Multi-cancer early detection test in symptomatic patients referred for cancer investigation in England and Wales (SYMPLIFY): a large-scale, observational cohort study

Brian D Nicholson, Jason Oke, Pradeep S Virdee, Dean A Harris, Catherine O'Doherty, John ES Park, Zaed Hamady, Vinay Sehgal, Andrew Millar, Louise Medley, Sharon Tonner, Monika Vargova, Lazarina Engonidou, Kaveh Riahi, Ying Luan, Sara Hiom, Harpal Kumar, Harit Nandani, Kathryn N Kurtzman, Ly-Mee Yu, Clare Freestone, Sarah Pearson, FD Richard Hobbs, Rafael Perera, Mark R Middleton

Summary

Background Analysis of circulating tumour DNA could stratify cancer risk in symptomatic patients. We aimed to evaluate the performance of a methylation-based multicancer early detection (MCED) diagnostic test in symptomatic patients referred from primary care.

Methods We did a multicentre, prospective, observational study at National Health Service (NHS) hospital sites in England and Wales. Participants aged 18 or older referred with non-specific symptoms or symptoms potentially due to gynaecological, lung, or upper or lower gastrointestinal cancers were included and gave a blood sample when they attended for urgent investigation. Participants were excluded if they had a history of or had received treatment for an invasive or haematological malignancy diagnosed within the preceding 3 years, were taking cytotoxic or demethylating agents that might interfere with the test, or had participated in another study of a GRAIL MCED test. Patients were followed until diagnostic resolution or up to 9 months. Cell-free DNA was isolated and the MCED test performed blinded to the clinical outcome. MCED predictions were compared with the diagnosis obtained by standard care to establish the primary outcomes of overall positive and negative predictive value, sensitivity, and specificity. Outcomes were assessed in participants with a valid MCED test result and diagnostic resolution. SYMPLIFY is registered with ISRCTN (ISRCTN10226380) and has completed follow-up at all sites.

Findings 6238 participants were recruited between July 7 and Nov 30, 2021, across 44 hospital sites. 387 were excluded due to staff being unable to draw blood, sample errors, participant withdrawal, or identification of ineligibility after enrolment. Of 5851 clinically evaluable participants, 376 had no MCED test result and 14 had no information as to final diagnosis, resulting in 5461 included in the final cohort for analysis with an evaluable MCED test result and diagnostic outcome (368 [6·7%] with a cancer diagnosis and 5093 [93·3%] without a cancer diagnosis). The median age of participants was 61·9 years (IQR 53·4–73·0), 3609 (66·1%) were female and 1852 (33·9%) were male. The MCED test detected a cancer signal in 323 cases, in whom 244 cancer was diagnosed, yielding a positive predictive value of 75·5% (95% CI 70·5–80·1), negative predictive value of 97·6% (97·1–98·0), sensitivity of 66·3% (61·2–71·1), and specificity of 98·4% (98·1–98·8). Sensitivity increased with increasing age and cancer stage, from 24·2% (95% CI 16·0–34·1) in stage I to 95·3% (88·5–98·7) in stage IV. For cases in which a cancer signal was detected among patients with cancer, the MCED test's prediction of the site of origin was accurate in 85·2% (95% CI 79·8–89·3) of cases. Sensitivity 80·4% (95% CI 66·1–90·6) and negative predictive value 99·1% (98·2–99·6) were highest for patients with symptoms mandating investigation for upper gastrointestinal cancer.

Interpretation This first large-scale prospective evaluation of an MCED diagnostic test in a symptomatic population demonstrates the feasibility of using an MCED test to assist clinicians with decisions regarding urgency and route of referral from primary care. Our data provide the basis for a prospective, interventional study in patients presenting to primary care with non-specific signs and symptoms.

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Nuffield Department of Primary Care Health Sciences (B D Nicholson DPhil, J Oke DPhil, P S Virdee DPhil, S Tonner PhD, M Vargova, L Engonidou MSc, L-M Yu DPhil, Prof F D R Hobbs MD, Prof R Perera DPhil), **and Department of Oncology** (C Freestone PhD, S Pearson BSc, Prof M R Middleton PhD), **University of Oxford, Oxford, UK; Swansea Bay University Health Board, Swansea, UK** (Prof D A Harris FRCS); **Mid and South Essex NHS Foundation Trust, Basildon, UK** (C O'Doherty MD); **Oxford University Hospitals NHS Foundation Trust, Oxford, UK** (J E S Park MD); **University Hospital Southampton NHS Foundation Trust, Southampton, UK** (Z Hamady PhD); **University College London Hospitals NHS Foundation Trust, London, UK** (V Seghal PhD); **North Middlesex Hospital NHS Foundation Trust, London, UK** (A Millar MD); **Torbay and South Devon NHS Foundation Trust, Torquay, UK** (L Medley MD); **GRAIL Bio UK, GRAIL, London, UK** (K Riahi BA, S Hiom MSc, H Kumar MBA); **GRAIL, Menlo Park, CA, USA** (Y Luan PhD, H Nandani MS, K N Kurtzman MD)

Correspondence to: Prof Mark R Middleton, Department of Oncology, University of Oxford, Oxford, OX3 7DQ, UK **mark.middleton@oncology.ox.ac.uk**

Introduction

The detection of cancer and subsequent intervention at the earlier stages of disease has the potential to greatly improve patient outcomes and reduce cancer-related mortality.[1] For most cancers, organised screening programmes have not been implemented, and most patients diagnosed with cancer first attend primary care with symptoms.[2] Expediting symptomatic cancer diagnosis can be achieved by having a high index of suspicion for cancer when the patient first contacts the health-care system, through the early use of appropriate diagnostic technologies, and with access to fast-track pathways for specialist assessment.[3,4]

Research in context

Evidence before this study

We searched PubMed from database inception to Feb 24, 2021 (the date of proposing the SYMPLIFY trial) for "multi-cancer screening" [title and abstract] OR "multi-cancer detection" [title and abstract] AND "symptoms" [any field] (and related terms) without language restrictions. From this search, we identified reports of the Circulating Cell Free Genome Atlas (CCGA) and DETECT-A studies, describing the development and clinical application of blood-based multi-cancer early detection (MCED) tests. The CCGA case-control study identified cell free DNA (cfDNA) analysis of methylation patterns as a basis for identifying up to 50 cancer types. Cases had an established cancer diagnosis and, with controls, were used to train and validate a classifier that delivered 99·3% specificity and 43·9% sensitivity in stage I–III cancer. Cancer site of origin was called in 96% of cases and accurate in 93% of cases. DETECT-A used a multi-analyte blood test incorporating DNA and protein biomarkers to screen women aged between 65 and 75 years, with a confirmatory test required to call a positive result. We found no studies examining the performance of an MCED test in patients presenting with symptoms requiring referral for cancer investigation, nor studies addressing their use in clinical decision making around specific symptoms. We repeated the PubMed search on April 10, 2023, and identified multiple additional studies reporting the development, validation, and performance of fragment and methylation-based cfDNA tests in diagnosing multiple or specific cancer types. We also searched abstracts for the American Association for Cancer Research Annual meeting, identifying further similar reports of assay development and one systematic review and meta-analysis of the accuracy and applicability of blood-based MCED tests in the general population. None of the reports assessed performance in a symptomatic population.

Added value of this study

To our knowledge, SYMPLIFY is the first study to consider the use of an MCED test in patients with symptoms that might be due to cancer. For cases in which the MCED test detects a cancer signal in this setting, the probability of a cancer diagnosis is greatly increased, and can identify cancers at sites other than those suspected at the original referral, reducing delays in diagnosis. A negative test assigns a lower probability of cancer, but not yet low enough to deflect the need for investigation, other than for upper gastrointestinal symptoms. MCED test results can help assess the risk that a symptom is due to cancer, providing a means for primary care physicians to decide whom to urgently investigate.

Implications of all the available evidence

Our results show that MCED tests have utility in identifying whom with symptoms to investigate for cancer. Current UK clinical guidelines for urgent referral for cancer investigation lead to cancer diagnoses in 4–8% of patients, and identify 55% of cancers. The current MCED test can help identify symptomatic patients for investigation who do not meet current referral criteria, subject to a confirmatory interventional trial. It might also be used to decide whom not to investigate, but in these cases, further work is needed to optimise negative predictive value.

Symptoms of cancer range from specific (eg, breast lump or rectal bleeding) to non-specific (eg, weight loss or abdominal pain).[5] The investigation of specific symptoms follows a relatively clear sequence of investigations, given that there is often only one cancer site under consideration.[6] The UK National Health Service (NHS) directs the rapid investigation of specific cancers in primary care according to defined clusters of patient demographics, symptoms, signs, and test results.[7] Diagnostic pathways are also known as 2-week wait pathways, reflecting the time within which investigation should happen.[8] The investigation of non-specific symptoms is more complex, because there are multiple potential causes.[9–11] Specialised clinics for non-specific symptoms have been rapidly introduced in high-income countries to investigate for cancer at multiple sites and to explain symptoms to patients.[12,13]

Symptoms have poor predictive value for cancer in low-prevalence settings such as primary care, where the tools for risk stratification remain sparse.[14,15] Only 7% of 2·07 million English 2-week wait referrals in 2020–21 resulted in a cancer diagnosis, accounting for 55% of cancer diagnoses that year.[16] Across five non-specific symptom pilots in England, 241 cancers were diagnosed following 2961 referrals, with a conversion rate of 8·1% spread across multiple cancer sites.[13]

More accurate triage tests in primary or secondary care are needed to spare patients without cancer unnecessary invasive and costly investigations.[17] Primary-care clinicians also report a desire to refer additional patients more urgently who do not meet current criteria.[18,19] A test that could discriminate between a high and low likelihood of cancer would be beneficial in this context. At present, only a few tests, such as cancer antigen 125 for ovarian cancer, are available in primary care to triage referrals for specific cancer sites.[20–22] Less specific blood tests, particularly components of the full blood count, liver function tests, and inflammatory markers, are used to assess risk in symptomatic patients,[23,24] but multiple common non-cancer diagnoses cause abnormal results. Novel approaches to risk stratification are urgently required.[1]

Blood-based multicancer early detection (MCED) tests measure cancer biomarkers, such as genetic and epigenetic changes in circulating tumour DNA or proteins produced by cancer cells.[25] Tests have primarily been used for screening in asymptomatic populations or in detecting recurrence following cancer treatment.[1] MCED tests could provide additional diagnostic information in the triage of symptomatic patients with suspected cancer, complementing existing cancer

diagnostics and pathways. We aimed to investigate the performance of a targeted methylation-based MCED test in symptomatic patients referred from primary care for urgent cancer investigation.

Methods

Study design and participants

We did a multicentre, observational study with prospective sample collection at NHS hospital sites in England and Wales. Patients were eligible for recruitment if they were aged 18 years or older, willing and able to give informed consent for participation, and were referred for urgent investigation for a possible gynaecological, lung, lower gastrointestinal, or upper gastrointestinal cancer or to a rapid diagnostic centre with non-specific symptoms that might be due to cancer. Referral criteria for each pathway were as summarised in the National Institute for Health and Care Excellence (NICE) Guideline 12 suspected cancer: recognition and referral (NG12) and the NHS rapid diagnostic centre specification (appendix pp 1–5).[8] Patients were excluded from the study if they had a history of invasive or haematological malignancy diagnosed within the preceding 3 years, had undergone definitive treatment for invasive or haematological malignancy in the past 3 years, were taking cytotoxic or demethylating agents that might interfere with test performance, or had participated in another study of a GRAIL MCED test. All patients were followed up until diagnostic resolution or 9 months. All patients provided written informed consent and the study was undertaken in accordance with the Declaration of Helsinki. The protocol was approved by the National Research Ethics Service (21/LO/0456 - London Central) and complied with UK regulations (appendix pp 40–64).

Procedures

Research staff collected up to 40 mL of blood from participants into Streck cfDNA tubes (Streck, Omaha, NE, USA) at the time of attending an appointment for urgent investigation. Samples were couriered to a central laboratory (Thermo Fisher, Bishop's Stortford, UK) for processing to plasma and freezing within 1 week, then stored until shipped to GRAIL in the USA for batch analysis. Participant data were collected in electronic case report forms including self-reported demographics (age and sex at birth [male or female]), clinical (smoking status, alcohol use, family history of cancer), and referral information (referral pathway, and symptom criteria). Site staff provided the outcome for standard of care investigations within 3 months of enrolment. For cases in which investigations had not been completed, updated information was sought within 9 months of enrolment. Participants were not included in the study if staff failed to collect sufficient blood for analysis, if they withdrew consent, if they were found to be enrolled in violation of the protocol, or if their sample did not pass quality control, in which case they were not followed-up.

Cancer diagnoses were recorded using the International Classification of Diseases 10th edition (ICD-10) and 3rd edition for Oncology (ICD-O-3) to capture cancer site, morphology, and behaviour; and staging was coded according to Union for International Cancer Control TNM or a cancer specific staging system, as appropriate. A modified National Disease Registration Service Routes to Diagnosis classification based on ICD-10 and ICD-O-3 codes was used to map all cancer diagnoses into one of 25 categories (appendix pp 21–39). To prevent bias, the MCED test (Galleri, GRAIL, as reported previously[26]) was run without knowledge of the clinical outcome. Test results included cancer signal detected (yes/no) and up to two predicted cancer signal origins. No MCED results were returned to study participants or clinicians responsible for their care. Electronic case report forms and MCED results were collated in a secure online OpenClinica

See Online for appendix

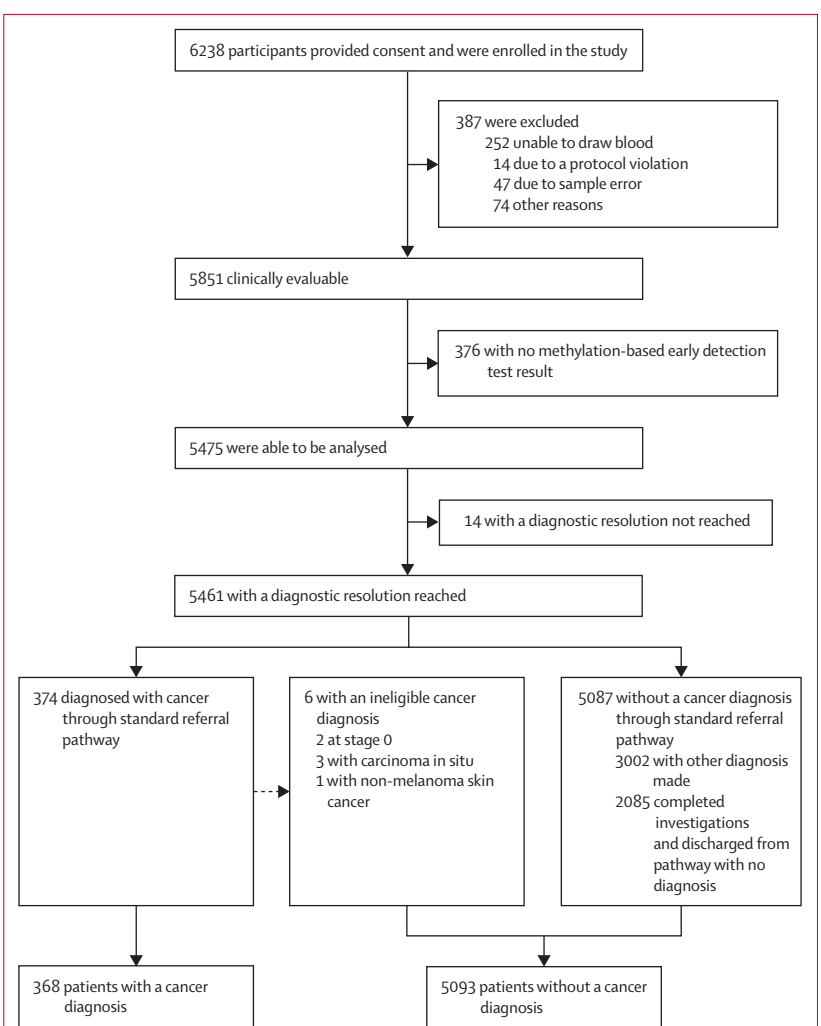


Figure 1: **Study profile**

research database hosted by the Nuffield Department of Primary Care Health Sciences.

Outcomes

The primary outcome was the diagnostic performance (sensitivity, specificity, positive predictive value, and negative predictive value, with 95% CIs) of the MCED test for the detection of new invasive cancer cases based on the cancer signal detection results of the MCED test and the outcome recorded at sites. Secondary outcomes were the same performance characteristics within each referral pathway, the accuracy of the top-one predicted cancer signal origin label from the MCED report with the diagnostic outcome recorded at sites, and the yield for the MCED test (defined as the number of true positives divided by the number of patients within each referral pathway). Prespecified exploratory endpoints are listed in the protocol (appendix p 50).

	Overall (n=5461)	Lung (n=299)	Gynaecological (n=1446)	Upper gastrointestinal (n=1021)	Lower gastrointestinal (n=2202)	Rapid diagnostic centre (n=493)
Cancer diagnosis						
Total	368 (6·7%)	89 (29·8%)	54 (3·7%)	46 (4·5%)	143 (6·5%)	36 (7·3%)
Age, years						
<50	925 (16·9%)	20 (6·7%)	322 (22·3%)	148 (14·5%)	385 (17·5%)	50 (10·1%)
50–59	1527 (28·0%)	54 (18·1%)	661 (45·7%)	226 (22·1%)	500 (22·7%)	86 (17·4%)
60–69	1268 (23·2%)	79 (26·4%)	270 (18·7%)	266 (26·1%)	529 (24·0%)	124 (25·2%)
70–79	1253 (22·9%)	109 (36·5%)	145 (10·0%)	275 (26·9%)	565 (25·7%)	159 (32·3%)
80+	488 (8·9%)	37 (12·4%)	48 (3·3%)	106 (10·4%)	223 (10·1%)	74 (15·0%)
Sex						
Female	3609 (66·1%)	139 (46·5%)	1446 (100·0%)	589 (57·7%)	1161 (52·7%)	274 (55·6%)
Male	1852 (33·9%)	160 (53·5%)	0	432 (42·3%)	1041 (47·3%)	219 (44·4%)
Ethnicity						
White	4938 (90·4%)	283 (94·6%)	1328 (91·8%)	892 (87·4%)	1986 (90·2%)	449 (91·1%)
Mixed	62 (1·1%)	1 (0·3%)	24 (1·7%)	13 (1·3%)	19 (0·9%)	5 (1·0%)
South Asian	200 (3·7%)	10 (3·3%)	30 (2·1%)	63 (6·2%)	87 (4·0%)	10 (2·0%)
Chinese	26 (0·5%)	0	4 (0·3%)	1 (0·1%)	16 (0·7%)	5 (1·0%)
African or Caribbean	171 (3·1%)	3 (1·0%)	49 (3·4%)	38 (3·7%)	71 (3·2%)	10 (2·0%)
Other	64 (1·2%)	2 (0·7%)	11 (0·8%)	14 (1·4%)	23 (1·0%)	14 (2·8%)
Smoking status						
Current smoker	810 (14·8%)	78 (26·1%)	164 (11·3%)	158 (15·5%)	314 (14·3%)	96 (19·5%)
Former smoker	1723 (31·6%)	128 (42·8%)	422 (29·2%)	313 (30·7%)	706 (32·1%)	154 (31·2%)
Non-smoker	2923 (53·5%)	93 (31·1%)	859 (59·4%)	550 (53·9%)	1178 (53·5%)	243 (49·3%)
Missing	5 (0·1%)	0	1 (0·1%)	0	4 (0·2%)	0
Symptoms*						
Abdominal pain	794 (14·5%)	2 (0·7%)	64 (4·4%)	87 (8·5%)	639 (29·0%)	2 (0·4%)
Anaemia	390 (7·1%)	1 (0·3%)	4 (0·3%)	65 (6·4%)	313 (14·2%)	7 (1·4%)
Appetite loss	116 (2·1%)	8 (2·7%)	1 (0·1%)	1 (0·1%)	5 (0·2%)	101 (20·5%)
Bloating	182 (3·3%)	0	57 (3·9%)	20 (2·0%)	77 (3·5%)	28 (5·7%)
Change in bowel habit	1199 (22·0%)	1 (0·3%)	0	8 (0·8%)	1184 (53·8%)	6 (1·2%)
Cough	129 (2·4%)	114 (38·1%)	0	8 (0·8%)	0	7 (1·4%)
Dyspepsia	195 (3·6%)	0	0	192 (18·8%)	1 (<0·1%)	2 (0·4%)
Dysphagia	482 (8·8%)	2 (0·7%)	0	477 (46·7%)	3 (0·1%)	0
Fatigue	180 (3·3%)	22 (7·4%)	3 (0·2%)	1 (0·1%)	17 (0·8%)	137 (27·8%)
Iron deficiency anaemia	193 (3·5%)	0	0	19 (1·9%)	171 (7·8%)	3 (0·6%)
Pain	580 (10·6%)	15 (5·0%)	45 (3·1%)	123 (12·0%)	260 (11·8%)	137 (27·8%)
Post-menopausal bleeding	875 (16·0%)	0	875 (60·5%)	0	0	0
Rectal bleeding	858 (15·7%)	0	0	2 (0·2%)	856 (38·9%)	0
Upper abdominal pain	183 (3·4%)	0	0	183 (17·9%)	0	0
Weight loss	1318 (24·1%)	51 (17·1%)	16 (1·1%)	395 (38·7%)	522 (23·7%)	334 (67·7%)

Data are n (%). Study population is categorised by referral pathway. *Symptoms are the 15 most common clinical features prompting referral with at least ten cancers diagnosed.

Table 1: **Baseline characteristics of study population**

Statistical analysis

NHS urgent cancer referrals data from 2018 were used to estimate the distribution by cancer type and stage within each pathway. Sensitivity estimates by cancer type and stage for GRAIL test data (v2.9 training and holdout cross-validated) using isotonic regression were used at a specificity of 99·4% to estimate the expected positive predictive value and negative predictive value for a given sample size, yielding the 6000 participant target. 6000 participants was predicted to yield 300 cancers. Recruitment by pathway was set between 500 and 2000, according to anticipated cancer prevalence. We permitted over-recruitment to take account of failures to acquire samples, and allowed the study to close before achieving 500 participants in the lung and rapid diagnostic centre pathways because the numbers of cancers diagnosed exceeded predictions.

Primary and secondary outcomes were analysed in participants with a valid MCED test result and diagnostic resolution. We calculated MCED test performance for the detection of invasive cancer as point estimates for sensitivity, specificity, positive predictive value, and negative predictive value, with 95% CIs. We further analysed cancer signal detected performance by referral pathway for the most commonly reported symptoms at referral and according to symptom clusters (as defined in NG12). We also calculated the sensitivity for cancer signal detected by cancer type and clinical stage. We then calculated post-test probabilities using positive and negative likelihood ratios for cancer in each study stratum from pre-test probabilities based on the prevalence of cancer reported in the study for each stratum. We compared the top-one predicted cancer signal origin label from the MCED report with the diagnostic outcome recorded at sites. Additional analyses are described in the statistical analysis plan (appendix p 65) and included assessment of cancer signal origin according to the primary care physician's choice of referral pathway data. All analyses were done in R (version 4.1.3). This study is registered as an International Standard Randomised Controlled Trial, number ISCRTN10226380.

Role of the funding source

The lead investigators (BDN and MRM), GRAIL, NHS England, and the University of Oxford designed SYMPLIFY together. The University of Oxford sponsored SYMPLIFY and was responsible for data collection, data analysis, and data interpretation. GRAIL provided the results of the MCED test, but had no role in the analysis and interpretation of the data. GRAIL authors contributed to the writing of the report.

Results

Between July 7 and Nov 30, 2021, 6238 participants consented to participate in the study across 44 NHS hospital sites in England and Wales. Staff were unable to draw blood from 252 and a further 135 were excluded due to sample errors, participant withdrawal, or identification of ineligibility after enrolment. Of the remaining 5851 clinically evaluable participants, no MCED test result was returned for 376, mainly due to failure to process samples within permitted parameters, and in 14, no information as to final diagnosis was

	Overall (n=368)	Lung (n=89)	Gynaecological (n=54)	Upper gastrointestinal (n=46)	Lower gastrointestinal (n=143)	Rapid diagnostic centre (n=36)
Cancer stage						
I	95 (25·8%)	22 (24·7%)	27 (50·0%)	4 (8·7%)	37 (25·9%)	5 (13·9%)
II	63 (17·1%)	10 (11·2%)	8 (14·8%)	7 (15·2%)	25 (17·5%)	13 (36·1%)
III	108 (29·3%)	21 (23·6%)	10 (18·5%)	17 (37·0%)	53 (37·1%)	7 (19·4%)
IV	86 (23·4%)	30 (33·7%)	8 (14·8%)	14 (30·4%)	25 (17·5%)	9 (25·0%)
Uncertain	16 (4·3%)	6 (6·7%)	1 (1·9%)	4 (8·7%)	3 (2·1%)	2 (5·6%)
Cancer site						
Colorectal	137 (37·2%)	0	6 (11·1%)	7 (15·2%)	119 (83·2%)	5 (13·9%)
Lung	81 (22·0%)	72 (80·9%)	1 (1·9%)	3 (6·5%)	2 (1·4%)	3 (8·3%)
Lymphoma	14 (3·8%)	2 (2·2%)	0	1 (2·2%)	2 (1·4%)	9 (25·0%)
Oesophago-gastric	22 (6·0%)	1 (1·1%)	0	20 (43·5%)	1 (0·7%)	0
Other*	47 (12·8%)	10 (11·2%)	6 (11·1%)	9 (19·6%)	10 (7·0%)	12 (33·3%)
Ovarian	14 (3·8%)	1 (1·1%)	12 (22·2%)	0	1 (0·7%)	0
Pancreas	12 (3·3%)	2 (2·2%)	1 (1·9%)	4 (8·7%)	2 (1·4%)	3 (8·3%)
Prostate	11 (3·0%)	0	0	2 (4·3%)	5 (3·5%)	4 (11·1%)
Uterus	30 (8·2%)	1 (1·1%)	28 (51·9%)	0	1 (0·7%)	0

Data are n (%). *Other includes the following cancer site categories: breast (seven cases), mesothelioma (six cases), anus (five cases), kidney (five cases), liver and bile duct (four cases), cervix (four cases), cancer of unknown primary (three cases), urothelial (three cases), vaginal (two cases), bladder (two cases), and one instance each of bone and soft tissue, CNS, gallbladder, head and neck, malignant immunoproliferative disease, and thyroid.

Table 2: **Cancers diagnosed by site and stage**

available, leaving 5461 as the cohort for analysis (368 with a cancer diagnosis and 5093 without a cancer diagnosis; figure 1).

The median age of the 5461 participants was 61·9 years (IQR 53·4–73·0), 3609 (66·1%) were female and 1852 (33·9%) were male, 2533 (46·4%) ever smokers, and 4938 (90·4%) of White ethnicity (table 1). The most commonly recorded symptoms leading to referral were unexpected weight loss in 1318 (24·1%) referrals, change in bowel habit in 1199 (22·0%), post-menopausal bleeding in 875 (16·0%), rectal bleeding in 858 (15·7%), abdominal pain in 794 (14·5%), and pain in 580 (10·6%) (table 1; appendix p 5–6).

Sites recorded 368 (6·7%) cancer diagnoses from standard of care investigations (table 2). Rates of diagnosis varied between referral pathways, with cancer identified in 89 (29·8%) of the 299 participants recruited from the lung pathway, 54 (3·7%) of 1466 gynae referrals, 46 (4·5%) of 1021 upper gastrointestinal referrals, 143 (6·5%) of 2202 lower gastrointestinal referrals, and 36 (7·3%) of 493 rapid diagnostic centre referrals (table 1). The most common cancer diagnoses were colorectal in 137 (37·2%) of 368 cancers, lung in 81 (22·0%), uterine in 30 (8·2%), and oesophago-gastric in 22 (6·0%; table 2). Just over half of cancers had evidence of nodal or metastatic spread at diagnosis, with 194 (53%) of 368 classified as stage III or IV.

	Total cancers (n=368)	Cancer signal detected (n=244)	Sensitivity (95%CI)
Overall	..	..	66·3% (61·2–71·1)
Cancer stage			
I	95	23	24·2% (16·0–34·1)
II	63	36	57·1% (44·0–69·5)
III	108	92	85·2% (77·1–91·3)
IV	86	82	95·3% (88·5–98·7)
Uncertain	16	11	68·8% (41·3–89·0)
Cancer stage group			
I–II	158	59	37·3% (29·8–45·4)
I–III	266	151	56·8% (50·6–62·8)
I–IV	352	233	66·2% (61·0–71·1)
II–IV	257	210	81·7% (76·4–86·2)
III–IV	194	174	89·7% (84·5–93·6)
Cancer site			
Colorectal	137	97	70·8% (62·4–78·3)
Lung	81	55	67·9% (56·6–77·8)
Lymphoma	14	8	57·1% (28·9–82·3)
Oesophagogastric	22	21	95·5% (77·2–99·9)
Other*	47	30	63·8% (48·5–77·3)
Ovarian	14	9	64·3% (35·1–87·2)
Pancreas	12	11	91·7% (61·5–99·8)
Prostate	11	1	9·1% (0·2–41·3)
Uterus	30	12	40·0% (22·7–59·4)

*Other includes the following cancer site categories: breast (seven cases), mesothelioma (six cases), anus (five cases), kidney (five cases), liver and bile duct (four cases), cervix (four cases), cancer of unknown primary (three cases), urothelial (three cases), vaginal (two cases), bladder (two cases), and one instance each of bone and soft tissue, CNS, gallbladder, head and neck, malignant immunoproliferative disease, and thyroid.

Table 3: **Sensitivity for cancer signal detected, by cancer stage and site**

The MCED detected a cancer signal in 323 cases, 244 of whom had a cancer diagnosed. The overall sensitivity of the MCED test in identifying the presence or absence of cancer across all 368 cancers was 66·3% (95% CI 61·2–71·1; table 3), the specificity was 98·4% (98·1–98·8), the positive predictive value was 75·5% (70·5–80·1), and the negative predictive value was 97·6% (97·1–98·0; figure 2A). Sensitivity varied by stage and cancer site (table 3). Sensitivity 80·4% (66·1–90·6) and negative predictive value 99·1% (98·2–99·6) were highest for patients with symptoms mandating investigation for upper gastrointestinal cancer (figure 2A, B).

Other than for upper gastrointestinal cancers, for which sensitivity was high across all stages, sensitivity was greater with more advanced stage (appendix p 7). Specificity exceeded 95% and positive predictive value exceeded 45% across all pathways, cancer specific symptom clusters, and for individual symptoms (figure 2A, B; appendix pp 8, 10–11).

Pre-test probabilities by SYMPLIFY sub-cohort ranged from 3·1% for participants in the endometrial symptoms cluster to 29·2% for participants in the lung 2-week wait pathway (figure 2C, D). The post-test probability of cancer after a cancer signal detected call ranged from 46·9% (95% CI 29·1–65·3) in the endometrial cancer symptom cluster to 100·0% (47·8–100·0) for the hepatobiliary symptom cluster. The largest increases in cancer risk after a cancer signal detected call were observed for the lower gastrointestinal pathway, the colon symptom cluster, and in participants with change in bowel habit, dyspepsia, pain or weight loss (appendix p 9–11). The post-test probability of cancer following a negative call ranged from 11·4% (95% CI 7·6–16·3) in the lung cancer symptom cluster to 0·0% (0·0–30·8) in participants with dyspepsia (appendix p 12). The probability of cancer after a negative test fell below 1% in the upper gastrointestinal pathway, gastric and oesophageal symptom clusters, and for bloating, dyspepsia, and dysphagia (appendix p 12). Modelling using hypothetical pre-test probabilities identified clusters of symptomatic patients for whom an MCED test might have the capacity to both rule-in and rule-out cancer (appendix pp 13–14).

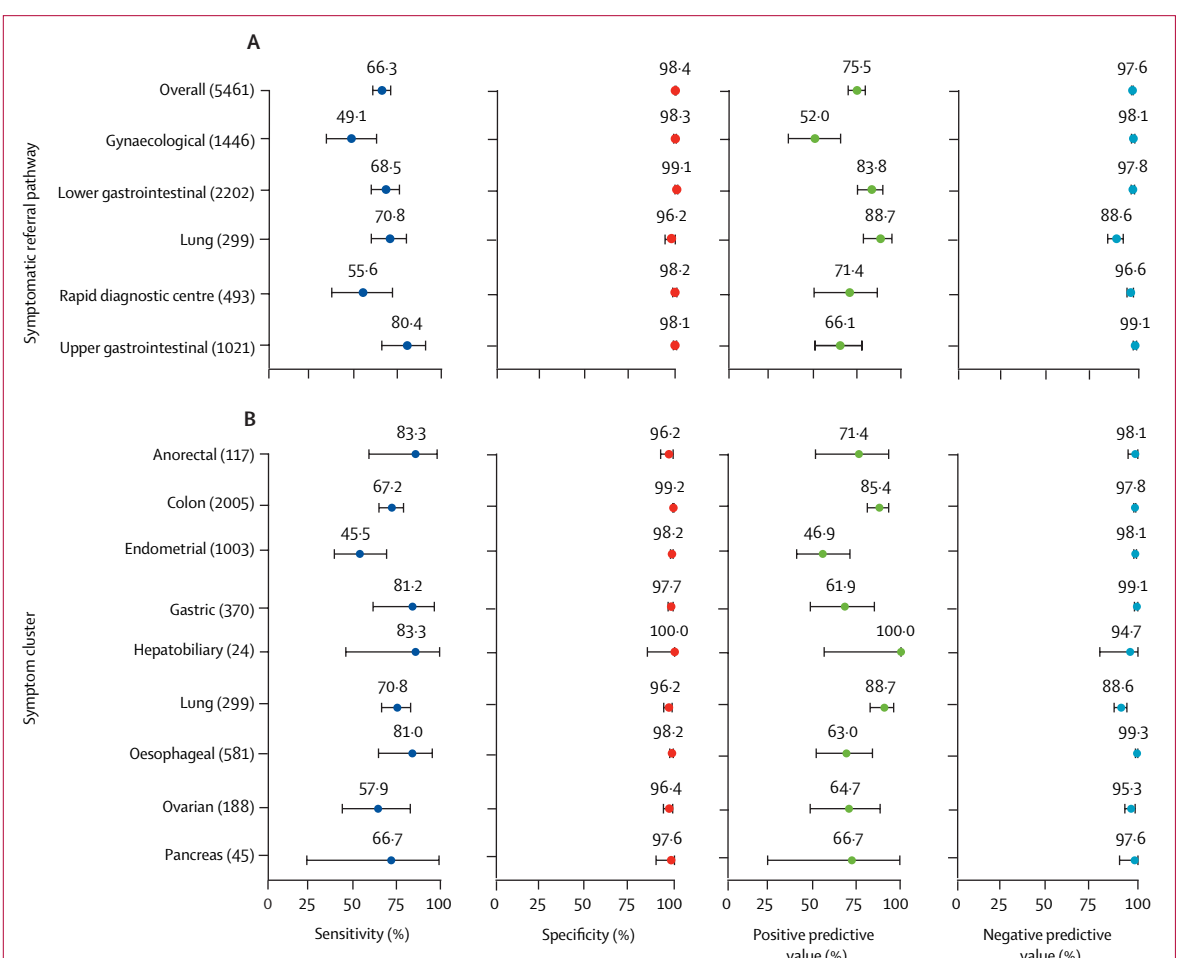
The overall accuracy of the top cancer signal origin prediction in cases for which a cancer signal was detected was 85·2% (95% CI 79·8–89·3; figure 3), ranging from 71·7% (58·4–82·2) for cancers diagnosed by the lung pathway to 93·8% (86·5–97·5) for the lower gastrointestinal pathway (appendix pp 15–19). Including the top two cancer signal origins, calls increased the overall cancer signal origin accuracy to 90·7% (95% CI 86·0–93·9). Cancer signal origin accuracy was higher for stage III–IV cancers, compared with stages I–II (88·2% *vs* 77·2%; appendix p 20).

Exploratory outcomes related to resource utilisation and to the completeness of data collected from central registries have not been presented and will be reported in future articles.

Discussion

We report findings of a large-scale prospective investigation of MCED test performance in symptomatic patients referred for cancer investigation in the NHS. We observed 66·3% (95% CI 61·2–71·1) sensitivity and 98·4% (98·1–98·8) specificity, similar to the findings of a 2023 systematic review[27] of the accuracy of blood-based MCED tests in the asymptomatic population found in ten case-control and six cohort studies, in which pooled sensitivity was 0·66 (95% CI 0·54–0·75) and specificity 0·98 (0·94–0·99) across ten studies selected for meta-analysis. Sensitivity was higher for advanced staged cancers, and sensitivity and specificity were unaffected by study type, sex at birth, or assay. Pooled accuracy of tumour origin prediction was 0·79 (95% CI 0·64–0·91) similar to the 85·2% (95% CI 79·8–89·3) we observed. These studies have largely been in mixed asymptomatic and symptomatic populations with poor characterisation of symptoms, and there are few data on MCED test performance in symptomatic patients.[3,12,25,27,28] In our well-characterised prospective symptomatic cohort, we show increased sensitivity for cancer detection with increasing cancer stage, consistent with previous reports, and additional variation in accuracy by referral pathway, symptom cluster, and for individual symptoms. This shows the importance of clinical context in interpreting the results of MCED tests and underlines the need for careful evaluation of each use case in its own right.

Because of the observational nature of the study, the NHS standard of care was relied upon at each recruiting site to ascertain cancer outcomes. Variation in clinical practice across recruiting sites was mitigated by recruiting from established, protocolised 2-week wait pathways that followed national standards. Investigations are directed to rule out the cancer linked to the symptoms driving referral, whereas the MCED test assesses whether there is a cancer signal detected and then predicts the cancer signal origin across 21 possible cancer classes. A false positive MCED result, following



(Figure 2 continues on next page)

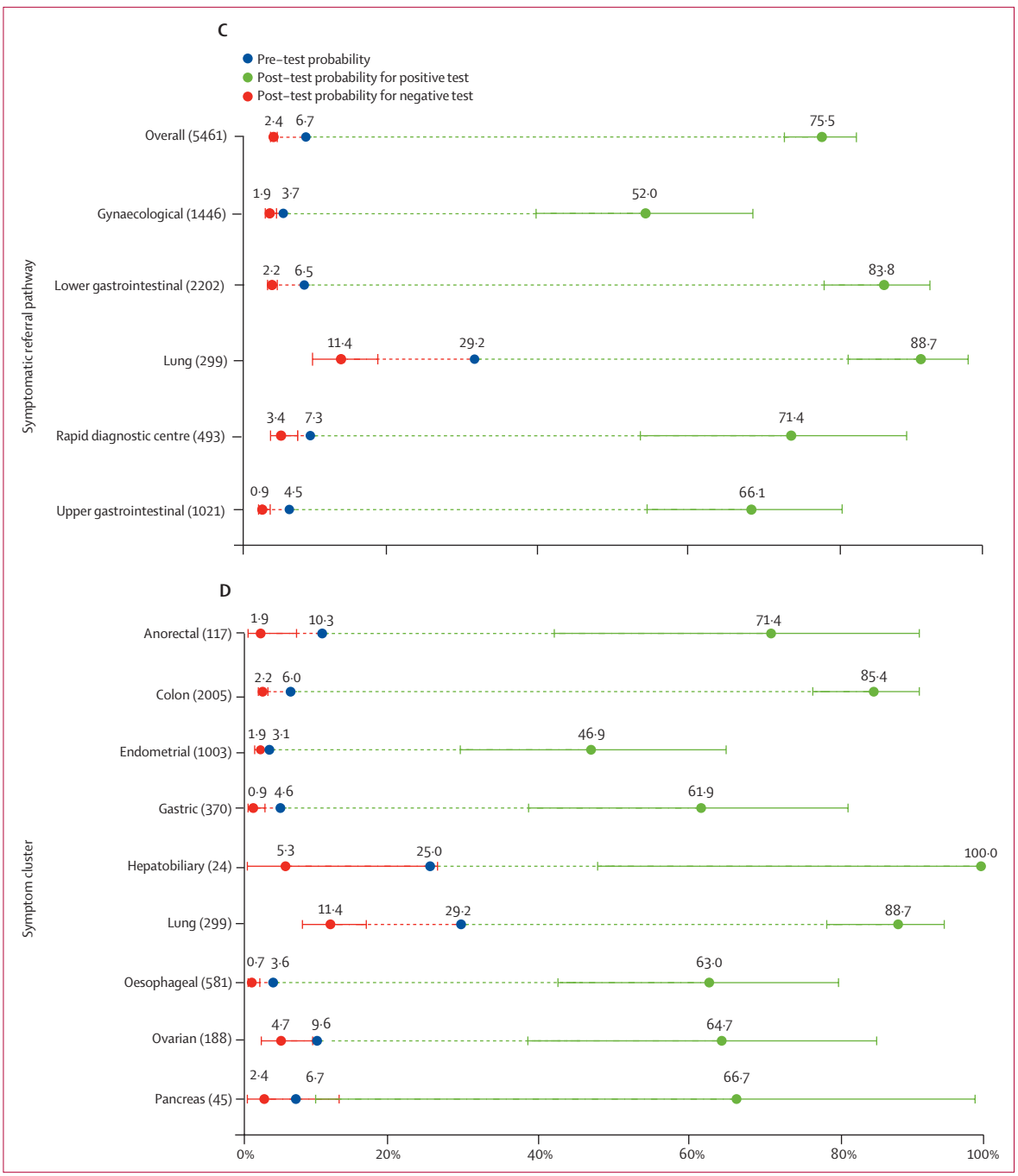


Figure 2: **Multi-cancer early detection test performance and effect on the probability of cancer**
Sensitivity, specificity, positive predictive value, and negative predictive value in participants with cancer signal detected, overall and by referral pathway (A) and by symptom cluster (B) and pre-test and post-test probabilities for cancer in participants with positive and negative cancer signal detected results, overall and by referral pathway (C) and by symptom cluster (D). The numbers in parentheses following the sub-cohort names show the number of participants. Error bars denote 95% CIs.

an appropriate investigative pathway based on guideline criteria, might therefore represent a true positive for a cancer that would not be diagnosed, leading to an underestimation of MCED accuracy. In mitigation, we asked sites to report delayed and subsequent cancer diagnoses after diagnostic resolution was reached for initial investigations. Even with these mitigations in place it remains plausible that the MCED test could detect early cancers that did not become clinically observable during the study period, albeit these cancers are unlikely to be related to the symptoms that led to the original referral for investigation.

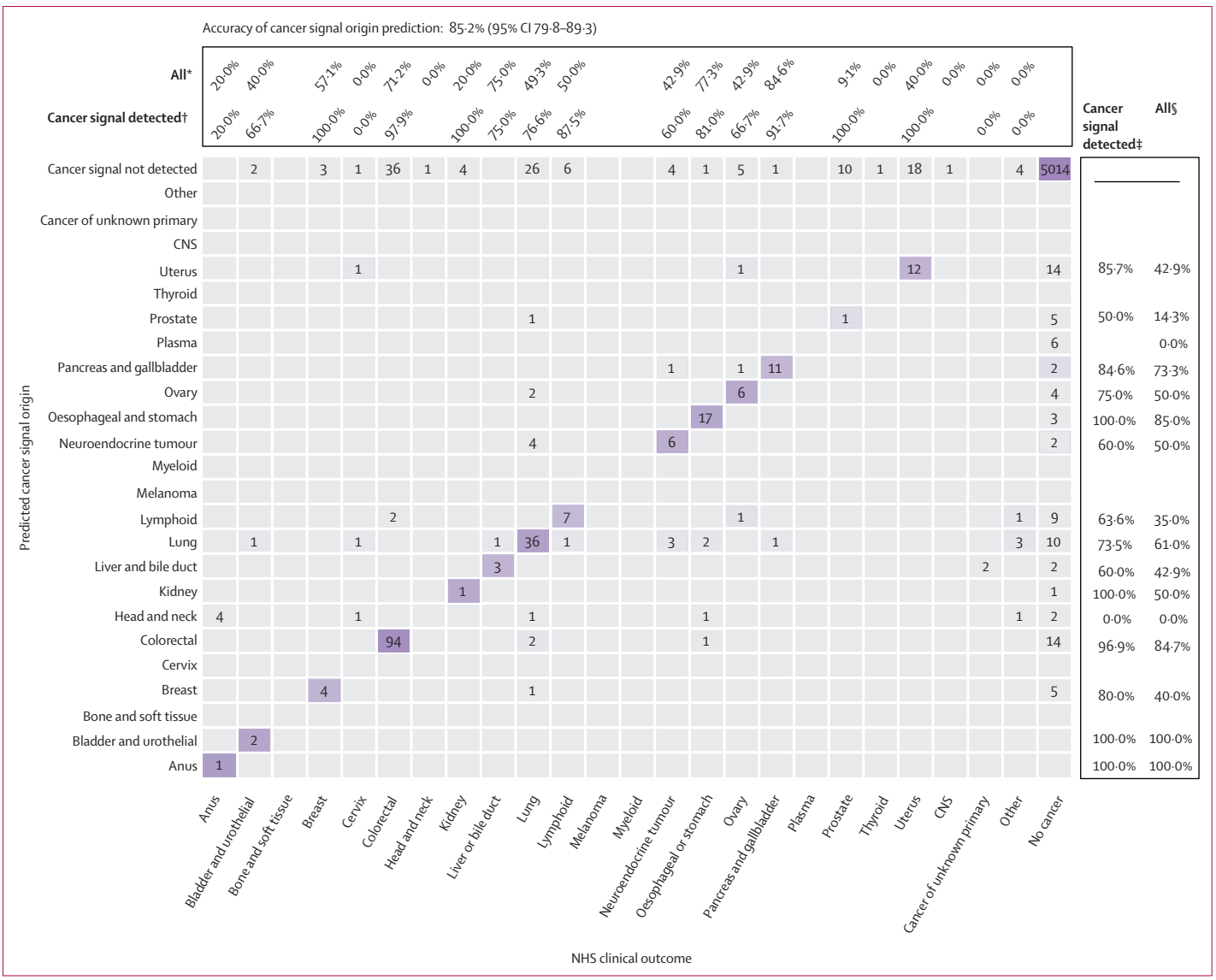


Figure 3: **Matrix of top cancer specific origin prediction and NHS cancer outcome.**
Boxes enumerate the percentages of correct cancer signal origin calls among all cancers diagnosed and all cancers diagnosed with a cancer signal detected on the multi-cancer early detection test. Purple boxes highlight the correct call with a deeper shade of purple shown for increasing numbers. NHS=National Health Service. *Denotes the percentage of correct site calls among all cancers diagnosed (calculated by dividing the number of correct calls by the sum of all the boxes in the relevant column). †Percentage of correct site calls among all cancers diagnosed and called as cancer signal detected (calculated by dividing the number of correct calls by the sum of all the boxes in the column other than the cancer signal not detected box). ‡Percentage of correct site calls among all cancers diagnosed and called as cancer signal detected (calculated by dividing the number of correct calls by the sum of all the boxes in the row other than the no cancer box). §Percentage of correct site calls in participants in whom cancer was diagnosed and this cancer site call was made (calculated by dividing the number of correct calls by the sum of all boxes in the row). The final column in the figure shows the cancer signal detected call and the site call when a cancer signal was detected in participants in whom no cancer was diagnosed by standard investigation.

To our knowledge, this is the largest prospective cohort study of symptomatic patients referred for cancer investigation. The wide geographical spread of the recruiting sites included a mix of large high-volume specialist centres and smaller hospital sites to capture the range of socio-economic statuses and ethnicities representative of the UK. The high resolution afforded by comprehensive characterisation of the cohort's demographics, presenting symptoms, referral route, cancer site, and cancer stage allows close inspection of symptom patterns and cancers with diagnoses that would most be impacted by MCED testing.

The high overall specificity, positive predictive values, and post-test probabilities for cancer signal detected reported across cancer types indicate that, if positive, the current MCED test could be used to confirm that referred patients should be investigated for cancer. The moderate overall sensitivity suggests that a negative result using the current classifier would not be sufficient to deflect patients who already qualify for investigation on the

basis of their clinical presentation, other than for upper gastrointestinal pathways. This is partly a function of the relatively high pre-test probability in our referred participants. Our results indicate that the current machine learning algorithm underlying the MCED test could play a role in the triage of symptoms in patients with a lower pre-test probability. Once the algorithm, which was optimised for positive predictive value in an asymptomatic population, is optimised using our data for negative predictive value in symptomatic patients it should have greater utility in ruling out the need for further investigations for cancer.

Considering the groups of patients included in this study, MCED test performance appeared most promising in patients referred for investigation of a possible upper gastrointestinal cancer. The post-test probabilities of cancer with a negative cancer signal detection in the upper gastrointestinal pathway or oesophageal symptom cluster were 0·9% and 0·7% respectively, limiting the MCED test's use in avoiding endoscopy in our symptomatic population. Were the test to be deployed in a lower risk population, these post-test probabilities would be lower and therefore of use in avoiding invasive investigations. Our results indicate that the MCED test might be useful in identifying a wider group of patients to be referred to the upper gastrointestinal 2-week wait pathway and are consistent with the notion that MCED tests might better be focused on clusters of non-specific symptoms that could represent a wide range of cancer types, retaining a broad field of target for cancer detection. MCED test performance in patients referred to rapid diagnostic centres was relatively poor. Further interrogation of the presenting features of the patients showed a wide range of indications for rapid diagnostic centre referral, which were not limited to non-specific symptoms. When individual non-specific symptoms were investigated, such as abdominal pain, anaemia, bloating, and weight loss, diagnostic performance was superior to the rapid diagnostic centre category overall. For example, in primary care, the positive predictive value of weight loss leading to a cancer diagnosis is about 2–3%. A negative MCED test with current performance reduces the post-test probability to around 0·5%, suggesting a non-specific symptom-based use case is worth exploring. More work is needed to define rule-out thresholds with patients, practitioners, and policymakers and will likely vary by health system. An interventional study in this setting, with patients whose symptoms do not trigger urgent investigations, might focus on the time to diagnosis, the stage at diagnosis, the proportion of patients receiving a delayed diagnosis, the resources used to achieve that diagnosis as early readouts, and on overall survival and mortality in the longer term.

Although conducted in the UK NHS, our results are more broadly applicable. International studies reporting the accuracy of symptoms in primary care were used to derive NICE guidance and the symptom clusters used in SYMPLIFY. These clusters reflect clinical practice and are used to guide referrals outside of the UK.[29,30] Nevertheless, the relevance of particular symptom clusters, and the likelihood of their occurrences being due to the presence of a cancer is expected to differ between health-care systems. For example, screening and investigation for oesophagogastric cancer differs substantially between Europe and North America, and Asia, meaning that the potential impact of the MCED test will need to consider the context in which it is deployed.

Many cancers were diagnosed at sites other than those inferred by the symptoms that led to referral. This was most pronounced in the upper gastrointestinal and gynaecological pathways, for which 47% and 25% of cancers, respectively, were incongruent with the referral pathway. This indicates the difficulty in primary care of achieving an efficient work-up and diagnostic resolution when cancer is suspected. Across the study, cancer signal origin was called correctly in 85% of cancers, with several erroneous calls explained by shared tumour biology. The high accuracy of cancer signal origin in patients with cancer signal detected could add valuable additional information to inform test sequencing and reduce the time to diagnosis and cost in patients referred for urgent cancer investigation.

Because an observational study can only model the impact of introducing the MCED diagnostic test on clinical decision making, resource utilisation, or clinical outcomes, an interventional study is required to evaluate these definitively. From our results it seems likely that, with the exception of gastrointestinal cancers, such a study is best conducted in a population in primary care, where the MCED test could be used to inform the decision to refer symptomatic patients for further cancer investigation.

Contributors

BDN, MRM, HK, SH, KR, RP, JO, YL, and HN designed the study. Data were collected by DAH, CO'D, JESP, ZH, VS, AM, LM, ST, MV, LE, L-MY, CF, and SP. Data analysis was done by JO, PSV, L-MY, RP, and HN. Data interpretation was done by BDN, MRM, HK, SH, KR, RP, JO, YL, HN, KNK, and FDRH. Data are held by the academic partner and were accessed and verified by JO, RP, and PSV. This report was written by the lead investigators and was reviewed and approved for publication by all co-authors, including those from the funder, and the sponsor. The authors vouch for the completeness and accuracy of the data and the data analyses. All authors had full access to all data in the study and had final responsibility for the decision to submit for publication.

Declaration of interests

BDN and MRM receive institutional research funding from GRAIL. MRM reports grants from Roche, Astrazeneca, BMS, Infinitopes, Immunocore, and study fees from BMS, Pfizer, MSD, Regeneron, BiolineRx, Replimune and Novartis outside of the submitted work. HK, KNK, SH, KR, YL, and HN are employees of GRAIL. HK, KNK, SH, KR, and YL hold stock in Illumina. HK reports a leadership position with GRAIL. DH reports a leadership role with CanSense, outside of the submitted work. All other authors declare no competing interests.

Data sharing

De-identified individual-level patient data can be provided to researchers upon written request 24 months after publication of the Article. Please send enquiries to the corresponding author. A detailed proposal for how the data will be used is required to allow assessment of the application.

The study protocol, statistical analysis plan, patient information sheets, and informed consent form are available with the publication in the appendix.

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