

PEER REVIEW HISTORY

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ARTICLE DETAILS

TITLE (PROVISIONAL)	The feasibility and acceptability of breath research in primary care: a prospective, cross-sectional, observational study
AUTHORS	Woodfield, Georgia; Belluomo, Ilaria; Boshier, Piers; Waller, Annabelle; Fayyad, Maya; von Wagner, Christian; Cross, Amanda; Hanna, George

VERSION 1 – REVIEW

REVIEWER	Haitham Amal Faculty of Medicine, Hebrew University, Israel
REVIEW RETURNED	01-Oct-2020

GENERAL COMMENTS	<p>The paper is dealing with an urgent need for a non-invasive test to diagnose cancer and specifically GI cancers. They showed that large-scale breath testing in primary care was feasible and acceptable. I think that this paper is ready to be accepted for publication. A few and minor comments are presented below:</p> <p>1- Why the patients were not required to follow any specific conditions, such as fasting, prior to breath sampling. Please explain the rationale?</p> <p>2- Why Phase 2 has a lower number of patients than phase 1?</p> <p>3- Can the authors give more interpretation on how they can overcome the "one location preference" in a real situation?</p> <p>4- How the authors concluded that "95% of the breath samples that analysed were deemed to contain adequate quantities of breath"? what is the threshold for saying this statement?</p> <p>5- the authors may need to extend the introduction and reviewing more studies in breath tests to diagnose GI cancers, here I suggest some: https://pubmed.ncbi.nlm.nih.gov/25869737/ https://pubmed.ncbi.nlm.nih.gov/29988892/</p>
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REVIEWER	Radu Ionescu Estonian University of Life Sciences, Estonia
REVIEW RETURNED	13-Oct-2020

GENERAL COMMENTS	Although the diagnostic performance of the breath test is not provided, the present manuscript presents a very interesting study that provides very useful hints for efficient patients recruitment for breath testing in primary care and is worth for publication in BMJ Open journal.
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	I suggest the acceptance of the manuscript in its present form, with just a little comment regarding the mention of the type of cancer assessed in publication number [11] that is referred in the background as follows: "A systematic review of breath testing in cancer identified distinctive VOCs signals for different tumour sites with pooled sensitivity and specificity of 79% and 89% respectively."
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REVIEWER	Niek de Wit Julius Center for Health Sciences and Primary Care University Medical Center Utrecht the Netherlands
REVIEW RETURNED	07-Dec-2020

GENERAL COMMENTS	<p>This is an interesting analysis of the conditions for implementation of breath tests facility for early diagnosis of cancer in primary care. It covers a relevant topic: breath analysis may be the route to earlier diagnosis of cancer among symptomatic patients, as current diagnostic information is often atypical and not discriminative. The manuscript reports on the feasibility and acceptance of breath testing facilities, preceding a RCT on its effectiveness. The paper is well structured, easy to read, and analyses are up standard.</p> <p>However, there are a number of issues that I want to bring forward:</p> <p>First of all, I was somewhat confused about the aim of the breath tests. So far, it is only used in clinical practice to detect <i>Helicobacter pylori</i>, and there are some studies reporting its effectiveness for <i>Helicobacter</i> detection in primary care. However, in this case the aim is early detection of cancer through innovative analyses of breath. However, the paper doesn't specify how this will work, for which types of cancer it will be used and in what diagnostic set-up? Is it to be used amongst symptomatic patients in combination with traditional diagnostic information, or for screening of those with chronic GI symptoms?</p> <p>Second, there is a difference between assessment of the clinical use of the breath test and that of the optimal implementation for research evaluation of its effectiveness. Although this may sound a bit theoretical, this difference may effect outcomes. Patients probably value the breath test differently when it is used 'real life' as part of their diagnostic pathway in caser they present with symptoms, as compared to the 'mock' application in the present study. I wonder how patients were informed in the present study, and if they were made aware of the clinical context in which the breath test is to be used in future. Especially the fact that it will be offered to patients with symptoms that maybe indicative of cancer may change their perception of its applicability. I can imagine that in some of these patients it will provoke anxiety, which does not appear in the neutral setting in which it was used in the present study. Symptomatic patients may consider a breath test as a waste of time once they hear that they have an increased risk of cancer, and want prompt imaging procedures instead.</p> <p>This difference between the clinical and research setting also has consequences for acceptability of the test for GP's. In clinical practice, they are not additionally paid for using the breath test, and they are not confronted with the decisions to be made based</p>
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	<p>on the test result in a real life patient. Authors do briefly refer to this in the discussion, but I would challenge them to go more into detail about the possible consequences of using breath test in real life setting on feasibility and acceptability of patients and GPs', as compared to the research purpose.</p> <p>In addition, during the study, accuracy of the breath test was not yet known, and both patients and GP's will take the test accuracy into account when evaluating its acceptability.</p> <p>Thirdly, two ways of organising the breath tests were compared; one through individual practices and the second one through a so-called 'hub'. There are more ways of introducing this diagnostic test, f.i. through an outreach program of a central laboratory facility, by referring patients to the hospital diagnostic service, or by a kind of 'self-management 'package. Why did authors chose for these two options?</p> <p>Detail questions:</p> <p>Page 10 line 53: what were the technical issues with the sampling equipment? Are these incidental, or structural problems that may recur?</p> <p>Page 17 line 31: the method of enrolment.....reflect the intended purpose of the test. What do authors mean by that ? I presume the test will be used for early detection of cancer, so it will be used in clinical practice for patinets with GI symptoms. Or do they also consider using the breathtest for screening of patients with chronic GI symptoms?</p>
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VERSION 1 – AUTHOR RESPONSE

REVIEWER 1

Reviewer comment: Why were the patients not required to follow any specific conditions, such as fasting, prior to breath sampling. Please explain the rationale

Author response: Patients were not required to follow any specific conditions prior to breath testing as there are currently no evidenced based guidelines for breath sampling in clinical practice. This study was intended to examine the feasibility and acceptability of the breath testing process itself with a focus on optimising patient enrolment strategies and sampling models. We acknowledge that future breath tests may require patients to adhere to specific conditions prior to the test, such a fasting, abstinence from smoking and vigorous exercise. As such the conditions of this study may not be fully representative of a future breath testing pathway. We have amended the discussion section to acknowledge this as a limitation of the study.

Page 17 paragraph 4: "Patients were not required to follow any specific conditions prior to the breath test, as there are currently no evidenced based guidelines for breath sampling in clinical practice. This means that the study may not be fully representative of a future breath testing pathway."

Reviewer comment: Why does Phase 2 have a lower number of patients than Phase 1?

Author response: the study had a recruitment target of 1000 patients over a one-year period. During the first 6 months of the study (Phase-1) our aims was to assess the feasibility of different methods of recruitment and engagement within single GP practices. This was intended to be an iterative process, informed by staff feedback. By comparison the second 6 months of the study (Phase-2) sought to test two specific, but potentially complementary, models for patient recruitment within a limited number of GP practices. During Phase-2 staff and patient acceptability of the test was also assessed.

Accordingly, an emphasis on patient recruitment in Phase-1 of the study meant that a greater number of patients were accrued. We do not feel that the difference in patient recruitment between each phase had a detrimental effect on the study's findings.

We have amended the discussion section to acknowledge this.

Page 16 paragraph 1: "The emphasis on patient recruitment during Phase-1 meant a greater number of patients were accrued during this period. This was not however felt to be detrimental to the findings of Phase-2."

Reviewer comment: Can the authors give more interpretation on how they can overcome the "one location preference" in a real situation?

Author response: It is true that attendance for the breath test may be less if patients are asked to travel to a non-preferred location. However, as this study showed >99% of patients did not express any difficulty or concern when asked to travel to a hub centre. This is indeed encouraging particularly as the patients knew they were taking part of a research study and would not receive any direct health or financial benefits by attending for the test.

We have amended the discussion to emphasise this point.

Page 16 paragraph 2: "It was hypothesised that attendance and attitudes towards the breath test may be negatively affected by having to travel to a central location. However in this study it was observed that centralising breath testing reduced staffing and equipment requirements with no discernible negative impact on patient feedback."

Reviewer comment: How the authors concluded that "95% of the breath samples that were analysed were deemed to contain adequate quantities of breath"? What is the threshold for saying this statement?

Author response: In the methods section, page 7, under the heading "Breath sampling quality control" we have referenced the criteria for determining sample quality. We have however amended this methods section to make this clearer to the reader.

Page 7 paragraph 6: "Breath samples within TD tubes were evaluated for quality based on detected levels acetone and isoprene (online supplementary data file S10). Acceptable thresholds for acetone and isoprene were dependent on analytical platform."

Reviewer comment: The authors may need to extend the introduction and review more studies on breath tests to diagnose GI cancers, here I suggest some.

Author response: We have amended the background section to include these importance references. Word restrictions however mean it is not possible to report the findings of those studies in detail.
Page 4 paragraph 3

REVIEWER 2

Reviewer comment: I suggest the acceptance of the manuscript in its present form, with just a little comment regarding the mention of the type of cancer assessed in publication number [11] that is referred in the background as follows: "A systematic review of breath testing in cancer identified distinctive VOCs signals for different tumour sites with pooled sensitivity and specificity of 79% and 89% respectively."

Author response: We have amended the manuscript to reflect the reviewer's comment. The sentence on page 4 now reads:

Page 4 paragraph 3: "A systematic review of breath testing in cancer identified distinctive VOCs signals for different tumour sites with pooled sensitivity and specificity of 79% and 89% respectively (including lung, breast, gastrointestinal, head and neck, prostate and gynaecological tumours).¹¹"

REVIEWER 3

Reviewer comment: First of all, I was somewhat confused about the aim of the breath tests. So far, it is only used in clinical practice to detect *Helicobacter pylori*, and there are some studies reporting its effectiveness for *Helicobacter* detection in primary care. However, in this case the aim is early detection of cancer through innovative analyses of breath. However, the paper doesn't specify how this will work, for which types of cancer it will be used and in what diagnostic set-up? Is it to be used amongst symptomatic patients in combination with traditional diagnostic information, or for screening of those with chronic GI symptoms?

Author response:

Breath testing offers an attractive approach to non-invasive disease detection and monitoring. One specific area that has attracted interest is the early detection of cancer. For the most part early cancers present with vague non-specific symptoms that are common to many benign (non-cancer) conditions. Accordingly, it is not feasible to refer all patients with such symptoms to undergo often invasive and expensive investigations (e.g. endoscopy, imaging) as the majority will not have cancer. Many patients with cancer are only first investigated when they have 'red flag' symptoms which are frequently associated with advanced incurable disease. We envisage that a future breath test could serve as a community triage test, for patients with vague symptoms that could be associated with cancer, but do not currently meet ('red flag') criteria for investigation. The test would identify those patients who would most benefit from expedited definitive investigation.

The current study emphasises the use of breath testing for the detection of gastrointestinal cancer.

We have amended the introduction and discussion of the manuscript to reflect the above comments and make the ultimate purpose of the breath test clearer to the reader.

Page 4, second sentence of introduction: "Patients with early oesophageal, gastric, pancreatic or colorectal cancers often have non-specific symptoms typical of many common benign conditions".

Page 4 paragraph 2: "A breath test could serve as a community triage test, for patients with vague symptoms that may be associated with cancer, but do not currently meet ('red flag') criteria for

investigation.”

Page 4 paragraph 2: “A breath test would support general practitioners (GPs) as well as other healthcare providers to determine which patients most warrant referral using existing gastrointestinal cancer diagnostic pathways.”

Page 4 paragraph 3: “Studies of different gastrointestinal tumour sites also showed different VOC biomarkers for oesophagogastric, pancreatic and colorectal cancers, providing the opportunity for a single breath test to diagnose different cancers based on their unique VOC signature, in a similar way to a single blood draw being used to assess for multiple diseases.12-15”

Page 16 paragraph 3: “The method of enrolment adopted in future trials and ultimately clinical practice will largely reflect the intended purpose of the test (for example triaging symptomatic patients or screening asymptomatic populations).”

Reviewer comment: Second, there is a difference between assessment of the clinical use of the breath test and that of the optimal implementation for research evaluation of its effectiveness. Although this may sound a bit theoretical, this difference may effect outcomes. Patients probably value the breath test differently when it is used ‘real life’ as part of their diagnostic pathway in caser they present with symptoms, as compared to the ‘mock’ application in the present study. I wonder how patients were informed in the present study, and if they were made aware of the clinical context in which the breath test is to be used in future. Especially the fact that it will be offered to patients with symptoms that maybe indicative of cancer may change their perception of its applicability. I can imagine that in some of these patients it will provoke anxiety, which does not appear in the neutral setting in which it was used in the present study. Symptomatic patients may consider a breath test as a waste of time once they hear that they have an increased risk of cancer, and want prompt imaging procedures instead.

Author response: We agree with the reviewers comment regarding the fundamental difference between research and established clinical practice. At the time of recruitment patients were informed that this was a research study investigating the feasibility of breath testing for the detection of gastrointestinal cancers in the community. It was made clear to patients that the study was focused on establishing new processes for cancer diagnosis, and not to develop a final test. The purpose of the study was carefully explained to patients both verbally and within an approved patient information sheet.

It is worth noting that in the absence of an appropriate reference test (e.g. endoscopy, imaging), something that was not available to this patient cohort in light of current NICE cancer guidelines, it was not possible to establish the diagnostic performance of the breath test.

We have amended the Methods of patient engagement section of the manuscript to reflect these comments:

Page 6 paragraph 2: “The purpose of the study was carefully explained to patients both verbally and within an approved patient information sheet prior to enrolment. All patients were told that the breath test will potentially be used in the future to detect gastrointestinal cancers, but that the current study was intended to investigate the process and feasibility of breath testing only.”

Reviewer comment: This difference between the clinical and research setting also has consequences for acceptability of the test for GP’s. In clinical practice, they are not additionally paid for using the breath test, and they are not confronted with the decisions to be made based on the test result in a

real life patient. Authors do briefly refer to this in the discussion, but I would challenge them to go more into detail about the possible consequences of using breath test in real life setting on feasibility and acceptability of patients and GPs', as compared to the research purpose.

In addition, during the study, accuracy of the breath test was not yet known, and both patients and GP's will take the test accuracy into account when evaluating its acceptability.

Author response: we have amended the discussion in response to the reviewer's valid comment.

Page 17 paragraph 3: "However, these factors may not apply outside of the research setting, potentially influencing acceptability of breath testing to GPs, particularly where responsibility for implementing testing, interpreting and actioning results may fall to them."

Reviewer comment: Thirdly, two ways of organising the breath tests were compared; one through individual practices and the second one through a so-called 'hub'. There are more ways of introducing this diagnostic test, f.i. through an outreach program of a central laboratory facility, by referring patients to the hospital diagnostic service, or by a kind of 'self-management' package. Why did authors chose for these two options?

Author response: The GP based hub and spoke model that was tested in this study can be considered analogous to a situation where the hub is a central hospital or other healthcare facility. For pragmatic reasons we adapted the existing GP network that we had established in Phase-1 of this study to form the hub and spoke model that was evaluated in Phase-2.

We have amended the discussion of the manuscript to reflect these comments:

Page 16 paragraph 2: "The hub and spoke model evaluated in Phase-2 of this study explored the concept of testing patients in a central location, in this instance a GP practice. Findings may be applicable to other centralised testing centres such as diagnostic centres and hospitals."

Reviewer comment: Page 10 line 53: what were the technical issues with the sampling equipment? Are these incidental, or structural problems that may recur?

Author response: The technical issues alluded to are detailed in supplementary data file S14. We have amended the Breath sampling and quality control section to better signpost this to the reader:

Page 9 paragraph 4: "Although there were minimal patient related limitations, technical issues with sampling equipment were reported. A summary of themes regarding feasibility and acceptability of the sampling process is detailed in online supplementary data file S14."

Reviewer comment: Page 17 line 31: the method of enrolment.....reflect the intended purpose of the test. What do authors mean by that ? I presume the test will be used for early detection of cancer, so it will be used in clinical practice for patients with GI symptoms. Or do they also consider using the breath test for screening of patients with chronic GI symptoms?

Author response: Please refer to the response to Reviewer 3's first comment above, with amendments detailed.

VERSION 2 – REVIEW

REVIEWER	Amal , Haitham Hebrew University of Jerusalem
REVIEW RETURNED	04-Jan-2021

GENERAL COMMENTS	The authors replied to all of my comments. I recommend accepting this paper.
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REVIEWER	Ionescu, Radu Estonian University of Life Sciences
REVIEW RETURNED	08-Jan-2021

GENERAL COMMENTS	The authors responded to my concern
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REVIEWER	de Wit, Niek University Medical Center Utrecht, Julius Center for Primary Care
REVIEW RETURNED	17-Jan-2021

GENERAL COMMENTS	In their rebuttle authors have adequately answered my initial questions and adressed specific queries.
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