, aligning with the preventative goals of MCED tests. This underscores the need for further research to clarify the role of MCED tests in identifying precancerous lesions and to establish optimal management strategies for these high-risk populations. The remaining 18 cases exhibited detectable ctDNA signals; however, no tumors or precancerous lesions were identified in subsequent diagnostic evaluations (Table 2). These cases may reflect early-stage cancers undetectable by current imaging technologies, cancers outside the detection scope of the SPOT-MAS panel, or "pseudo-signals" potentially associated with non-cancerous conditions [37]. Longer-term follow-up is essential to determine the true cancer status of these individuals, which could provide critical insights into refining the SPOT-MAS test's predictive capacity and clinical application.

Nevertheless, SPOT-MAS demonstrates higher specificity compared with existing single-cancer screening tests, which typically yield false positive rates of 5 to 15% per screening episode [38]. A low false positive rate reduces the number of individuals without cancer who are referred for cancer investigations, thereby directly impacting resource allocation and costs. Importantly, SPOT-MAS successfully detected cancer signals across 11 cancer types, including six that lack established SOC-recommended screening (Fig. 4). Cancers without SOC screening programs account for more than 60% of all cancer diagnoses and approximately 71% of cancer deaths [39]. These results highlight the potential of the SPOT-MAS test to complement current SOC screening programs, thereby enhancing the effectiveness of multicancer early detection.

The median time to achieve diagnostic resolution after receiving SPOT-MAS results was 12 days. This short period could significantly alleviate patients' anxiety and expedite necessary interventions for those diagnosed with precancerous conditions. However, one patient (K2409) experienced the longest diagnostic resolution time of 187 days. This patient, asymptomatic at the time of receiving SPOT-MAS results and living in a rural Vietnamese province without advanced imaging diagnostic facilities, initially declined the recommended colon endoscopy based on the TOO prediction. After 6 months, the patient developed clinical symptoms of colon cancer, including rapid weight loss and bloody diarrhea. A subsequent colonoscopy detected cancer (Additional file 1: Table S3). This case highlights the importance of a post-test consultation procedure when applying the SPOT-MAS test in clinical practice.

Our stratification analysis revealed higher PPV in older individuals ($\geq$50 years), male patients, and those classified as high-risk (Additional file 1: Table S6). These findings underscore the importance of accounting for clinical characteristics and demographic differences in screening populations when evaluating test performance and outcomes. The decreased PPV observed in women raises legitimate concerns regarding the utility and reliability of the test in this group. This is likely attributed to the lower sensitivity of the test for breast cancer, which is more prevalent in women. The reduced sensitivity for breast cancer may stem from the lower ctDNA shedding by breast tumors [17, 40] and the confounding influence of benign breast lesions on methylation patterns [41]. We are actively researching and developing new strategies to address this limitation and improve the sensitivity of SPOT-MAS for breast cancer. Our participants' demographic characteristics (Table 1) closely mirror the distribution of risk factors in the general population of Vietnam, including 19.8% alcohol consumption [27], 22.5% smoking [28], 10.5% hepatitis B infection [29], and 5.4% diabetes [30]. These similarities suggest that our study cohort is representative of the Vietnamese screening population, indicating a potential for equivalent performance when using SPOT-MAS in clinical practice in Vietnam.

In Vietnam, routine health check-ups generally do not incorporate age-appropriate standard-of-care (SOC) cancer screening tests, such as those recommended by international guidelines (e.g., colorectal cancer screening for individuals over 50). This gap can be attributed to three primary factors: (i) the absence of a comprehensive national cancer screening program, (ii) the exclusion of SOC screening tests from national health insurance coverage, and (iii) limited public awareness and understanding of cancer [42]. Consequently, most asymptomatic participants in the K-DETEK study did not undergo the recommended age-appropriate screening tests during their routine checkups. The lack of SOC screening test results hinders an accurate estimation of the incidence of precancerous lesions in the test-negative group and the sensitivity of our test in detecting these lesions across the entire cohort. During follow-up, we identified a subset of 538 SPOT-MAS-negative participants who underwent colonoscopy as part of a premium health check-up package provided by their employers. Among these, 534 participants (99.26%) had no abnormalities, while four (0.74%) were found to have precancerous lesions. However, this subset of participants is not representative of the entire study cohort, and the observed incidence of precancerous lesions in this group cannot be extrapolated to the whole K-DETEK study due to the biased selection criteria.

In the K-DETEK study, a true positive rate of 0.19% (17 true positives in 9024 cases) was observed in a cohort of asymptomatic, moderate- to high-risk individuals, which is higher than the cancer incident rate in the general

Vietnamese population (0.18%) [43]. The observed true positive rate of 0.19% is lower than the expected cancer incidence rate of 0.26% for the Vietnamese population aged 40 and above, as reported by GLOBOCAN 2022 (International Agency for Research on Cancer (IARC)) [44]. This difference can be attributed to the strict inclusion criteria of the K-DETEK study, which focused on a population of healthy individuals with no cancer-related symptoms or prior cancer diagnosis who underwent routine health check-ups at primary healthcare screening units rather than specialized cancer diagnostic centers.

The distribution of cancer types identified in the K-DETEK study is as follows: lung (17.6%), breast (17.6%), colorectum (17.6%), liver (5.9%), gastric (5.9%), and others (35.3%). Collectively, the five investigated cancer types account for 64.7% of the total cases. This distribution aligns closely with the incidence rates reported for the Vietnamese population aged 40 and above, which is 61.5% according to GLOBOCAN 2022 [44]. However, the specific proportion of each cancer type detected in the K-DETEK study does not fully correspond to their prevalence in the general population. This discrepancy may be due to the test's sensitivity, which varies across different cancer types [20].

Comparing the findings from K-DETEK to other clinical studies requires careful consideration of population risk variations and their potential impact on test performance. The lower cancer incidence rate observed in K-DETEK compared to DETECT-A [45] and PATHFINDER [15] can likely be attributed to the younger study population (starting at age 40) and the lower overall cancer incidence rate in Vietnam (0.18%) [43, 44] compared to the USA (0.71%) [44, 46]. These distinctions underscore the importance of tailoring early detection test evaluations to the specific demographic and epidemiological characteristics of the target population. Notably, this incident rate was estimated using all cancer types but with detection methods differing from SPOT-MAS. Our study focused on moderate- and high-risk participants with an elevated chance of developing cancer. The resulting PPV for detecting invasive cancer was comparable to that observed in the PATHFINDER study, which evaluated the Galleri MCED test in a similar-risk population, achieving a PPV of 38% [47]. It is worth noting that our study detected a wide range of solid tumors whereas the majority of cancer patients identified in PATHFINDER had hematologic cancers (48.57%). The lower cancer incidence observed in the test negative group in the K-DETEK study, compared to DETECT-A [45] and PATHFINDER [15], may be attributed to differences in population demographics, test performance variantions, and the follow-up protocol implemented for the test negative participants.

The multimodal approach of SPOT-MAS, integrating methylation, fragment length profile, DNA copy number aberration, and end motif in a single library reaction, could explain these differences compared to the Galleri test, which focused primarily on methylation makers [20, 48]. Furthermore, the SPOT-MAS test uses a unique approach that combines shallow, genome-wide sequencing (0.55X) with an efficient, all-in-one protocol. This allows it to simultaneously capture multiple ctDNA signatures in the bloodstream. By taking this innovative approach, the SPOT-MAS test not only reduces the high costs typically associated with deep sequencing but also streamlines the analytical process. Therefore, SPOT-MAS offers a cost-effective solution for healthcare systems, particularly in resource-limited settings [20].

Successful implementation of the SPOT-MAS test in clinical settings requires comprehensive training programs for clinicians, focusing on result interpretation and follow-up management. Prior to the launching of SPOT-MAS, training sessions were conducted to educate clinicians on how to interpret the complex outputs of the SPOT-MAS test and integrate these results into clinical decision-making. As part of this training, a Standard Operating Procedure (SOP) was also developed for post-test counseling and follow-up [25]. This SOP provided tailored guidelines corresponding to different categories of ctDNA results, facilitating diagnostic resolution during a 12-month follow-up period. All members of the research teams at participating hospitals and institutions have been trained on this protocol.

The clinical care pathway is represented in process diagrams, outlining consultations, patient support, and recommended diagnostic tests based on standard of care practices and SPOT-MAS results. This structured approach ensures timely diagnostic resolution while providing comprehensive patient support throughout the ctDNA analysis process.

Furthermore, addressing challenges related to technological infrastructure, cost, and workflow integration is crucial for successful clinical implementation. To support this, we are launching pilot programs in healthcare institutions and establishing centralized testing partnerships to facilitate the seamless integration of the SPOT-MAS test into both high-resource and resource-limited healthcare settings. This ensures that clinicians are well-prepared to manage the test's implementation and that logistical and infrastructural challenges are addressed to promote broader accessibility.

Our study has some limitations. First, the number of true positive cases (17 cases, 0.19%) with confirmed malignant lesions is relatively small due to the rigorous selection of patients without cancer-related symptoms. To address this, we are conducting another clinical trial

aiming to evaluate SPOT-MAS performance in symptomatic participants. Second, it remains unclear whether a MECD test such as SPOT-MAS could enhance survival. Future randomized clinical trials are needed to address this question and provide valuable insights into the effectiveness of the test and its impact on patient outcomes. To confirm the cancer-free status of participants who tested negative for SPOT-MAS, we used a symptom-based questionnaire consistent with the PATHFINDER study by Schrag et al. [15]. This follow-up approach, relying primarily on self-reports and clinical diagnoses during the follow-up period, may fail to capture asymptomatic or undetected cancers, particularly in early stages. However, mandating uniform screening tests or advanced diagnostic procedures for such large cohorts is impractical. Certain procedures, such as endoscopies, are invasive and can cause anxiety and inconvenience, potentially reducing participant compliance. This challenge is a common limitation across MCED studies with large, geographically dispersed populations (9911 cases for DETECT-A [45], 6621 cases for PATHFINDER [15], and 9024 cases for K-DETEK). For K-DETEK, a multi-center clinical validation study designed to enhance the generalizability of findings across diverse populations, including those in urban and remote areas, telephone follow-ups at 6 and 12 months using a specialized questionnaire represented a considerable effort to accurately assess participants' health statuses over time. Given these limitations, the SPOT-MAS test should not be considered a replacement for existing screening programs but rather a complementary tool to enhance surveillance. Additionally, the absence of ctDNA should be interpreted as an indicator of low risk, not the complete absence of risk.

Conclusions

Our study provides compelling evidence supporting the clinical utility of SPOT-MAS as a multi-cancer blood test for early detection in Vietnam, where national cancer screening programs are not available. Ongoing research is evaluating the performance of the SPOT-MAS test across diverse ethnicities, healthcare systems, and cancer prevalence profiles. These studies will also determine whether the machine learning model used by SPOT-MAS is applicable beyond the Vietnamese population and will guide any necessary adjustments to enhance broader applicability.

Abbreviations

MCED	Multi-cancer early detection
ctDNA	Circulating tumor DNA
SPOT-MAS	Screening for the Presence Of Tumor by DNA Methylation And Size
SOC	Standard-of-care
TOO	Tissue of origin
PPV	Positive predictive value
NPV	Negative predictive value
USPSTF	United States Preventive Services Task Force
LB	Liquid biopsy
CTC	Circulating tumor cells
cfDNA	Cell-free DNA
cfRNA	Circulating cell-free RNA
NCCN	The National Comprehensive Cancer Network
FDR	First-degree relatives
SOP	Standard Operating Procedure

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12916-025-03929-y.

Additional file 1 Table S1-S6. Table S1-Demographic details of eligible 9024 participants enrolled in K-DETEK study. Table S2-Standard of care imaging tests used for diagnostic resolution. Table S3-Standard of care imaging test results of true positive cases. Table S4-Standard of care imaging test results of false negative cases. Table S5- Standard of care imaging test results of false positive cases. Table S6-Sensitivity, specificity, positive predictive value, negative predictive value of SPOT-MAS test in different demographic groups.

Additional file 2 Appendix 1. Follow-up survey form at 6 and 12 months post SPOT-MAS test.

Additional file 3 Appendix 2. Details of the diagnostic outcomes for participants with cancerous lesions.

Additional file 4 Figures S1. The diagnostic results of 17 true positive cases. Histological images are shown for 16 cases, while a CT scan is provided for case K0339 due to no indication for biopsy or resection. This patient received transarterial chemoembolization (TACE) and radiofrequency ablation (RFA) for early-stage hepatocellular carcinoma (stage IB). CRC: colorectal cancer, PC: prostate cancer, BDC: bile duct cancer, LC: lung cancer, GC: gastric cancer, EC: endometrial cancer, BC: breast cancer, HCC: hepatocellular carcinoma, LM: lymphoma, CC: cervical cancer, TC: thyroid cancer.

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Authors' contributions

LHDN, THHN, NMP, BLT, DHV, THT, TDN, VTCN, YTL, THN, VUT, MPL, TMTT, MNN, TTVV, ANN, TTN, HTPN, CTTC perfomed formal analysis. LHDN, THHN, VHL, VQB, LHN, NHP, THP, HTN, VST, CVB, VKV, PTNN, HHPD, VDP, VTC, BLT, TTN, HTPN, CTTC, VTN, TLQL, TLAL, TKPD, TTD, CDP, TXN, NTP, BTN, TTTP, HLL, CTT, TXJ, MCL, VBP, QBT, THLT, MTH, TQT, STN, VT, VKT, HNN, DSN, TVP, TTTD, DKT, HST perfomed patient consultancy and screening. LHDN, THHN, NNTD, HTN, PLD, LAKH, TAN performed data curation. DSN, TVP, TTTD, DKT, HST, MDP, HG, HNN, LST performed the methodology. HNN, DSN, TVP, TTTD, DKT, HST, MDP, HG, HNN, LST perfomed conceptualization. LHDN, THHN, GTHN, LST performed writing-original draft. LHDN, GTHN, MDP, HG, HNN, LST perfomed writing-review and editing. All authors read and approved the final manuscript. LHDN, THHN, VHL, VQB, LHN, NHP, THP, HTN, VST, CVB, VKV, PTNN, HHPD, VDP, VTC, BLT, TTN, HTPN, CTTC, VTN, TLQL, TLAL, TKPD, TTD, CDP, TXN, NTP, BTN, TTTP, HLL, CTT, TXJ, MCL, VBP, QBT, THLT, MTH, TQT, STN, VT, VKT, HNN, DSN, TVP, TTTD, DKT, and HST perfomed patient consultancy and screening. LHDN, THHN, NNTD, HTN, PLD, LAKH, and TAN performed data curation. DSN, TVP, TTTD, DKT, HST, MDP, HG, HNN, and LST performed the methodology. HNN, DSN, TVP, TTTD, DKT, HST, MDP, HG, HNN, and LST perfomed conceptualization. LHDN, THHN, GTHN, and LST performed writing-original draft. LHDN, GTHN, MDP, HG, HNN, and LST perfomed writing-review and editing. All authors read and approved the final manuscript.

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Data availability
The data that support the findings of this study are available from the corresponding authors upon reasonable request.

Declarations

Ethics approval and consent to participate
This research received approval from the Ethics Committee of the University of Medicine and Pharmacy, Ho Chi Minh City, Vietnam (approval number: 192/HĐĐĐ-ĐHYD). Each participant provided written informed consent, adhering to the Declaration of Helsinki.

Consent for publication
Not applicable.

Competing interests
The authors including LST, HNN, HG, MDP, HHN and DSN hold equity in Gene Solutions. NHN, HG, MDP and LST are inventors on the patent application (USPTO 17930705). LHDN, THHN, NMP, BLT, GTHN, DHV, THT, TDN, VTCN, THN, VUT, MPL, TMTT, MNN, TTVV, ANN, TTN, NNTD, HTN, PLD, LAKH, TAN, HTPN, YTL, CTTC, TVP, TTTD, DKT, and HST received salaries from the project funded by the Medical Genetics Institute, Ho Chi Minh City, Vietnam. We confirm that this does not alter our adherence to journal policies on sharing data and materials. VHL, VQB, LHN, NHP, THP, HTN, VST, CVB, VKV, PTNN, HHPD, VDP, VTC, BLT, TTN, HTPN, CTTC, VTN, TLQL, TLAL, TKPD, TTD, CDP, TXN, NTP, BTN, TTTP, HLL, CTT, TXJ, MCL, VBP, QBT, THLT, MTH, TQT, STN, VT, VKT declare that they have no competing interests.
VHL, VQB, LHN, NHP, THP, HTN, VST, CVB, VKV, PTNN, HHPD, VDP, VTC, BLT, TTN, HTPN, CTTC, VTN, TLQL, TLAL, TKPD, TTD, CDP, TXN, NTP, BTN, TTTP, HLL, CTT, TXJ, MCL, VBP, QBT, THLT, MTH, TQT, STN, VT, and VKT declare that they have no competing interests.

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