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ORIGINAL ARTICLE

A multi-centre performance evaluation of a commercially developed liquid biopsy for the earlier detection of brain tumours

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Background: Delayed diagnosis of brain cancer leads to two-thirds of patients receiving a diagnosis only after presenting to the emergency department with more severe symptoms or neurological deficits. A simple, rapid, liquid biopsy implemented in primary care could enable more efficient triage of patients with non-specific symptoms potentially related to brain cancer, prioritising patients for urgent brain imaging, and accelerating diagnosis.
Patients and methods: Presented is the international, multi-centre, observational Early and tiMely detection of BRAin CancEr (EMBRACE) study. Patients were prospectively recruited across seven sites in Europe, from the United Kingdom, Belgium, Sweden and Switzerland. The target population consisted of patients with symptoms potentially associated with brain cancer. Blood serum samples were analysed by the Dxcover® Brain Cancer Liquid Biopsy Platform. Test performance was assessed by comparison of the liquid biopsy result to diagnostic imaging.
Results: Two thousand five hundred and fifty-four patients were enrolled across the seven collection sites; 2324 were deemed eligible and taken forward for test assessment. There were 697 brain tumours in total, of which 395 were malignant, and 1627 non-tumour diagnoses. Overall diagnostic performance for the primary objective was 86% sensitivity for brain cancer detection with 99% negative predictive value (NPV). Sensitivity for all brain tumours combined (malignant and benign) was 77%. Notably, for the most prevalent and most aggressive brain cancer, glioblastoma, 86% of cases were successfully identified. Additionally, 94% of patients with central nervous system lymphoma, and 90% of brain metastases were predicted correctly as having tumours.
Conclusions: Existing symptom-based referral pathways are ineffective for the detection of brain cancer, and there is an urgent need for new tests to help with clinical decision making. With a NPV of 99%, the Dxcover Liquid Biopsy test could assist in primary care for efficient stratification of patients toward diagnostic imaging.
Key words: brain tumour, brain cancer, liquid biopsy, early detection

INTRODUCTION

In 2022, ∼321 731 individuals worldwide were diagnosed with a primary malignant brain tumour.[1] Unfortunately, mortality rates closely mirror incidence rates because of the aggressive nature of these tumours. In 2019, the global age-standardized mortality rate for all malignant brain tumours was 3.9 per 100 000 in males and 2.8 per 100 000 in females.[2] Earlier diagnosis is an important consideration for

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improving patient prognosis and quality of life. It was recently reported that in the UK up to two-thirds of brain tumour patients receive a diagnosis via the emergency department (ED).[3] This is despite there being opportunities for earlier detection in the diagnostic pathway, as many patients will have visited their primary care team several times before their tumour diagnosis.[4] Across international health care systems, the challenge of brain tumour diagnosis in patients with non-specific symptoms can contribute to marked diagnostic delay. It is evident that the early diagnosis of brain tumours remains a challenge across Europe.[5,6]

A significant barrier to achieving earlier detection is that symptoms of brain tumours are usually non-specific and a non-tumour diagnosis is more likely. The positive predictive value (PPV) of symptom-based referral guidelines for suspected brain cancer, for the presence of 'symptoms related to the central nervous system,' is 2.9%.[7] However, few patients present with such well-differentiated symptoms. The PPV for the symptoms most commonly associated with brain tumours, such as headache, is only 0.09%.[8] Unsurprisingly then, only 1.3% of brain tumour patients diagnosed in England in 2020 were identified following urgent referral from their primary care team.[9] Similarly, <2% of patients referred for brain imaging to exclude a possible brain tumour diagnosis are actually found to have a brain tumour.[7]

Innovative approaches are needed to support decision making in primary care. A simple, rapid blood test could enable more efficient triage of patients with non-specific symptoms. The most at-risk patients could be fast-tracked for urgent brain imaging while those at lower risk could be managed expectantly or forwarded to another type of diagnostic work-up. The Dxcover® Brain Cancer Liquid Biopsy Platform is a qualitative *in vitro* spectroscopic human blood serum test intended to assist triage of suspected brain tumours in the symptomatic population, supporting clinical decision making. The Dxcover test is well-suited to standard clinical pathways, and capable of a rapid test turnaround time of 1 day.

The test has been in development since 2012, starting with feasibility studies on retrospective biobank samples.[10-22] The first prospective study, BRAIN-ED1, in a targeted population of patients referred by their primary care doctor for urgent brain imaging, demonstrated 92% sensitivity for the most common brain tumour, glioblastoma, and 81% sensitivity for all brain tumours, with 80% specificity.[23,24] The subsequent BRAIN-ED2 study reported a sensitivity-tuned model achieving 96% sensitivity with 45% specificity [negative predictive value (NPV) 99.3%], and identified 100% of glioblastoma patients.[25] When tuned for a higher specificity, the model reported sensitivity of 47% with 90% specificity (PPV 28.4%). Before commencing an external clinical performance evaluation, an internal verification study was carried out to determine the analytical performance of the test; a requirement in the development of an *in vitro* diagnostic (IVD) device under the *in vitro* diagnostic regulation (IVDR).[26] The design and development of the Dxcover Platform, including diagnostic algorithm, was finalised following design control standards (ISO 13485). The cut-off value of the test, which denotes the probability threshold generated by the diagnostic algorithm above which a case is classified as positive, was locked down before beginning the study. In a cohort of 415 patients, the sensitivity for malignant brain tumours was 88%, and specificity was 51%. The sensitivity for all brain tumours (benign and malignant) was 80%.

Here the results of the world's largest brain tumour-focused liquid biopsy study is presented. It is an observational, prospective, multi-centre clinical performance evaluation study. The diagnostic algorithm has remained locked down for this study. There were seven recruitment sites across the UK and Europe. The Dxcover Liquid Biopsy Platform was installed for analysis in three of these sites, to mimic routine use of the test. The primary objective was to determine the clinical performance of the test for patients with suspected brain tumour, focused on diagnostic sensitivity and specificity for malignant brain tumours. The secondary objective was to determine the test performance for all brain tumours (malignant and benign).

PATIENTS AND METHODS

This report presents a clinical validation of a brain tumour liquid biopsy through an observational, prospective, multi-centre study.

Target population

The target population consisted of patients presenting with head-related symptoms that could be associated with a brain tumour, from two patient pathway cohorts:

Cohort 1. Participants presenting acutely to hospital for assessment, where the differential diagnosis included a brain tumour, and where the assessing clinician identified a need for a diagnostic investigation [e.g. computed tomography (CT) or magnetic resonance imaging (MRI)].

Cohort 2. Participants with a recent, newly established diagnosis of a brain tumour, before instigation of surgery, chemotherapy or radiotherapy.

Study overview

The primary objective of this study was to determine the clinical performance of the test for patients with a brain tumour, in terms of diagnostic sensitivity and specificity for malignant brain tumours. Brain tumour status was defined as participants definitively diagnosed with a brain tumour via medical imaging and confirmed through histopathology (where available). The secondary objective was to determine the clinical performance of the test for all brain tumours, including both malignant (cancerous) and benign (non-cancerous) tumours.

Before the study, a power calculation was conducted by Edinburgh Clinical Trials Unit to determine the most appropriate sample size. The primary aim of the study was to estimate the parameters of sensitivity and specificity

with a pre-defined precision, namely a 95% confidence interval with a half-width of 2.5%. Desired statistical power was 90%. The prevalence of brain cancer in the population was assumed to be 10%. The target sensitivity and specificity were 96% and 45% respectively, based on results from the BRAIN-ED2 study.[25] Applying a normal approximation for the error distribution and calculating sample size following the method of Buderer,[27] a total sample size of ~2200 patients including at least 237 brain cancer cases, was estimated to be required. Based on expected disease prevalence, recruiting from cohort 1 alone would not provide the required number of brain tumour positive samples to be statistically relevant. Cohort 2 was therefore included to enrich the number of positive cases.

A summary of the clinical performance evaluation study, and the principal investigator information for each contributing site, can be found in Supplementary Tables S1 and S2, available at https://doi.org/10.1016/j.esmoop.2025.105938, respectively. The study was conducted in accordance with ethical principles as outlined by the Declaration of Helsinki and the International Conference on Harmonisation Tripartite Guideline for Good Clinical Practice. All necessary ethical approvals were obtained from each contributing site (Supplementary Table S3, available at https://doi.org/10.1016/j.esmoop.2025.105938). There were seven recruitment sites involved in this study: the Royal Infirmary of Edinburgh; The Walton Centre National Health Service Foundation Trust (Liverpool); Antwerp University Hospital; St James' University Hospital (Leeds); Sahlgrenska University Hospital (Gothenburg); University Hospital Zurich; Royal Preston Hospital. The site investigators were responsible for ensuring informed consent was obtained before any study-specific procedures were carried out. Participants always received adequate oral and written information. The oral explanation to the participant was carried out by trained site staff. All patient facing documents (informed consent form and patient information sheet) were specific to the respective institutions and were prepared according to local requirements and review processes.

Sample collection

Blood samples were obtained during routine venepuncture using S-Monovette Serum Gel Z collection tubes (Sarstedt, Germany) and anonymized for processing and analysis. Each sample was gently inverted and allowed to clot at 2-8°C. Samples were centrifuged for 15 min at 2200 g and stored in a −80°C freezer. Data interpretation was blinded to brain imaging and histological diagnosis. Imaging outcomes were recorded from the formal radiological report and histological tumour diagnosis when available for patients that underwent surgery.

Study data were collected and managed using REDCap (Research Electronic Data Capture) tools hosted at the Edinburgh Clinical Trials Unit.[28,29] REDCap is a secure, web-based software platform designed to support data capture for research studies, providing (i) an intuitive interface for validated data capture; (ii) audit trails for tracking data manipulation and export procedures; (iii) automated export procedures for seamless data downloads to common statistical packages; and (iv) procedures for data integration and interoperability with external sources. The database meets standards on data security and participant privacy and confidentiality.

Sample analysis

Patient serum samples were analysed using the Dxcover Brain Cancer Liquid Biopsy Platform. For further details we direct the reader past publications.[23-25] Briefly, patient whole blood samples were collected and processed on-site via standard clinical sample preparation procedures before brain imaging or brain tumour surgery. The resultant serum samples were stored at −80°C until the date of analysis. The serum samples were allowed to thaw for up to 30 minutes at room temperature (18-25°C) and inverted three times to ensure sufficient mixture and thawing. For each patient sample, 3 µl of serum was pipetted into each of three sample wells of the Dxcover® Sample Slides (Dxcover, Glasgow, UK). Each patient sample was analysed by the Dxcover Brain Cancer Liquid Biopsy Platform, which takes 15 minutes to produce a test result. The cut-off value of the test, which denotes the threshold above which predicted scores (probabilities) generated by the diagnostic algorithm are classified as positive, was 0.3234. Anonymized samples were reported as positive or negative according to test results.

Dxcover Platforms were installed at three sites: Edinburgh, Liverpool and Antwerp. The samples were analysed by the research team at each of the analysis sites, who were fully trained by Dxcover staff. The three analysis sites analysed samples collected at their own facility. The samples collected in Gothenburg and Zurich were shipped to Antwerp for analysis. In the UK, Preston and Leeds transferred their samples to Edinburgh for analysis. All analysts were blinded to the true diagnoses during the analysis period. Dxcover as the study sponsors were blinded to the REDCap database until all analyses were complete. Once the Dxcover test results were recorded in the study database, the true diagnoses (i.e. outcome from medical imaging via CT/MRI) were unblinded for data analysis by the study's co-ordinating investigator (P.M.B.).

Data analysis

Patients were categorised into 'malignant' and 'benign' groups based on a consensus approach, led by coordinating investigator and lead clinician (P.M.B.). To assign a benign or malignant label to each case, the following general rules were applied: (i) grade 1 and 2 tumours were labelled as benign; (ii) grade 3 and 4 were labelled as malignant; (iii) cysts were labelled as non-tumour, except epidermoid cysts, which were grouped with the benign tumours. Where a tumour diagnosis was made based on brain imaging, but

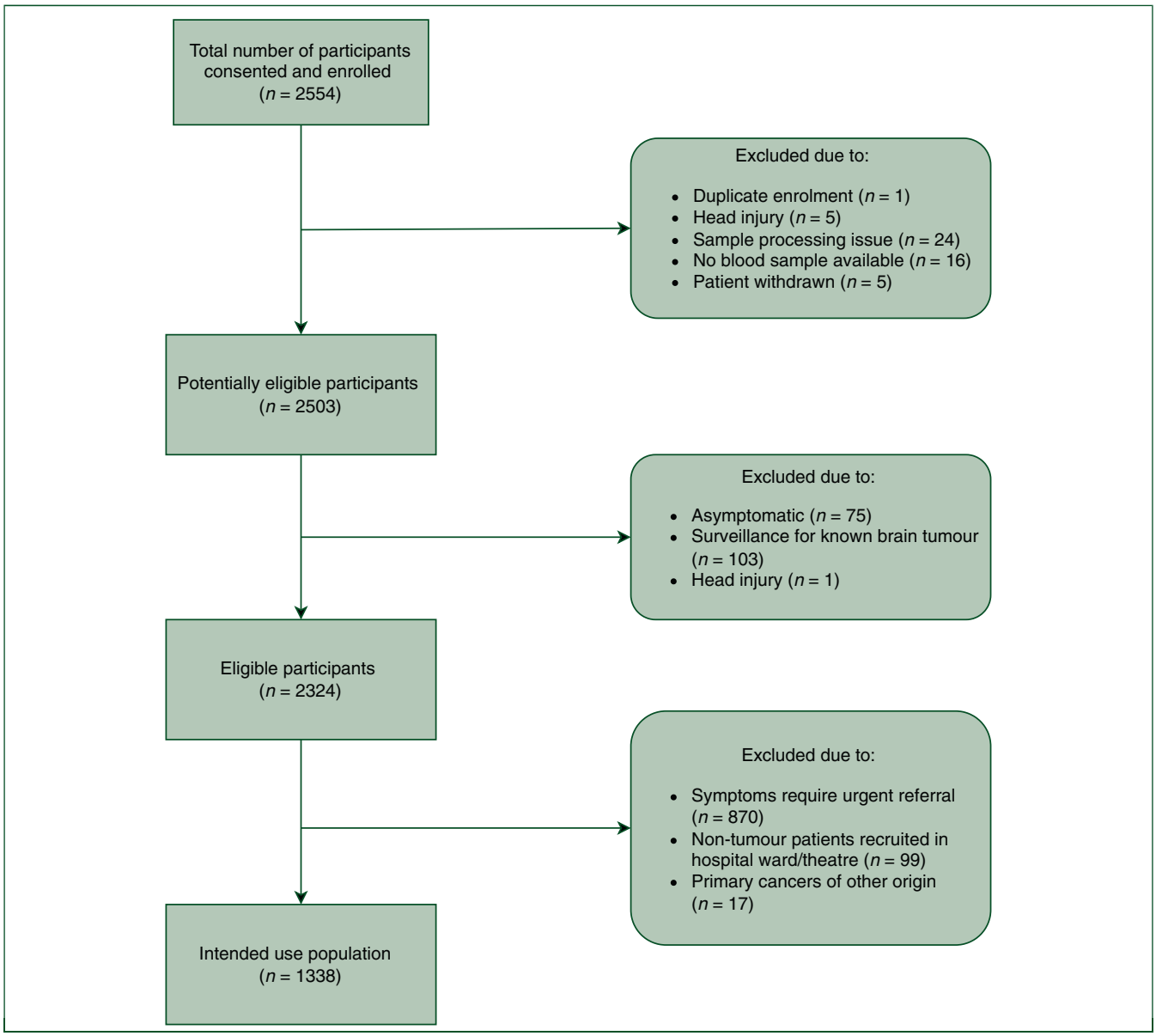

Figure 1. **Flowchart overviewing patient eligibility and study inclusion.**

it could not be determined whether the tumour was benign or malignant, the diagnosis was labelled as 'unclear'.

Participants without imaging findings suggestive of a tumour were considered to have a non-tumour status and the medical notes were reviewed by each site after 3 months to ensure that there was no delayed tumour diagnosis. If no definitive determination could be reached as to whether a patient had a tumour, a final label was assigned (by PMB), based on the available information.

In order to assess test performance in the intended use population, eligible participants were also assigned a symptom group label—non-specific or urgent—based on the nature and severity of their presenting symptoms. Patients with symptoms that met the criteria for urgent referral for suspected stroke, for example via a Face Arm Speech Test (FAST) pathway,[30] would be eligible for direct brain imaging from primary or secondary care, and would not be suitable candidates for a triage blood test.

Additionally, we utilised the Kernick referral criteria[31] to categorise patients into non-specific and urgent groups. Current guidelines in the UK to support primary care doctors in identifying patients most at-risk of having a brain tumour include the Kernick referral criteria, which utilize a red/orange/yellow flagging system to reflect three levels of patient 'risk' for having a brain tumour based on symptoms (Supplementary Table S4, available at https://doi.org/10.1016/j.esmoop.2025.105938).[31] Red flags warrant urgent investigation (papilloedema, new epileptic seizure, significant alterations in consciousness, memory, confusion or coordination, etc.). The orange and yellow flags have a low threshold for investigation but require monitoring/management. These are typical non-specific symptoms, such as headache,

Table 1. Eligible patient cohort characteristics

		Tumour				Non-tumour	Total
		Malignant	Benign	Unclear	All tumour		
Sex	Male	235	131	4	370	794	1164
	Female	160	160	7	327	833	1160
Age, years (deciles)	10	—	—	—	0	9	9
	20	7	18	1	26	112	138
	30	21	30	1	52	163	215
	40	42	49	1	92	203	295
	50	77	69	4	150	302	452
	60	148	63	2	213	338	551
	70	90	46	1	137	325	462
	80	10	14	1	25	154	179
	90	—	2	—	2	21	23
Collection site	Edinburgh	87	17	2	106	948	1054
	Liverpool	80	76	2	158	40	198
	Preston	41	15	0	56	347	403
	Leeds	47	64	5	116	16	132
	Antwerp	19	35	2	56	131	187
	Gothenburg	66	48	—	114	56	170
	Zurich	55	36	—	91	89	180
Overall		395	291	11	697	1627	2324

confusion, migraine, weakness or motor loss, memory loss and personality change. Patients with non-specific symptoms are the intended target population of the Dxcover test, as those are the patients who should be triaged into imaging.

Participants with urgent/red-flag symptoms, those with a known primary cancer, and those non-tumour patients recruited from inpatient secondary care facilities, e.g. hospital ward or theatre, (Figure 1) were not included in the intended use population.

RESULTS

Recruitment overview

Two thousand five hundred and fifty-four patients enrolled in this study across the seven collection sites: Edinburgh ($n = 1082$); Liverpool ($n = 259$) Preston ($n = 418$) Leeds ($n = 229$) Gothenburg ($n = 173$) Antwerp ($n = 208$) Zurich ($n = 185$). Of the 2554 recruited participants, 2324 were determined to meet the eligibility criteria. Figure 1 overviews the flow of participants in the study, as per the Strengthening the Reporting of Observational studies in Epidemiology (STROBE) reporting guidelines.

Fifty-one patients were removed due to withdrawals, sampling issues and ineligibility highlighted by site staff after the initial enrolment. Of the remaining 2503 patients, 179 participants were removed after clinical information review, where patients did not meet the inclusion criteria, for example because they had no symptoms or because they were under surveillance of a known tumour rather than presenting with a new or recent tumour. Some patients had no known primary malignancy but were diagnosed with brain metastasis. They were included in the analysis if they were symptomatic when diagnosed with the brain tumour. Two thousand three hundred and twenty-four participants were therefore determined to be eligible for the study and were included in the primary analysis. A sub-group of patients was determined based on those who presented with non-specific symptoms, who then formed the intended use population ($n = 1338$).

Characteristics of the eligible patient cohort ($n = 2324$) are detailed in Table 1; the dataset is sex-matched with an approximate 50 : 50 split of males and females, with a similar number of tumours and non-tumours in each sex. Forty percent of participants were aged between 50 and 80 years. There were 697 patients with brain tumours, and 1627 non-tumour diagnoses, resulting in a tumour prevalence of 30%. Of the tumours, 395 were malignant (17% of total cohort).

Eligible patients for primary analysis

The primary objective was estimation of diagnostic performance (sensitivity and specificity) for the detection of malignant tumours. Supplementary Table S5, available at https://doi.org/10.1016/j.esmoop.2025.105938, overviews test sensitivity and specificity for each individual site, and the overall test performance for all sites combined. The overall sensitivity for malignant brain tumour detection was 86% (339/395) with 39% specificity (632/1627). The sensitivities for the malignant cases were consistent across collection sites, with five of seven sites reporting sensitivities >80%.

The secondary objective was to assess test performance for all brain tumours (malignant and benign). With the inclusion of benign brain tumours, overall sensitivity decreased slightly (77%). There was greater variance in test specificity values between sites, yet Edinburgh, Liverpool, Preston and Antwerp have values close to the overall specificity. The specificity for Leeds was higher than average, at 81%. The correct determination of non-tumour samples was significantly less in patients recruited in Zurich and Gothenburg. The PPV and NPV were calculated. These metrics are highly dependent on prevalence. Based on

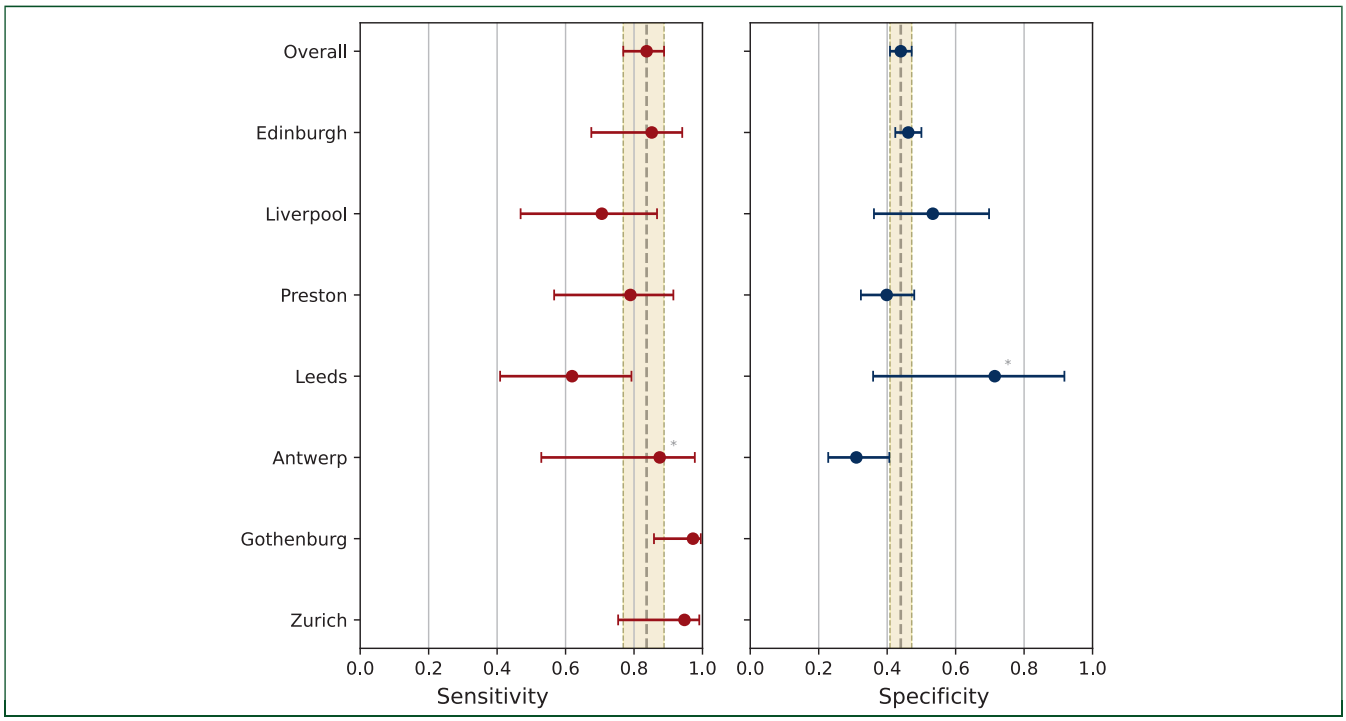

Figure 2. Test performance for the intended use cohort ($n = 1338$), split by collection site, with 95% Wilson confidence intervals. The dashed line indicates the overall sensitivity and specificity, and the yellow band shows the confidence intervals. Note that specificity was not determined for Gothenburg and Zurich, because no non-tumour patient samples from these sites were eligible for inclusion in this analysis.
*A group with <15 patients; there were only 12 tumours from Antwerp and 7 non-tumours from Leeds in this cohort.

disease prevalence in this enriched cohort, the PPV is 22% and NPV is 93% for the primary objective (Supplementary Table S6, available at https://doi.org/10.1016/j.esmoop.2025.105938).

Detection rate by brain tumour type

Detection rates were stratified by tumour type (e.g. glioma, brain metastases, pituitary tumours, meningioma, etc.) and subtype (e.g. glioblastoma, astrocytoma, oligodendroglioma, etc.) (Supplementary Table S7, available at https://doi.org/10.1016/j.esmoop.2025.105938). In general, malignant tumours were detected more often than benign brain tumours. For the most prevalent and most aggressive brain cancer, glioblastoma, 86% of cases were successfully identified. Additionally, 94% of central nervous system lymphoma, 90% of brain metastases, 75% of astrocytomas, 70% of pituitary tumours and 66% of meningiomas were predicted correctly.

Intended use population analysis

We assessed test performance in the intended use population, focusing on patients who presented with non-specific symptoms (as described in Figure 1). Participants who had other primary cancers but did not have a brain metastasis, patients who would warrant an urgent scan based on symptoms, and non-tumour patients who were recruited from inpatient secondary care facilities, e.g. hospital ward or theatre, were not included in this analysis.

One thousand three hundred and thirty-eight patients were included. Four hundred and one patients had a brain tumour, of which 199 were malignant. The test performance for this dataset is summarised in Supplementary Table S8, available at https://doi.org/10.1016/j.esmoop.2025.105938, and the sensitivities and specificities for the primary objective are illustrated in Figure 2. The overall diagnostic performance for detection of malignant brain tumours was 86% sensitivity (171/199) with 44% specificity (412/937). For the detection of all brain tumours (benign and malignant), the sensitivity was 73% (294/401). Based on the malignant brain tumour prevalence in this cohort of 1338 patients (15%), the PPV was 21% and NPV was 95% (Supplementary Table S6, available at https://doi.org/10.1016/j.esmoop.2025.105938). The number of brain tumours in this study is higher than the expected disease prevalence in the target population for the test. Table 2 shows the PPV and NPV values at various estimated prevalences for a primary care population, ranging from 0.5% to 3%. Since the prevalence is low in this population, naturally the PPV will be significantly lower than the NPV. That said, the NPV is ∼99% for each case, which is imperative for triage tests.

DISCUSSION

This is the largest reported international multi-centre study for performance evaluation of a brain tumour liquid biopsy test, which has now validated the performance of the technology. In the intended use population, the overall test performance was 86% sensitivity for brain cancer detection with 44% specificity. Importantly, the NPV was ≥99% across a range of estimated primary care prevalences. The sensitivity was 73% for all brain tumours, and 86% for the most common form of malignant brain cancer, glioblastoma.

Table 2. PPV and NPV with fixed sensitivity and specificity at different prevalences for the intended use population ($n = 1338$)

	Sensitivity, %	Specificity, %	Prevalence, %	PPV, %	NPV, %
Primary objective (malignant only)	86	44	0.5	0.8	99.8
			1.0	1.5	99.7
			1.5	2.3	99.5
			2.0	3.0	99.4
			2.5	3.8	99.2
			3.0	4.5	99.0
Secondary objective (all brain tumours)	73	44	0.5	0.7	99.7
			1.0	1.3	99.4
			1.5	2.0	99.1
			2.0	2.6	98.8
			2.5	3.2	98.5
			3.0	3.9	98.1

NPV, negative predictive value; PPV, positive predictive value.

The Dxcover Brain Cancer Liquid Biopsy Platform is intended for use in primary care to support clinical decision making in identification of patients with a suspected brain tumour to prioritise for urgent brain imaging (CT or MRI scans) (Figure 3).

In the intended use population, patients for whom the primary care team would propose serial clinical review rather than immediate imaging referral, there is clinical uncertainty as to whether a brain cancer is likely based on non-specific symptom presentation. Patients who require urgent brain imaging because of the severity of their symptoms, for example where an acute stroke is suspected, are not suitable candidates for the Dxcover test. Most patients with non-specific symptoms where a brain tumour is in the differential diagnosis will actually have an alternate diagnosis, usually of a lesser severity. The liquid biopsy triage test needs to identify patients to prioritise for urgent brain imaging within this 'low-risk' population.

A negative Dxcover test result can provide reassurance to patients and to family physicians that a brain tumour diagnosis is unlikely. Ongoing care is based on clinical judgment. There are important diagnoses that could be evident on brain imaging, but which are not brain tumours. A negative liquid biopsy triage test is not intended to prevent all brain imaging, which should still be requested when clinically indicated. However, for many patients with a low clinical suspicion of brain cancer, a negative test result offers definitive reassurance, and their symptoms resolve spontaneously over time. There becomes no need for brain imaging. Future clinical studies will investigate the effect that introducing the test into clinical practice has on the number of CT/MRI scans, health care costs, and time-to-diagnosis for tumour patients. If the triage test reduces unnecessary brain imaging, fewer incidental abnormalities will be detected that might otherwise prompt further investigations or unnecessary treatments, and additional cost.[17,32]

A positive test result will accelerate access to diagnostic imaging for a patient in whom there was a low clinical suspicion of cancer. That low suspicion would otherwise have contributed to diagnostic delay. Bringing forward the date of diagnosis by even a few weeks matters, because identifying tumours earlier, when they are smaller, significantly improves patient outcomes.[33]

The study population was 'enriched' through the recruitment of patients with a recent new brain cancer diagnosis, to ensure there were sufficient positive cases for adequate assessment of test performance. Recruiting patients in a primary care setting was considered, but because of the low prevalence it was determined to be unfeasible to consent and recruit the required number of eligible patients, at a non-prohibitive cost. Liquid biopsy test performance will need to be similar in the target primary care population to the patients recruited into this study. We expect this will be the case. Evidence from previous studies indicates that there is not a difference in diagnostic performance based on tumour size. In a study of test performance in a cohort of high and low grade

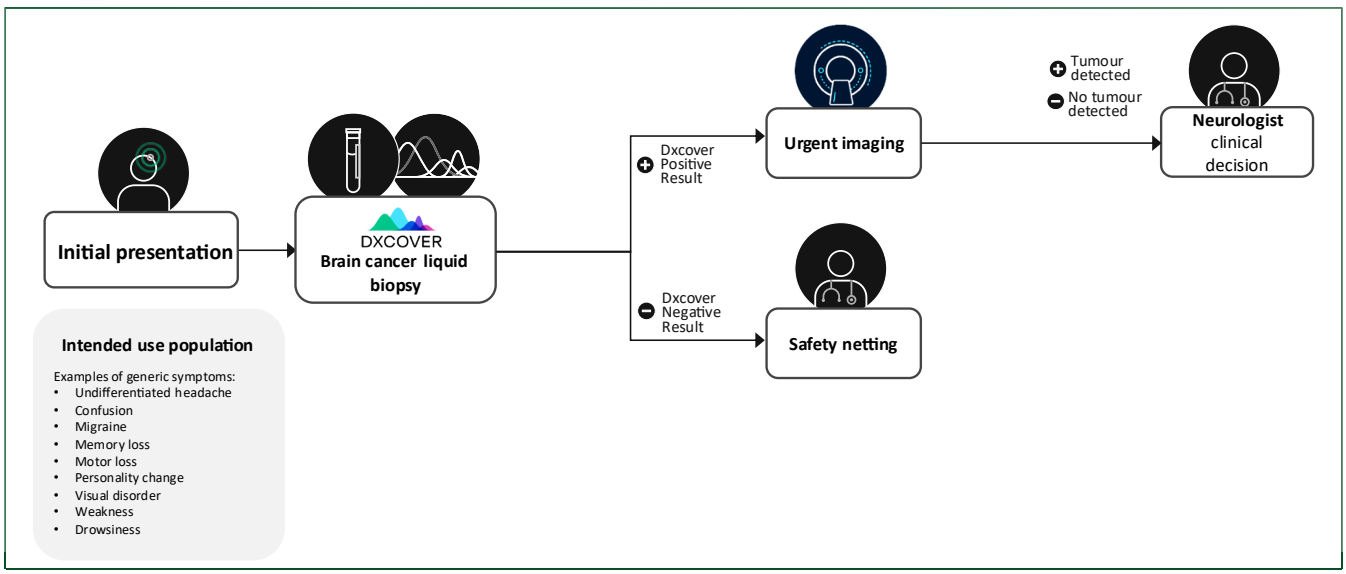

Figure 3. Proposed brain tumour diagnostic pathway with the implementation of the triage blood test in primary care.

gliomas, where the tumour volume was assessed by a radiologist, tumours as small as 0.2 cm^3 were detected irrespective of tumour grade.[34]

However, the disease prevalence will change in the primary care population. PPV and NPV are heavily dependent on disease prevalence. The best estimate of brain cancer prevalence in a primary care population has previously been reported as ∼1.6%.[7] The brain tumour prevalence in this study was higher than the estimated prevalence of 10%, which was used for the power calculation. Some of the collection sites had difficulty identifying eligible non-tumour patients for the study, which explains the inflated brain cancer prevalence in the dataset. For example, Leeds ($n = 16$) and Liverpool ($n = 40$) recruited fewer non-tumour patients than expected. At the lower estimated prevalence in primary care, the NPV of the Dxcover test will nevertheless be ≥99% (Table 2). A high NPV is crucial for triage tests, particularly for the early detection of cancer, as it defines the probability that the patients with a negative test result truly do not have the disease. This ensures that true negative patients are 'ruled-out' and removed from the urgent scanning pathway.

The specificity of the test should be interpreted in the context of its intended clinical application. A specificity of 44% means that just under half of patients who do not have a brain tumour would correctly receive a negative result and could be triaged to a less urgent pathway. The remaining 56% of patients without a brain tumour would be incorrectly triaged to urgent imaging. This may have a psychological impact on the patient while they await the diagnosis, but the urgent nature of the diagnostic imaging that ensues will minimise the duration of this. In any case, we expect that in standard care many of these patients would have received brain imaging on a routine basis at some point. In current symptom-based referral pathways for suspected brain tumour and brain imaging, the true positive rate is substantially lower.[35] This differs from the impact of false positives in other cancer screening tests that detect multiple types of cancer, where false positives can result in uncertain or inconsistent follow-up procedures. As a result, the introduction of this blood test and false positive result poses minimal additional risk to patients and to the health care systems. As a comparison, existing primary care referral pathways for direct access imaging for suspicion of a brain tumour detect fewer than two tumours in every hundred scans.[7]

Study limitations

The number of patients recruited and ratio of tumour: non-tumour participants, varied between centres. Some sites did not meet their predicted recruitment target, or the projected balance of non-tumour to tumour patients, mainly because of difficulty recruiting directly from the ED. In Gothenburg and Zurich, non-tumour patients were recruited from inpatient wards after admission, rather than the ED, contrary to the study protocol. Most of these patients had presented with severe red-flag symptoms for conditions, such as stroke, who would warrant an immediate brain scan. This led to their exclusion from the intended indication analysis.

There were some variations in test performance between collection sites. However, this may be reflective of the total recruitment number from each site. For example, the test performance in Edinburgh (the site with the highest number of patients recruited) closely matches the overall statistics, whereas the sensitivity (or specificity) for the sites with fewer tumours (or non-tumours) was greatly influenced by a few incorrect predictions. Although these limitations affected the homogeneity of recruitment across the participating centres, the overall utility of the test remains clear.

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DISCLOSURE

JMC, DE, HJB, AS, AL, GA, DSP, MJB are employed by Dxcover Ltd and/or have stock in Dxcover Ltd. EG is a consultant for Dxcover Ltd. The University of Edinburgh receives consultancy fees from Dxcover Ltd on behalf of work carried out by PMB. All other authors have declared no conflicts of interest.

DATA SHARING

Study data, including patient metadata and test results, may be made available upon reasonable request.

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