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ORIGINAL ARTICLE

Canine Olfaction and Electronic Nose Detection of Volatile Organic Compounds in the Detection of Cancer: A Review

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Olfactory cancer detection shows promise as an affordable, precise, and noninvasive way to screen for cancer. This review focuses on two methods of olfactory cancer detection: first, the ability of canines to differentiate between cancerous and healthy individuals through the use of biological samples and second, electronic nose technology that uses chemical sensors to detect known biomarkers in exhaled breath. This review summarizes and critiques past research and outlines future directions to improve understanding of both canine olfaction and electronic nose technology.

Keywords: Biochemical markers, Detection/diagnosis, Cancer biomarkers

INTRODUCTION

Cancer is one of the leading causes of death in the developed world, with an estimated 1.6 million diagnosed cases in 2014 (1). Early diagnosis and treatment are crucial, and while there are some fairly effective methods of detection available depending on the type of cancer, many are invasive and costly. Further, with specificity rates of only 47% for prostate cancer (2,3) and between 50 and 60% for initial colorectal cancer tests (4), it is crucial that more effective detection techniques are developed. Numerous studies have shown that canine olfactory detection can be as effective as biopsies in diagnosing multiple types of cancers, with sensitivity rates ranging from 64 to 99% and specificity ranging from 29 to 99% (5,6). One study even found sensitivity and specificity rates of 100% (7). Therefore, the utilization of canines is a promising future direction for highly specific human cancer detection.

For centuries, bloodhounds have been used to track individuals (8). More recently, dog been used to detect explosives, drugs, and even bed bugs (9). The idea of canines for disease detection can be traced back to 1989, when a woman noticed her dog constantly sniffing a mole on her leg that later turned out to be malignant melanoma (10). It should be noted that this dog was not specifically trained to detect cancer or any substances, but did belong to an obedience trainer.

It is now known that dogs can detect specific volatile organic compounds (VOCs) in urine, breath, blood, and stool (5,6,7,11,12), and that VOC levels are varied in cancer patients (13). Individuals with cancer release certain VOCs in expired air, urine, blood and stool, most of which are alkanes and benzene derivatives (14). Several hundred of these proteins and volatile compounds have been discovered in the breath of those with ovarian, lung, prostate, bladder, and colorectal cancer (15–18). Limited evidence suggests that the concentration of VOCs in expired air may increase as cancer progresses (19). Due to their complex olfactory system and ability to detect volatile compounds in concentrations of parts per trillion, dogs may be capable of detecting cancer earlier and more effectively than blood and tissue analysis or imaging techniques (15). Consequently, dogs may be a valuable asset in a field where treatment efficacy depends so heavily on catching the disease early.

Recent and developing technology has allowed scientists to olfactorily detect cancer without relying on dogs, which are costly to train and keep. An example is the electronic nose (e-nose), a device that analyzes patterns of VOCs in expired breath using gas chromatography and chemosensation (20–24). E-nose technology shows promise as an inexpensive, noninvasive and more controlled way of detecting cancer.

In this review, we will discuss evidence for the existence and potential mechanisms of cancer detection in humans focusing on two techniques, canine detection and e-nose technology. Further, we will explore VOCs as probable biomarkers in ovarian, lung, prostate, bladder, and colorectal cancers. We will review the canine olfactory system and dogs ability to detect cancer before discussing future directions including the utilization of e-nose technology as a more reliable and cost effective method of cancer detection.

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BACKGROUND INFORMATION

When discussing the potential for human cancer detection by dogs, it is important to be familiar with the chemical signals and pathways involved in detection. Dogs have up to 300 million olfactory receptors and an olfactory brain region 40 times greater than ours (25). The canine olfactory system is highly specialized to detect and interpret semiochemicals, chemical substances used for communication (26), and detection is accomplished through the vomeronasal organ (VNO) in their accessory olfactory system (27). Interestingly, however, recent research has suggested that the ability of canines to detect cancer is most likely due to their ability to detect VOCs through the main olfactory system (28–30).

Before we describe studies on canine detection of VOC's, we will first give a brief description of the anatomy of the olfactory system. When an odor enters the nostrils of a dog, it is directed ipsilaterally toward the nasal epithelium, which contains olfactory receptors that project to olfactory nerve cells in the olfactory bulb of the same hemisphere as the detecting nostril (31, 32). From there, olfactory nerve cells form glomeruli with mitral cells, allowing olfactory information to be transmitted to the olfactory cortex of the brain through the olfactory tract. It is important to note that each olfactory receptor only projects to one olfactory cell, allowing each odor to produce a unique spatial map of excitation in the olfactory cortex (33, 34).

Further research has shown that canines initially use the right nostril for novel stimuli before moving to left nostril use as odor becomes familiar (32). An exception to this behavior is that in the presence of arousal stimuli such as adrenaline or sweat odorants, canines preferentially continue to use the right nostril and thus the right hemisphere. These behaviors are consistent with the idea that the hypothalamic-pituitary-adrenal (HPA) axis, responsible for strong emotion and the fight or flight mechanism, is primarily controlled by the right hemisphere (32).

METHOD

A literature search was conducted using the empirical databases PubMed, EBSCOHost, and ScienceDirect. Separate searches were performed for the two main foci of this review: canine cancer detection and electronic nose technology.

Searches for articles involving canine cancer detection included a combination of the terms 'canine,' 'dog,' 'detection,' 'cancer,' and 'volatile organic compound' or 'VOC.' Searches for articles involving electronic nose technology included 'electronic nose,' 'technology,' 'e-nose,' 'cancer,' 'VOC,' 'detection,' and 'olfaction.' In addition, five review articles were found that related to the research question and were subsequently inspected for relevant sources. Overall, 11 empirical studies of canine cancer detection and 16 empirical studies of e-nose cancer detection were identified. Due to the relatively small body of research found, all studies were included regardless of the quality of their experimental designs. The authors noted any perceived flaws or shortcomings in the discussion of the article. For the purpose of this review, the authors felt it appropriate to divide the articles involving dogs into three sections: initial studies, prostate cancer studies, and alternative experimental designs. Further, articles involving the e-nose were also divided into three sections: breath-based cancer biomarkers, electronic nose detection methods, and proteomics and applications.

INITIAL STUDIES

Willis et al. (2004) were the first to show that dogs could be reliably trained to discriminate between urine from controls and cancer patients (35). Six dogs that had completed basic obedience training, but had no previous scent detection training, underwent 7 months of operant conditioning clicker training to differentiate between the urine of cancer patients and controls. After being exposed to novel urine samples, these dogs correctly selected urine from bladder cancer patients 41% of the time compared with an expected rate of 14% by chance alone. Notably, this study incorporated patients with other diseases such as diabetes and chronic cystitis in the control group in order to strengthen their conclusion that dogs were specifically detecting cancer and not simply general illness in the patient or participant.

McCulloch et al. (2006) were among the first to examine the use of canine olfaction to detect lung and breast cancer (5). The study recruited 55 patients with lung cancer, 31 patients with breast cancer, and 83 volunteers with no history of cancer. The exhaled breath of the participants was collected and presented to household dogs that were trained to differentiate between healthy breath samples and malignant breath samples. Among lung cancer patients the test had overall sensitivity of 99% and specificity of 99%. Among the breast cancer patients, the test had an overall sensitivity of 88% and specificity of 99%. These results show promise in using dogs to detect cancer, but, as the researchers suggest, do not yet indicate that the test is sufficient in differentiating between benign and malignant tumors, or those patients who may be at risk of developing cancer.

PROSTATE CANCER STUDIES

Gordon et al. (2008) studied the abilities of four dogs to detect prostate cancer (PCa) and found that only two performed better than chance in specificity, but were no better than chance in sensitivity (36). However, this particular study was flawed and thus caution is urged in the interpretation of results. Some of the primary shortfalls were that one of the trainers was not certified to train dogs, some had never worked with detection dogs before, and all were allowed to use their own unique training tactics with their individual dogs in their own homes. In addition, training and experimentation took place over a 12–14 month period, so it was possible that dogs learned to remember individual odor signatures as opposed to broader cancer VOCs. This study had poor internal validity due to these methodological issues, but highlights potential flaws in canine cancer detection and

the need for careful analysis and refinement of experimental design.

Cornu et al. (2011) were the first to show that a dog could be trained to detect PCa in human urine (37). This study utilized only one dog that was extensively trained using operant conditioning with clicker training. The dog was exposed to 66 samples (33 from healthy individuals and 33 from cancer stricken individuals), and it correctly identified 30 of 33 samples from each group with sensitivity of 91% and specificity of 91%. The experimenters postulated that PCa may have a unique odor signature based on multiple VOCs, and suggested that the next step should be identifying the compounds with gas chromatography or mass spectroscopy.

In contrast to previous work on urine and PCa, Elliker et al. (2014) failed to find significant detection patterns among three highly trained dogs (38). In Stage I, they trained 10 dogs to indicate positive PCa samples using positive reinforcement with food control, however, only three dogs were consistent enough to be moved on to the second training stage. Stage II was effective in training these three dogs to distinguish between PCa and healthy controls, but relied on previously used samples so cancer odor detection could not be confirmed due to the likely role of olfactory memory. When the testing stage commenced, the dogs were presented with unfamiliar urine samples, and their correct detection rates (sensitivity and specificity) were found to be no greater than chance. This suggests that the dogs were able to distinguish between specific urine samples, but only after repeated exposure to a familiar stimuli. It may be that the dogs were not exposed to enough samples during training and testing, and thus held specific olfactory memories for each sample instead of developing a sense for a more general cancer odor.

However, in the most recent study on canine detection of PCa in humans, Taverna et al. (2015) found averages of 98.2% specificity and 99.3% sensitivity across two dogs (11). This study had 902 individual trials, as well as the inclusion of non-healthy, non PCa controls. Interestingly, this is one of few studies which used dogs that had previously been trained in other types of odor detection (explosives), suggesting that prior training may enhance detection abilities. Dogs deemed appropriate and trained for explosive detection or other law enforcement use may be the most effective for detection of cancer in humans and future studies should take this onto account.

ALTERNATIVE EXPERIMENTAL DESIGNS

Colorectal cancer is currently detected either through an invasive colonoscopy or using a fecal occult blood test that has a positive predictive value of only 12% (39). Therefore, there is a clear demand for a more economical, noninvasive and efficient detection method. Sonoda et al. (2011) used one Labrador retriever that had previously been trained for water rescue, and was retrained as a cancer detection dog using a reward based approach (7). Using both breath and watery stool samples of colorectal cancer patients and healthy controls, they found 91% sensitivity and 99% specificity for breath samples, and 97% sensitivity and 99% specificity for watery stool samples. However, the experimenters noticed an age discrepancy between control group and cancer groups whereby the control group was approximately 5 years younger than the cancer group, potentially impacting health and confounding the results. The experimenters reanalyzed samples from patients and controls using only those under 80 years of age, and found 99% sensitivity and 95% specificity for breath samples, and 100% sensitivity and 100% specificity for stool samples. These results support the notion that watery stool samples could allow for higher detection rates due to their proximity to the physical location of the cancer in the body.

Instead of using dogs to discriminate between healthy individuals and individuals known to have cancer, Amundsen et al. (2014) used canine olfaction to differentiate between malignant and benign tumors in individuals with suspected lung cancer (6). The dogs in this study were initially trained by learning the odor signature of lung cancer through exposure to malignant tumors and to urine of lung cancer patients. They were then trained to distinguish between benign tumor odor and the malignant odor that they had been familiarized with. At this stage, dogs exposed to breath samples achieved 64.7% sensitivity and 8.3% specificity, and dogs exposed to urine samples achieved 73.6% sensitivity and 25.0% specificity, both of which were lower than researchers expected and less than the rates established by previous studies such as McCulloch et al. (2006) (5). Due to these initial results, dogs were retrained in a more rigorous and intensive manner, then re-exposed to malignant and benign breath and urine samples. After intensive training, sensitivity decreased from 64.7% to 56% for breath while specificity increased from 8.3% to 33.3%. In the urine samples, sensitivity decreased from 73.6% to 64.2%, while specificity increased from 25.0% to 29.2%. It is possible that the relatively low sensitivity and specificity compared to other studies was due to the presence of a tumor, regardless of its malignancy, which confused the dogs.

Horvath et al. ran two separate studies (2010, 2013) examining canine olfactory detection of ovarian cancer in blood (12,40). In the initial work, the researchers trained two canines to differentiate cancerous blood and ovarian tissue samples from healthy ovarian tissue, and from male and female blood plasma. When exposed to tissue samples, the dogs had 100% sensitivity and 95% specificity, and when exposed to blood samples they had 100% sensitivity and 98% specificity (40). Crucially, the dogs were able to distinguish between ovarian cancer patients and patients with other gynecological malignancies, suggesting that a characteristic odor of ovarian cancer exists and is present in both blood and tissue.

In their second study, the researchers obtained blood samples from healthy individuals and patients with ovarian cancer before treatment, during chemotherapy treatment, and after completing six courses of chemotherapy (12). In series 1, the dogs were exposed to control blood as well as blood samples of patients before treatment and reached 97% sensitivity and 99% specificity (12). In series 2, dogs were exposed to the blood of a subset of patients 3 and 6 months after completion

of chemotherapy. Both dogs positively indicated five patients using the 3-month samples and four of these patients went on to relapse. The fifth had a rare form of cancer known as small cell ovarian cancer which may be expressed differently in the blood and thus appeared unfamiliar to the dogs. Using the 6-month samples, both dogs indicated positively to four patients, two of which were the same as two of the relapse patients from the 3-month samples. The other two patients were not positively identified by the dogs in the 3-month samples, and had not had recurrences of their cancer by the time of publication. However, based on the accuracy of the dogs in the 3-month samples, patients whose blood is positively indicated by both dogs in the 6-month samples may have an increased risk of recurrence. This study presents promising results in using dog detection to assess the likelihood of cancer recurrence in patients following the completion of treatment.

Ehmann et al. (2012) investigated the ability of dogs to detect lung cancer in breath samples (41). The dogs were trained to differentiate the breath of healthy individuals and patients with confirmed lung cancer. During the testing phase, the dogs were also exposed to the breath of patients with chronic obstructive pulmonary disease (COPD). COPD changes exhaled biomarker levels in the breath, and having COPD increases one's likelihood of getting lung cancer. The dogs showed rates of 71% sensitivity and 93% specificity. in addition, the detection was independent from COPD, and the dogs were better at distinguishing between patients with COPD and patients with lung cancer than patients with lung cancer and healthy control participants. Perhaps different VOCs are present in the exhaled breath of COPD patients, making it easier for the dogs to tell that the breath sample is not consistent with that of lung cancer patients. The differentiation of various diseases and illnesses is another potential use for canine olfaction testing. An earlier and more effective way to diagnose COPD would lead to earlier treatment, and fewer occurrences of lung cancer due to the disease. See Table 1 for a summary of these canine cancer detection studies.

BREATH-BASED CANCER BIOMARKERS

Studies of VOCs in expired breath have identified molecules specific to the breath of individuals with cancer (18,42,43). Many of the identified VOCs are alkanes, alkane derivatives, and benzene derivatives. Combinations of these VOCs can serve as fingerprints for different types of cancer. For example, lung cancer patients repeatedly show a telltale combination of 22 specific VOCs in expired breath (18), while breast cancer patients exhale a separate combination of 3 different VOCs (43). These fingerprints, which seem to be unique to each type of cancer, can be compiled in databases and cross-referenced with VOCs in collected e-nose samples, allowing efficient, noninvasive, precise identification of cancer. See Table 2 for a complete list of known VOCs associated with specific cancers.

ELECTRONIC NOSE DETECTION METHODS

The first e-nose, proposed by Persaud & Dodd (1982), was designed to loosely mimic the human olfactory pathway (Figure 1), and e-noses developed since then have used similar designs (42,44,45). E-noses typically run a sample through three phases: a sensor, a preprocessor, and a microchip containing data-analysis software, each of which is discussed below.

VOC Sensors: Sensors come in various forms. Most are based on the same principle: when a VOC passes through the sensor, it interacts preferentially with specific parts of the sensor in a pattern that is unique to that molecule. The result is that each VOC has a specific chemical signature by which it can be identified. Many types of sensors are used (for an excellent summary of the different types and their chemical mechanisms, see Turner & Magan, 2004) (46).

Preprocessors: The binding patterns of VOCs to sensor arrays are coded by a preprocessor. The preprocessor aggregates all of the individual sensory inputs to determine the unique chemical signature of the VOCs before passing it along to a comparative database for identification (44).

Data Analysis Software: Chemical signatures of VOCs are compared to databases of signatures of thousands of compounds, stored in a microchip within the e-nose. The software highlights matches and produces an output, identifying which VOCs are present in the sample (44).

PROTEOMICS AND APPLICATIONS

In addition to the detection of volatile compounds, researchers have begun exploring olfactory detection of non-volatile compounds in expired breath. The main focus of the research has been protein analysis. Proteomics is the study of the structure and function of proteins and how they interrelate on a fundamental level. A better understanding of protein function has the potential to elucidate mechanisms of different diseases, which can translate to early detection of the disease. Understanding the role of proteins as they relate to disease could allow doctors to better treat the disease.

In the study and treatment of cancer, proteomics could serve to identify functional pathways in cancer cells for treatment or diagnostic reasons. Research has found that cancer cells release certain chemical compounds that are unique to cancer cells. The identification of groups of these compounds has allowed e-nose technology to development. New protein biomarkers can be trained into the e-nose's detection system so that it can identify the compound in the future. These proteins are identified through mass spectrometry, gas chromatography, electrophoresis gel runs, and protein assays. See Conrad et al. (2008) for a review of different techniques used for identifying proteins (47).

In addition to proteins and VOCs, another avenue of e-nose research includes the ability of the e-nose to detect and analyze non-volatile compounds that might be exhaled in breath from the lungs. So far, researchers have been able to successfully detect several forms of cancer with high sensitivity. To date, lung, prostate, bladder, ovarian, breast,

Table 1. Summary of Canine Cancer Detection Studies

Authors and Year	Type of Cancer	Sample Type	# of Dogs Used	# of Cancer Samples	Sensitivity	Specificity	Notes
Willis et al., 2004	Bladder	Urine	6	54	N/A	41%	1st proof of principle
McCulloch et al., 2006	Breast	Breath	—	116	88%	99%	
	Lung			574	99%	99%	
Gordon et al., 2008	Prostate	Urine	4	33	—	—	Flawed design
	Breast		6	18	—	—	
Horvath et al., 2010	Ovarian	Tissue	2	40	100%	95%	
		Blood	2	40	100%	98%	
Cornu et al., 2010	Prostate	Urine	1	33	91%	91%	
Sonoda et al., 2011	Prostate	Stool	1	37	100%	100%	Patients under 80 years old
		Breath	1	33	95%	99%	
Ehmann et al., 2012	Lung	Breath	4	25	71%	93%	Compared with COPD
Horvath et al., 2013	Ovarian	Blood	2	42	97%	99%	
Amundsen et al., 2014	Lung	Urine	3	59	73.6%	29.2%	Benign vs. malignant
		Breath			64.7%	33.3%	
Elliker et al., 2014	Prostate	Urine	3	16	—	—	Not better than chance
Taverna et al., 2015	Prostate	Urine	2	362	99.3%	98.2%	

and colorectal cancer have been positively identified through the use of electronic nose technology (21,24,47–49).

DISCUSSION

Since the first proof of principle study in 2004 showed that dogs could detect cancer at a better rate than chance (35), at least six follow up studies have confirmed these findings. Through the use of blood, urine, feces and breath, it seems clear that dogs possess the ability to detect cancer in human bodily fluids. However, dogs can be expensive to train and maintain, difficult to manage, are prone to illness, and generally inconsistent. While some of the organic compounds that dogs presumably use to detect cancer-associated odors have been identified, there are no doubt more that have yet to be found. Using current technology such as gas chromatography and mass spectroscopy, it is possible to identify additional odorous compounds in cancer patients. New technology such as the electronic nose is being developed that aims to match and eventually supersede the detection abilities of dogs.

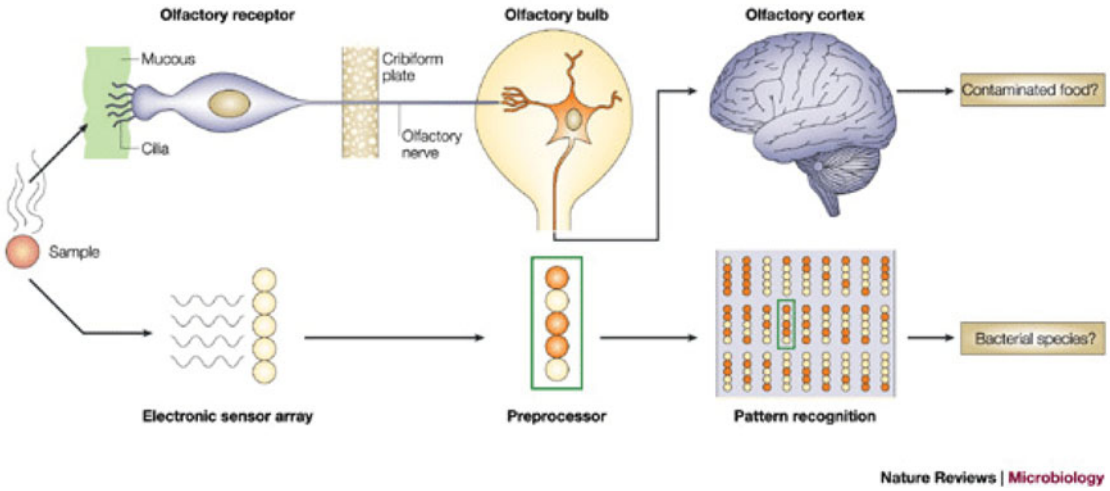

Figure 1. Electronic nose processing compared to human olfactory processing (41).

Table 2. Known Volatile Organic Chemicals by Associated Cancer Type

Lung cancer	**Prostate cancer**
1-methyl-4-(1-methylethyl)benzene	Toluene
Toluene	2-amino-5-isopropyl-8-methyl-1-azulenecarbonitrile
dodecane	p-xylene
3,3-dimethyl pentane	2,2-dimethyl decane
2,3,4-trimethyl hexane	formaldehyde
Styrene (ethenylbenzene)	**Breast Cancer**
Heptane, 2,2,4,6,6-pentamethyl	3,3-dimethyl pentane
Heptane, 2-methyl	2-amino-5-isopropyl-8-methyl-1-azulenecarbonitrile
Decane	5-(2-methylpropyl)nonane
Benzene, propyl-	2,3,4-trimethyl decane
Undecane	6-ethyl-3-octyl ester 2-trifluoromethyl benzoic acid
Cyclopentane, methyl-	Nonane
Cyclopentane, 1-methyl-2-pentyl-	Tridecane, 5-methyl
Methane, trichlorofluoro	Undecane, 3-methyl
Benzene	Pentadecane, 6-methyl
Benzene, 1,2,4-trimethyl-	propane, 2-methyl
1,3-butadiene, 2-methyl- (isoprene)	nonadecane, 3-methyl
Octane, 3-methyl-	dodecane, 3-methyl
1-hexene	Octane, 2-methyl
Nonane, 3-methyl-	Dimethyl trisulphide
1-heptene	**Colon Cancer**
Benzene, 1,4-dimethyl	2,3,4-trimethyl hexane
Heptane, 2,4-dimethyl	1,3-dimethyl benzene
Hexanal	1-iodo nonane
Cyclohexane	[(1,1-dimethylethyl)thio] acetic acid
Benzene, 1-methylethenyl-	4-(4-propylcyclohexyl)-40 -cyano[1,10 -biphenyl]-4-yl ester benzoic acid
Heptanal	2-amino-5-isopropyl-8-methyl-1-azulenecarbonitrile
hydrazine-carboxamide	Nonanal
methyl-hydrazine	4-methyl-2-pentanone
ethyl alcohol	Decanal
o-Xylene	2-methylbutane
Ethylbenzene	1,2-pentadiene
dimethyl ether	2-methylpentane
butylated hydroxytoluene	3-methylpentane
Carbonic dihydrazide	Methylcyclopentane
1-methyl-2-(1-methylethyl)-benzene	Cyclohexane
1-methyl-3-(1-methylethyl)-benzene	methylcyclohexane
1,3,5-Cycloheptatriene	4-methyloctane
3-Methyl-hexane	1,4-dimethylbenzene
1,3,5,7-cyclooctatetraene	A (4-methylundecane)
Bicyclo[4.2.0]octa-1,3,5-triene	B (trimethyldecane)
2,6-Bis(1,1 = dimethylethyl)-4-methyl-methylcarbamate phenol	4-methylphenol*
2,4-Dimethyl-heptane	pentanoic acid*
4,7-Dimethyl-undecane	indole*
2,4,6-Tris(1,1-dimethyl-ethyl)-4-methylcyclohexa-2,5-dien-1-one	2 or 3-methylfuran*
2,6,6-Trimethyl octane	Dimethylsulfide*
2-Butanone	Carbon disulfide*
1,3-Pentadiene	Butanoic acid*
3,3-dimethyl-hexane	Benzaldehyde*
2-methyl-hexane	Ethanoic acid*
3-ethyl-hexane	6-methyl-5-hepten-2-one*
2,2,3-Trimethyl-hexane	2-Pentanone*
Ethylidene cyclopropane	2-Butanone*
4-methyl-octane	2,3-Butanedion*
2-ethyl-1-hexanol	Acetaldyhyde*
2-ethyl-4-methyl-1-pentanol	Acetone*
2,3,4-trimethyl-pentane	2-Heptanone*
2,3-Dimethyl-hexane	Propanal*
3-ethyl-3-methyl-2-pentanone	Hexanal*
2-methyl-4,6-octadiyn-3-one	3-methylbutanal*
2-propyl-1-pentanol	Butanal*
6,10-dimethyl-5,9-dodecadien-2-one	Ethanol*
formaldehyde	2-methylbutanal*
	Propanoic acid*
	Head and Neck Cancers
	Dimethyl trisulphide

These compounds were sourced from (14,19,40,52–55). *refers to a compound discovered in headspace and not expired breath. No asterisk indicates VOC's found in expired breath.

One of the primary concerns of canine studies reviewed here is the method of training and stimulus presentation. As mentioned earlier, the dogs used in the Elliker et al. (2014) study were able to distinguish between familiar stimuli but were unable to discriminate between unfamiliar cancer and control urine samples (38). Older research had suggested the potential for a similar pattern of odor signature memorization among dogs trained for both PCa and breast cancer (36). This leads to concern about the potential confounding elements of olfactory memory and the possibility that dogs are able to distinguish individual scents, but that they are not using cancer biomarkers to do so, and are instead just remembering which samples they were rewarded for. To control for this, Elliker et al. strongly encouraged the implementation of double blinded studies with novel urine samples (38).

While many studies show high sensitivity and specificity, it is important to note the following confounds that were present in some of the studies. First, many articles reviewed in this paper compared cancer patients to healthy controls and negated to include any patients with other illnesses, failing to control for the possibility that dogs were simply detecting poor health, comorbid issues or symptoms that may not be unique to cancer. This is of particular concern in the work of Sonoda et al. (2014), because without knowing the method of training it is reasonable to assume that the canines were simply able to detect gastrointestinal changes that may have been due to disease but not necessarily cancerous growth (7). Further research should include patients with other illnesses, including those that affect the same anatomical structures as the cancer in question, such as the work done by Ehmann et al. (2012) (41).

Second, the breeds of the dogs and their training backgrounds were highly varied. Dogs that had been previously trained for other scent detection work, such as explosives, were highly effective at correctly identifying cancer (11), while those without any previous experience in scent detection generally performed worse (38). While some research has shown that varying dog breeds did not significantly alter performance (6), it should be further studied, especially due to the high levels of specialization attainable by breeds such as German Shepherds and Basset Hounds that are preferred by law enforcement. In addition, training methods are highly varied across studies, with some particularly flawed studies using inexperienced, even non-certified trainers who were free to train their dogs however they saw fit before being aggregated into one study (36). It is crucial to eliminate as much variability as possible in training and experimentation through the use of consistent and detailed protocols.

Finally, presentation of stimuli is paramount and extensively varied across studies, with some dogs being allowed or even encouraged to physically touch the samples with their noses (41), while others were unable to see the stimuli and had to rely solely on odor (38). In addition, in the first proof of principle study of human cancer detection by canines, dogs that had been trained with wet urine performed better (50% correct) than those trained with dry urine (22% correct), highlighting the importance of sample storage, preparation and presentation (35).

Dog training and maintenance are both time consuming and expensive, and dogs have varying scent ability and concentration levels on a day to day basis, leading to concerns about the practicality of using dogs in cancer detection. Many of the articles reviewed here utilize just one or two dogs, minimizing the power of the study and the significance of results. Future studies should include more dogs per experiment in order to improve consistency under one experimental design.

Previous studies have suggested that dogs possess an ability to detect estrous cycles in cows (50), as well as nematode infections in sheep, (51) yielding more evidence for the ability of dogs to detect VOCs in other animals. Finally, dogs are the only other large mammal that commonly develop certain cancers, including prostate cancer (52), making cancer detection a potential evolutionary benefit for reproductive success and lending more support to the ability of dogs to detect cancer.

While e-nose screening also shows promise as a method of detecting cancer, there are shortcomings in the field. As with canine cancer detection research, most studies using e-noses have compared cancer patients to healthy controls. It is possible that VOC patterns in the breath change as the result of poor health in general, and not specifically because of cancer. Wang et al. (2014) addressed this concern with promising results using patients with breast cancer, cyclomastopathy, and mammary gland fibroma, as well as healthy controls (43). Through VOC analysis, they found three VOCs that differentiated breast cancer patients from all other groups. Future research should adopt similar methodologies to confirm the uniqueness of VOC patterns to specific forms of cancer.

E-nose technology is progressing rapidly. New cancer fingerprints are being researched and catalogued (43,53), as are possible fingerprints for diagnosing other medical conditions, including Alzheimer's disease (54), asthma (55), and bacterial infections (56). However, accuracy is still an issue. For example, an advanced and effective electronic nose, called the NaNose (Technion, Haifa, Israel), uses nanotechnology to increase accuracy of cancer detection and give readings in real time. The NaNose has undergone successful clinical testing, showing detection of early-stage malignant lung tumors with accuracy of sensitivity and specificity greater than 80% (23). The lab developing the product expects it to undergo evaluation by the Food and Drug Administration (FDA) within 1–2 years, and says that it could reach health-care markets before 2020. Real-time, noninvasive screening for cancer could become a standard part of routine doctor visits, drastically decreasing mortality rates by catching the disease early. At present, more clinical research and technological fine-tuning is required to ensure the accuracy and efficacy of the electronic nose. In the meantime we may have to continue to rely on man's best friend, not just as a companion but as a critical aspect of a cancer-free lifestyle.

The authors recognize the need for a more comprehensive and systematic review of olfactory cancer detection methods in the coming years. Such a review was published in 2010 (57), and a great deal of research has been performed since.

This general review attempts to fill a gap between older reviews and a more systematic future review.

DECLARATION OF INTEREST

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the article.

REFERENCES

1. Siegel R, Ma J, Zou Z, Jemal A. Cancer statistics, 2014. CA: Cancer J Clin 2014;64(1):9–29.
2. Parpart S, Rudis A, Schreck A, Dewan N, Warren P. Sensitivity and specificity in prostate cancer screening methods and strategies. J. Young Invest. 2007 Apr.
3. Tanday S. Targeted biopsy boosts detection of high-risk prostate cancer. Lancet Oncol. 2015;16(3):e107.
4. Winawer SJ. Colorectal cancer screening. Best Pract Res Clin Gastroenterol 2007;21(6):1031–1048.
5. McCulloch M, Jezierski T, Broffman M, Hubbard A, Turner K, Janecki T. Diagnostic accuracy of canine scent detection in early- and late-stage lung and breast cancers. Integrat Cancer Therap 2006;5(1):30–39.
6. Amundsen T, Sundstrøm S, Buvik T, Gederaas OA, Haaverstad R. Can dogs smell lung cancer? First study using exhaled breath and urine screening in unselected patients with suspected lung cancer. Acta Oncol 2014;53(3):307–315.
7. Sonoda H, Kohnoe S, Yamazato T, Satoh Y, Morizono G, Shikata K, Morita M, Watanabe A, Morita M, Kakeji Y, Inoue F, Maehara Y. Colorectal cancer screening with odour material by canine scent detection. Gut 2011;60(6):814–819.
8. Schoon A, Haak R. K9 suspect discrimination: Training and practicing scent identification line-ups. Calgary, AB: Brush Education; 2002.
9. Vaidyanathan R, Feldlaufer MF. Bed bug detection: current technologies and future directions. Amer J Trop Med Hyg 2013;88(4):619–625.
10. Williams H, Pembroke A. Sniffer dogs in the melanoma clinic? Lancet 1989;333(8640):734.
11. Taverna G, Tidu L, Grizzi F, Torri V, Mandressi A, Sardella P, La Torre G, Cocciolone G, Seveso M, Giusti G, Hurle R, Santoro A, Graziotti P. Highly-trained dogs' olfactory system detects prostate cancer in urine samples. J Urol 2015;193(4):1382–1397.
12. Horvath G, Andersson H, Nemes S. Cancer odor in the blood of ovarian cancer patients: a retrospective study of detection by dogs during treatment, 3 and 6 months afterward. BMC Cancer 2013;13(1):1–7.
13. Arasaradnam RP, McFarlane MJ, Ryan-Fisher C, Westenbrink E, Hodges P, Thomas MG, . . . Covington JA. Detection of colorectal cancer (CRC) by urinary volatile organic compound analysis. PloS One 2014;9(9):e108750.
14. O'neill HJ, Gordon SM, O'Neill MH, Gibbons RD, Szidon JP. A computerized classification technique for screening for the presence of breath biomarkers in lung cancer. Clin Chem 1988;34(8):1613–1618.
15. Banks R, & Selby P. Clinical proteomics—insights into pathologies and benefits for patients. Lancet 2003;362(9382):415–416.
16. Carpagnano GE, Foschino-Barbaro MP, Resta O, Gramiccioni E, Carpagnano F. Endothelin-1 is increased in the breath condensate of patients with non-small-cell lung cancer. Oncology 2004;66(3):180–184.
17. Okano T, Kondo T, Kakisaka T, Fujii K, Yamada M, Kato H, . . . Hirohashi S. Plasma proteomics of lung cancer by a linkage of multi-dimensional liquid chromatography and two-dimensional difference gel electrophoresis. Proteomics 2006;6(13):3938–3948.
18. Phillips M, Gleeson K, Hughes JMB, Greenberg J, Cataneo RN, Baker L, McVay WP. Volatile organic compounds in breath as markers of lung cancer: a cross-sectional study. Lancet 1999;353(9168):1930–1933.
19. Fu XA, Li M, Knipp RJ, Nantz MH, Bousamra M. Noninvasive detection of lung cancer using exhaled breath. Cancer Med 2014;3(1):174–181.
20. Szulejko JE, McCulloch M, Jackson J, McKee DL, Walker JC, Solouki T. Evidence for cancer biomarkers in exhaled breath. Sensors J IEEE 2010;10(1):185–210.
21. Asimakopoulos AD, Del Fabbro D, Miano R, Santonico M, Capuano R, Pennazza G, . . . Finazzi-Agrò E. Prostate cancer diagnosis through electronic nose in the urine headspace setting: a pilot study. Prostate Cancer Prostatic Dis 2014;17(2):206–211.
22. Di Natale C, Macagnano A, Martinelli E, Paolesse R, D'Arcangelo G, Roscioni C, Finazzi-Agrò A, D'Amico A. Lung cancer identification by the analysis of breath by means of an array of non-selective gas sensors. Biosensors Bioelectronics, 2003;18(10):1209–1218.
23. Peled N, Hakim M, Haick H, Bunn PA, Miller YE, Kennedy TC, . . . Hirsch FR. Use of a nanoparticle-based artificial olfactory system, NaNose, to distinguish malignant from benign pulmonary nodules. ASCO Ann Meeting Proc 2010;28(15):10521.
24. Peng G, Hakim M, Broza YY, Billan S, Abdah-Bortnyak R, Kuten A, . . . Haick H. Detection of lung, breast, colorectal, and prostate cancers from exhaled breath using a single array of nanosensors. Brit J Cancer 2010;103(4):542–551.
25. Tyson P. Dogs' dazzling sense of smell. NOVA [Internet]. 2012 Oct 4 [Retrieved 2014 Oct 23];NOVA scienceNOW. Available from: http://www.pbs.org/wgbh/nova/nature/dogs-sense-of-smell.html
26. Wyatt T. Pheromones and Animal Behaviour Communication by Smell and Taste. Cambridge, UK: Cambridge University Press, 2003.
27. Adams DR, Wiekamp MD. The canine vomeronasal organ. J Anatomy 1984;138(Pt 4):771.
28. Pickel D, Manucy GP, Walker DB, Hall SB, Walker JC. Evidence for canine olfactory detection of melanoma. App Animal Behav Sci 2004;89(1):107–116.
29. Bjartell AS. Dogs sniffing urine: a future diagnostic tool or a way to identify new prostate cancer markers?. Eur Urol 2011;59(2):202–203.
30. de Boer NK, de Meij TG, Oort FA, Ben Larbi I, Mulder CJ, van Bodegraven AA, van der Schee MP. The scent of colorectal cancer: detection by volatile organic compound analysis. Clin Gastroenterol Hepatol 2014;12(7):1085–1089.
31. Reece WO. Functional Anatomy and Physiology of Domestic Animals. Singapore: John Wiley & Sons; 2013.
32. Siniscalchi M, Sasso R, Pepe AM, Dimatteo S, Vallortigara G, Quaranta A. Sniffing with the right nostril: lateralization of response to odour stimuli by dogs. Animal Behaviour, 82(2), 399–404.
33. Buszewski, B., Rudnicka, J., Walczak, M., & Jezierski, T. A new approach to identification of biomarkers for early cancer stage detection. Nova Biotechnologica et Chimica 2014;13(1): 21–27.
34. Ressler KJ, Sullivan SL, Buck LB. Information coding in the olfactory system: evidence for a stereotyped and highly organized epitope map in the olfactory bulb. Cell 1994;79(7): 1245–1255.
35. Willis CM, Church SM, Guest CM, Cook WA, McCarthy N, Bransbury AJ, Church MR, Church JC. Olfactory detection of human bladder cancer by dogs: proof of principle study. BMJ 2004;329(7468):712.
36. Gordon RT, Schatz CB, Myers LJ, Kosty M, Gonczy C, Kroener J, . . . Zaayer J. The use of canines in the detection of human cancers. J Alternat Complement Med 2008;14(1):61–67.
37. Cornu JN, Cancel-Tassin G, Ondet V, Girardet C, Cussenot O. Olfactory detection of prostate cancer by dogs sniffing urine: a step forward in early diagnosis. Eur Urol 2011;59(2): 197–201.

38. Elliker KR, Sommerville, BA, Broom DM, Neal DE, Armstrong S, Williams HC. Key considerations for the experimental training and evaluation of cancer odour detection dogs: lessons learnt from a double-blind, controlled trial of prostate cancer detection. BMC Urol 2014;14(1):22.
39. Steele RJ, McClements PL, Libby G, Black R, Morton C, Birrell J, . . . Fraser CG. Results from the first three rounds of the Scottish demonstration pilot of FOBT screening for colorectal cancer. Gut 2009;58(4):530–535.
40. Horvath G, Andersson H, Paulsson G. Characteristic odour in the blood reveals ovarian carcinoma. BMC Cancer 2010;10(1):643.
41. Ehmann R, Boedeker E, Friedrich U, Sagert J, Dippon J, Friedel G, Walles T. Canine scent detection in the diagnosis of lung cancer: revisiting a puzzling phenomenon. Eur Respirat J 2012;39(3):669–676.
42. Poli D, Carbognani P, Corradi M, Goldoni M, Acampa O, Balbi B, . . . Mutti A. Exhaled volatile organic compounds in patients with non-small cell lung cancer: cross sectional and nested short-term follow-up study. Respiratory research, 2005;6(1):71.
43. Wang C, Sun B, Guo L, Wang X, Ke C, Liu S, . . . Li E. Volatile organic metabolites identify patients with breast cancer, cyclomastopathy, and mammary gland fibroma. Scientific Reports 2014;4:5383.
44. Persaud K, Dodd G. Analysis of discrimination mechanisms in the mammalian olfactory system using a model nose. Nature 1982;299:352–355.
45. Phillips M, Altorki N, Austin JH, Cameron RB, Cataneo RN, Greenberg J, . . . Schmitt P. Prediction of lung cancer using volatile biomarkers in breath. Cancer Biomark 2007;3(2):95–109.
46. Turner AP, Magan N. Electronic noses and disease diagnostics. Nature Rev Microbiol 2004;2(2):161–166.
47. Conrad DH, Goyette J, Thomas PS. Proteomics as a method for early detection of cancer: a review of proteomics, exhaled breath condensate, and lung cancer screening. J General Internal Med 2008;23(1):78–84.
48. Horvath G, Chilo J, Lindblad T. Different volatile signals emitted by human ovarian carcinoma and healthy tissue. Future Oncol 2010;6(6):1043–1049.
49. Roine A, Veskimäe E, Tuokko A, Kumpulainen P, Koskimäki J, Keinänen TA, . . . Oksala NK. Detection of prostate cancer by an electronic nose: a proof of principle study. J Urol 2014;192(1):230–234.
50. Hawk HW, Conley HH, Kiddy CA. Estrus-related odors in milk detected by trained dogs. J Dairy Sci 1984;67(2):392–397.
51. Richards KM, Cotton SJ, Sandeman RM. The use of detector dogs in the diagnosis of nematode infections in sheep feces. J Veterin Behavior 2008;3(1):25–31.
52. Keller JM., Schade GR, Ives K, Cheng X, Rosol TJ, Piert M, . . . Keller ET. A novel canine model for prostate cancer. Prostate 2013;73(9):952–959.
53. Wang C, Li P, Lian A, Sun B, Wang X, Guo L, Chi C, Liu S, Zhao W, Luo S, Guo Z, Zhang Y, Ke C, Ye G, Xu G, Zhang F, Li E. Blood volatile compounds as biomarkers for colorectal cancer. Cancer Biol Therapy 2014;15(2):200–206.
54. Koczulla AR, Gold M, Hattesohl A, Lubbe D, Mengel D, Schmid S, . . . Bach JP. An application of electronic nose technology for diagnosis of Alzheimer's disease. Eur Respirat J 2013;42(Suppl 57): P1275.
55. Dragonieri S, Schot R, Mertens BJ., Le Cessie S, Gauw SA, Spanevello A, . . . Sterk PJ. An electronic nose in the discrimination of patients with asthma and controls. J Allergy Clin Immunol 2007;120(4):856–862.
56. Thaler ER, Hanson CW. Use of an electronic nose to diagnose bacterial sinusitis. Amer J Rhinol 2006;20(2):170–172.
57. Moser E, McCulloch M. Canine scent detection of human cancers: A review of methods and accuracy. J Veterin Behav 2010;5(3):145–152.
58. Altomare DF, Di Lena M, Porcelli F, Trizio L, Travaglio E, Tutino M, . . . De Gennaro G. Exhaled volatile organic compounds identify patients with colorectal cancer. Brit J Surg 2013;100(1):144–150.
59. Hakim M, Billan S, Tisch U, Peng G, Dvrokind I, Marom O, . . . Haick H. Diagnosis of head-and-neck cancer from exhaled breath. Brit J Cancer 2011;104(10):1649–1655.
60. Phillips M, Cataneo RN, Ditkoff BA, Fisher P, Greenberg J, Gunawardena R, . . . Wong C. Volatile markers of breast cancer in the breath. Breast J 2003;9(3):184–191.
61. Ulanowska A, Ligor T, Michel M, Buszewski B. Hyphenated and unconventional methods for searching volatile cancer biomarkers. Ecol. Chem. En 2010;17:9–23.