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Short Review Article

Breath analysis as a diagnostic tool for lung cancer

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ABSTRACT

The early diagnosis of lung cancer is key to effectively treating this common and deadly disease. In this review we consider whether non-invasive breath-based detection of volatile biomarkers offers a means to screen populations for the presence of the disease. We find that many potential volatile cancer biomarkers have been reported, including some in multiple studies, and that many are biologically plausible. The field lacks prospective studies, however, which are needed to determine whether such markers are of use for the detection of lung cancer in its pre-symptomatic stages. This,and a lack of knowledge about the various endogenous and exogenous sources of such potential cancer markers, hampers the clinical development of this testing paradigm despite its undeniable appeal. Further work is therefore required before any firm conclusions can be drawn regarding the utility of breath analysis for the diagnosis of lung cancer.

Keywords:breath, cancer, volatile chemical

INTRODUCTION:

Background

Of all types of cancer, lung cancer has the highest worldwide incidence. According to a recent report there were almost 2 million new cases of lung cancer worldwide in 2012, comprising approximately 13% of all new cancers. Lung cancer has a higher than average mortality (compared to other cancers). Indeed, the disease led to approximately 1.6 million deaths worldwide which equates to around 19% of all cancer mortality [1]. Moreover, the burden of disease is gradually shifting to less developed countries where the prevalence of lung cancer continues to climb, and where it putsincreased stress on already overstretched healthcare services [2]. The high prevalence of lung cancer is primarily due to environmental exposure to substances such as arsenic, polycyclic hydrocarbons and asbestos [3,4], and those related to lifestyle, predominantlyto smoking. [5]. Due to these causative factors being so common lung cancer is likely to remain a significant global mortality risk for the foreseeable future [6].

The high prevalence of lung cancer is compoundedby the fact that the 5 year mortality rate of lung cancer, from the time of presentation, is very high, estimated to be about 85-90%. This occurs even though surgical and chemotherapeutic treatments are available and commonly used [7]. Encouragingly, many reports suggest that if the lung cancer is detected at its early stage it can be more easily treated [8 -10].

The problem of early and timely diagnosis of lung cancer.

Patients with lung cancer are frequently symptomatic for a long time before they seek medical attention [11]. They generally present with numerous symptoms including coughing, hemoptysis, shortness of breath or breathing changes, wheezing, chest pain, weight loss, and fatigue [12,13]. The fact that patients frequently ignore the initial symptoms obviously delays diagnosis but also worsens the prognosis, whereas enhancing patient awareness of lung cancer symptoms results in diagnosis at an earlier stage of the disease [11-14]. This is important sinceearly diagnosis generally increases the effectiveness of treatment, reducing mortality and morbidity, since the tumour can be treated at a stage when it has caused minimal tissue damage, and before it has metastasized[14]. Ideally a diagnostic test to achieve this should be accurate, cause no

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discomfort or risk to health, and be non-invasive [15].

Beginning in the early 20th century various techniques have been used for detecting the presence of lung cancer, including chest radiography, histological assessment, and sputum cytology. These tools are not suitable for population-based screening due to the risk associated with these invasive procedures such as radiation exposure, and/or the involvement of technically difficult and expensive techniques such as gas chromatography mass spectrometry. Thus, they are not widely used for the early detection of cancer [16]. Morphological abnormalities can of course be used to diagnose the illness following tissue biopsy, but such techniques are of little use for early detection unless the disease can be screened for using a less invasive method [17].

Most recently, Computed Tomography (CT) scans have proven to be useful for the early diagnosis of lung cancer compared to traditional radiography. In CT X-rays are used to form 3 dimensional images of the body which assists in the detection of small early stage tumours. Recently, CT has been augmented by Positron Emission Tomography, which increases the diagnostic accuracy [10]. An advanced form of CT imaging called 'spiral' or 'helical' CT scan, which provides more accurate images of internal organs, has allowed for the detection of tumours as small as 1mm [8] Although this is an effective technique to detect lung cancer, it is expensive, and associated with some risk due to radiation exposure. This makes techniques,such as CT, unsuitable for the sort of regular 'health check' screening which would revolutionize the early diagnosis of lung cancer. There is therefore a need for new diagnostic techniques to be developed, such as those utilizing so-called cancer 'biomarkers', particularly when used as a pre-imaging screening procedureto select those who should undergo further testing.

Biomarker based cancer tests.

A chemical biomarker can usefully be defined as a molecule that is associated with a physiological state, including pathological disease states; for example, plasma glucose concentration is a biomarker of diabetes [18]. Genomic based tests can estimate cancer risk but cannot detect the presence of illness [19]; rather markers indicating the actual occurrence of a tumour are needed, based on altered gene transcription, protein translation, or the resulting metabolic changes [18]. Ideally disease biomarkers would specifically and sensitively reflect a pathological state which could be utilized for diagnosis, estimating prognosis, treatment selection, and/or for monitoring the efficacy of treatment [18] While their development for clinical use is not without difficulties they have great appeal given they are relatively simple and inexpensive to use. Volatile compounds found in the breath are a type of metabolic change-based biomarker. They are particularly attractive since they can be quantified using an entirely non-invasive process [20]. Specifically, 'breath analysis' involves analyzing the chemical composition of trace gas volatile inorganic and organic compounds (VC) in the exhaled breath. The technique is based on the idea that VC are end products of metabolic processes which may be able to tell us something about physiological and pathological states [20]. Moreover, such testing may be especially appropriate for diseases of the lungs and airways including lung cancer.

Volatile Organic and Inorganic Compounds as a type of biomarker.

Volatile Compounds (VC) have a high vapour pressure at room temperature under normal pressure conditions and therefore exist, to varying degrees, in the gas phase. They can be aliphatic or aromatic organic compounds, such as acetone, or inorganic such as nitric oxide. Consisting of nitrogen, oxygen, carbon dioxide, inert gases and water, breath also contains approximately 1000 trace VC. The concentrations of trace VC in the breath range from parts per million (PPM) to parts per trillion (PPT) with some of the most abundant being isoprene, acetone, acetone, ammonia, and propanol [21]. It has been proposed that VC can be used a disease markers which have the potential to form the basis of diagnostic tests, particularly when exhaled in the breath, with a growing body of evidence supporting that claim [22].

Use of VC as disease biomarkers.

To date the only 'breath test' in common clinical use is used to diagnose the presence of *Helicobacter pylori* in the stomach. In that test ingestion of isotopically labelled urea is

catabolized by urease present in the bacterium. This leads to the release of labeled carbon dioxide which can be detected in the breath [23]. Another less common, but commercialized application, measures nitric oxide as a measure of airway inflammation [24], while other applications are still in development such as the detection of hydrogen cyanide as a marker of lung *Pseudomonas aeroginosa*infection [25].The catabolism of isotopically labelled erythromycin to carbon dioxide has been used to estimate the clearance rate of the chemotherapeutic drug docetaxel. This is done as a means to detect hypo-metabolisers who will experience severe toxic reactions [26]. Finally, the catabolism of glucose to hydrogen has been assessed using a breath test to determine bacterial growth rates in the gastrointestinal tract as may occur in several bowel disorders [27].

Putative VC as diagnostic or screening test for cancer.

Such results are encouraging to researchers attempting to develop a simple test for lung cancer based on volatile biomarkers. To investigate the state of the field we searched the literature for studies of volatile biomarkers found in the breath of lung cancer patients, while excluding those that used only post mortem tissue or blood fractions, or those which speculated on possible markers based on the analysis of cell culture headspace,as summarized in Table 1. From these reports, we have summarized the breath volatiles suggested to be markers of the disease (Table 2). As can be seen many potential biomarkers have been identified although none have been developed into a routinely used clinical test. The most established, from a commercial perspective at least, emanate from the research group lead by Philips who have made use of Gas Chromatography Mass Spectrometry (GC/MS) to identify lung cancer markers. This group has reported that these markers can detect lung cancer with an approximate sensitivity and specificity of 80% [29-32].

Many of the cancer biomarker studies have a fairly low sample size, which reduces their statistical power and hence the robustness of their findings, possibly explaining the large variety of potential biomarkers reported, although the range of detection modalities used also likely contributes to this heterogeneity. The putative markers cover a wide range of chemical classes, predominantly aromatic compounds and alkanes, although alcohols, aldehydes and ketones, esters, sulphides and halo hydrocarbons have been reported (Table 2). To identify potential 'lead' markers we also counted the number of apparently independent investigations which found the same marker VC. This is a crude approach given that, as mentioned, the methodology used, and the interests of the researchers, necessarily bias such an analysis. However, keeping such limitations in mind, it is notable that some compounds have been reported multiple times. These include benzene, isoprene and propanol as the most identified, followed by styrene, pentane, decane, heptene, hexanal, and heptanal, while a variety of mainly alkanes and aromatic compounds which have been identified at least twice. For some of these compounds, such as benzene, toluene, and styrene, any possible endogenous source remains obscure thereby decreasing their plausibility as biomarkers. Indeed, an exogenous source for these compounds is morelikely, with increased breath concentrations relating to their altered body absorbance occurring due to changed respiratory function, or perhaps by the tumour itself. This does not rule out the use of such compounds as disease markers but it would make their use much more complex as it would depend upon the makeup of ambient air. Others, such as isoprene, propanol and acetone, are well characterised metabolicallyand, hence, are somewhat more credible biologically as endogenous cancer markers [38-41]. Even so many of these compounds deserve closer examination. Isoprene is formed during the metabolism of mevalonate as part of cholesterol biosynthesis predominantly in the liver [38] while acetone is formed from fatty acidsvia the decarboxylation of excess acetyl-CoA. Propanol can be derived from the reduction of acetone (as well as being derived from gut flora) with both acetone and propanol rising in concentration in ketonemic individuals, [40, 41]. For all three (isoprene, acetone, and propanol), dietary or metabolic changes can therefore produce altered volatile concentrations [38,42-44]. It must therefore be considered that nutritional or metabolic changes which occur in symptomatic cancer may contribute to altered breath concentrations in patients with lung cancer.

Furthermore, many of the other compounds mentioned at least twice in the literature are either aldehydes or alkanes. Both classes of compounds can be produced as secondary products of unsaturated fatty acid peroxidation. In particular, pentane, hexanal, and propanal, are common oxidation products, although a range of other members of these compound classes can also be produced due to the complexity of reactions involved [45,46]. Given that oxidative stress is known to be increased in rapidly growing tumours, alkanes and aldehydes represent plausible lung cancermakers. This is particularly so since their concentrations appear to be much more influenced by the extent of oxidative stress than the availability, and hence dietary intake, of their fatty acid precursors [47-49]. On the other hand, it cannot be ruled out that secondary effects of cancer related to dietary intake of antioxidants are responsible for their altered breath concentrations [50].

In general, the difficulties encountered in the interpretation of these studies occurs due to the use of symptomatic patients as study subjects. This raises the question of whether the marker is present in the pre-symptomatic period when they would be most useful, or whether they are 'epiphenomena' occurring subsequent to the primary pathophysiological process (such as cachexia, or changes in nutritional status due to therapeutic interventions). To further investigate the utility of any of the putative markers requires researchers to conduct so-called prospective investigations in either general or 'at risk' populations. This would allow it to be determined if any of the potential biomarkers identified can detect lung cancer in the pre-symptomatic stage of the disease, a requirement for any useful screening test. Prospective studies are very expensive to perform given they require large sample sizesto achieve the statistical power necessary to determine the utility of any marker, this being due to the low incidence of the disease in any study group,the high participant drop-out rates, and to the long follow-up times needed, a fact that likely explains the lack of such data in the literature. A less costly alternative, aimed at generating evidence that would justify the cost of a prospective analysis, is to use a cross-sectional design that includes multiple cancer types. While one cannot conclude that only observing altered breath concentrations of a putative marker in a single type of cancer means that it is a primary disease marker, at least one can conclude that it is not general marker of persons with cancer which increases the likelihood of the compound being an epiphenomenonrelated tosymptomatic cancer. Such studies, those that include multiple cancer types, would be useful additions to the literature.

Sources of VC in breath as a factor complicating clinical use.

The actual clinical interpretation of breath-based diagnostics is also complicated by the fact that the compounds one breathes out have several sources of origin, any of, and frequently all of which,can be present simultaneously. Many of the VC in the breath are exogenous in origin, that is, what is breathed out derives from what is breathed in. Indeed, atmospheric air has been identified as the main source of breath VC originating from both natural and human-made sources [51]. For example, chemicals including trichloroethene, toluene and tetrachloroethylene are commonly found in the bloodstream but are thought to be exclusively exogenous in origin [51]. Aside from occupational chemical exposure applications such exogenous compounds are not of great interest as biomarkers. Endogenous VC, on the other hand, may be much more useful as they derive from metabolic processes taking place in the body including the airways, bloodstream (cells and plasma), and other tissues [52]. Unfortunately, most common breath VC also occur exogenously making the interpretation of breath concentrations difficult [51]. Indeed, many of the compounds commonly reported to be putative biomarkers of lung cancer highlighted have both exogenous and endogenous sources. For example, although, as described above, aldehydes and alkanes, acetone, propanol, and isoprene can all be produced by various cellular processes, they are also found in ambient air deriving from a variety of human-made and natural processes [38, 39, 51, 53, 54] Adding to this complexity, endogenous compounds are not always formed in the patients' own tissues. For example, ethanol and methanol in the breath derive from intestinal or oral cavity microorganisms [55,56]. Breath sulphur containing compounds can originate in the liver and lungs, but predominantly derive from the gastrointestinal tract andthe oral cavity [56-58].

Similarly, while ammonia in the breath can indicate kidney failure, the gas mostly originates from microorganisms present in the mouth [56,59]. Moreover, the relationship between the VC in each body "compartment" (such as the circulation and various body tissues) is unclear even though much of the diagnostic potential of breath testing relies assumes there is a direct relationship between VC in the diseased tissue and VC in breath. It is therefore difficult to determine the actual source of breath VC and, hence, what any changes in their concentration may mean. The impact of such considerations cannot be underestimated and must be taken into account if breath analysis is to be used clinically for the diagnosis of lung cancer.

Conclusion.

As such, many putative volatile lung cancer markers have been identified, some of which have been replicated multiple times. While this is encouraging the investigation of the diagnostic utility of these markers in 'at risk' patient groups is needed before a definitive conclusion can be drawn about their use as early-stage screening tools for this common and deadly form of cancer.

Table 1: Studies of breath VC in lung cancer.

Patient group	n	Age	Method	Sensitivity %	Specificity %	Ref.
LC	29	>50	SPME-GC/MS	86	69	28
LC	60	67	GC/MS	100	81	29
LC	178	64	GC/MS	90	82	30
LC	193	66	GC/MS	85	80	31
LC	193	NC	GC/MS	85	81	32
LC	28	60	Gas Sensor array	85	100	33
NSCLC	40	68	SPME-GC/MS	NC	NC	34
LC	14	64	Sensor array	71	92	35
NSCLC	36	67	GC/MS	72	94	36
LC	17	62	PTR/MS	54	99	37

Values are taken directly from each paper; age is the mean age if reported. The specificity and selectivity are calculated from the study population using the biomarkers described in Table 2. Abbreviations: LC: all lung cancer, NSCLC: non-small cell lung cancer only, NC: not calculated.

Table 2: Putative volatile biomarkers of lung cancer found in human breath.

Name	Number	Class	References
benzene	4	aromatic	28, 29, 35, 36
isoprene	4	alkene	31, 34, 35, 36
propanol	4	alcohol	31, 32, 35, 37
styrene	3	aromatic	29, 34, 36
decane	3	alkane	28, 29,31
pentane	3	alkane	30, 35, 36
1-heptene	3	alkene	29, 30, 36
heptanal	3	aldehyde	28, 29, 34
hexanal	3	aldehyde	28, 29, 34
1,2,4-trimethylbenzene	2	aromatic	28, 36
2,3-dihydro-1,1,3-trimethyl-3-phenyl-1-H-indene	2	aromatic	31, 32
2,5-dimethyl-furan	2	aromatic	31, 32
ethyl-4-ethoxybenzoate	2	aromatic	31, 32
o-toluidine	2	aromatic	33, 37
propylbenzene	2	aromatic	28,29
toluene	2	aromatic	35, 36
3-methyl-octane	2	alkane	29, 36
butane	2	alkane	30,35
methyl-cyclopentane	2	alkane	29, 33
undecane	2	alkane	28, 29
1-hexene	2	alkene	28, 29
acetone	2	ketone	35, 37
2-methyl-,1-(1,1-diamethylethyl)-2-methyl-1,3-propanediyl ester	2	UC	31, 32
1,1-(1,2-cyclobutanediyl)bis-,cis-benzene	1	aromatic	32
1,1-[1-(ethylthio)propylidene]bis-benzene	1	aromatic	32
1,1-ethylidene-bis[4-ethyl]-benzene	1	aromatic	32
1,2,3,4-terahydro-9-propyl-anthracene	1	aromatic	32
1,2,4,5-3,3,6,6-tetraphenyl-tetroxane	1	aromatic	32
1,2,4-trimethyl-benzene	1	aromatic	29
1,4-dimethyl-benzene	1	aromatic	29
10,11-dihydro-5H-dibenzo-(B,F)-azepin	1	aromatic	31
1-methylethenyl-benzene	1	aromatic	29
1-oxybis-benzene	1	aromatic	31
2,2-diethyl-1,1-biphenyl	1	aromatic	31
2-ethyl-9,10-anthracenediol	1	aromatic	32
aniline	1	aromatic	33
benzophenone	1	aromatic	32
diethylbenzene-1,2-dicarboxylate	1	aromatic	31
xylene	1	aromatic	36
1-methyl-2-pentyl-cyclopropane	1	alkane	29
2,2,4,6,6-pentamethyl-heptane	1	alkane	29
2,4-dimethyl-heptane	1	alkane	29

Name	Number	Class	References
2-methylheptane	1	alkane	29
2-methylhexane	1	alkane	30
2-methyl-pentane	1	alkane	36
3-methyl-hexane	1	alkane	30
3-methyl-nonane	1	alkane	29
3-methyltridecane,	1	alkane	30
4-methyl-decane	1	alkane	31
4-methyl-octane	1	alkane	30
5-methyl-decane	1	alkane	30
7-methyl-tridecane	1	alkane	30
cyclohexane	1	alkane	29
methylcyclopropane	1	alkane	28
pentamethylheptane	1	alkane	36
1,3-butadiene,2-methyl-isoprene	1	alkene	29
1,5,9-trimethyl-1,5,9-cyclododecatriene	1	alkene	31
2,3-hexadiene	1	alkene	32
5,5-dimethyl-1,3-hexadiene	1	alkene	32
ethylbenzene	1	alkene	36
2,4-dimethyl-3-pentanone	1	ketone	31
2-methyl-3-hexanone	1	ketone	32
α-isomethyl ionone	1	ketone	32
2,5-dimethyl-2,4-hexanedione	1	aldehyde	31
butanal	1	aldehyde	34
formaldehyde	1	aldehyde	37
nonanal	1	aldehyde	34
octanal	1	aldehyde	34
pentanal	1	aldehyde	34
propanal	1	aldehyde	34
2,2,4-trimethyl-pentan-1,3-dioldiisobutyrate	1	ester	31
propanoicacid,2,2,4-trimethyl-3-carboxyisopropyl,isobutylester	1	ester	32
2-methoxy-2-methyl-propane	1	ether	32
1,1,2-trichloro-1,2,2-trifluoro-ethane	1	halohydrocarbon	32
trichlorofluoro-methane	1	halohydrocarbon	29
1-(methylthio)-(E)-1-propene	1	organosulphur	32
carbondisulfide	1	organosulphur	30
dimethylsulfide	1	organosulphur	35
2,2,4-Trimethyl-1,3-pentanediol diisobutyrate	1	UC	32
2,2,7,7-tetramethyltricyclo[6.2.1.0(1,6)]undec-4-en-3-one	1	UC	32
2,5-2,6-bis(1,1-dimethylethyl)-cyclohexadiene-1,4-dione	1	UC	31
2,6-bis(1,1-dimethylethyl)-4-ethylidene-2,5-cyclohexadien-1-one	1	UC	32
5-(Ethoxycarbonyl)bicyclo[3.2.2]nonane-1-carboxylic acid	1	UC	32

Name	Number	Class	References
5-isopropenyl-2-methyl-7-oxabicyclo[4.1.0]heptan-2-ol	1	UC	32
7,7-trimethyl-(1S)-bicyclo[2.2.1]heptan-2-one	1	UC	32
camphor	1	UC	32
trans-caryophyllene	1	UC	29
α,α-4-trimethyl-3-cyclohexene-1-methanol	1	UC	32
4-penten-2-ol	1	alcohol	32
ethanol	1	alcohol	35
methanol	1	alcohol	35
p-menth-1-en-8-ol	1	alcohol	32

Putative volatile biomarkers identified in the literature are listed in order of the number of time in the literature the compound has been identified, and grouped by chemical class (UC = unclassified).

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