Canine Scent Detection of Lung and Breast Cancers

Diagnostic Accuracy of Canine Scent Detection in Early-and Late-Stage Lung and Breast Cancers

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***Background*:** Lung and breast cancers are leading causes of cancer death worldwide. Prior exploratory work has shown that patterns of biochemical markers have been found in the exhaled breath of patients with lung and breast cancers that are distinguishable from those of controls. However, chemical analysis of exhaled breath has not shown suitability for individual clinical diagnosis. ***Methods*:** The authors used a food reward-based method of training 5 ordinary household dogs to distinguish, by scent alone, exhaled breath samples of 55 lung and 31 breast cancer patients from those of 83 healthy controls. A correct indication of cancer samples by the dogs was sitting/lying in front of the sample. A correct response to control samples was to ignore the sample. The authors first trained the dogs in a 3-phase sequential process with gradually increasing levels of challenge. Once trained, the dogs' ability to distinguish cancer patients from controls was then tested using breath samples from subjects not previously encountered by the dogs. The researchers blinded both dog handlers and experimental observers to the identity of breath samples. The diagnostic accuracy data reported were obtained solely from the dogs' sniffing, in double-blinded conditions, of these breath samples obtained from subjects not previously encountered by the dogs during the training period. Results: Among lung cancer patients and controls, overall sensitivity of canine scent detection compared to biopsy-confirmed conventional diagnosis was 0.99 (95% confidence interval [CI], 0.99, 1.00) and overall specificity 0.99 (95% CI, 0.96, 1.00). Among breast cancer patients and controls, sensitivity was 0.88 (95% CI, 0.75, 1.00) and specificity 0.98 (95% CI, 0.90, 0.99). Sensitivity and specificity were remarkably similar across all 4 stages of both diseases. ***Conclusion*:** Training was efficient and cancer identification was accurate; in a matter of weeks, ordinary household dogs with only basic behavioral "puppy

training" were trained to accurately distinguish breath samples of lung and breast cancer patients from those of controls. This pilot work using canine scent detection demonstrates the validity of using a biological system to examine exhaled breath in the diagnostic identification of lung and breast cancers. Future work should closely examine the chemistry of exhaled breath to identify which chemical compounds can most accurately identify the presence of cancer.

Keywords: dogs; canine scent detection; breast cancer; diagnosis; lung cancer, diagnosis

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Early detection of cancer is a desirable goal as it often allows treatment with lower toxicity and predicts longer survival. In many cancers, however, the limited capabilities of existing diagnostic methods may contribute to high cancer mortality.

When lung cancer is detected in early stages, surgical resection alone can achieve 5-year survival rates as high as 50%.1 However, chest x-ray and sputum cytology have a high false-negative rate and therefore fail to detect many early-stage cases.1 Furthermore, computed tomography (CT) scan, able to detect lesions as small as 1 mm in diameter, produces many false positives, which lead to unnecessary surgery for biopsy.2 The high false-positive rate of the CT scan can be reduced through confirmation with positron emission tomography scan.3

In breast cancer, although evidence suggests mammography screening reduces breast cancer mortality4,5 and allows treatment with lower toxicity,6 there is still uncertainty

due to variable quality of studies7 and inconsistency of results across studies.8 Mammography screening detects noncancerous lesions, leading to unnecessary testing, treatment, and anxiety.9 In addition, mammography is more likely to fail to detect cancers in women with dense breast tissue.10

An alternate approach to these traditional diagnostic tools has been the search to identify biomarkers that could be identified through testing blood samples with analytical chemistry methods.

However, these methods have significant drawbacks. In lung cancer, many biomarkers, when considered individually, have not shown suitability for use as screening tests, and it is not yet clear what panel of tests used together will provide sufficient diagnostic sensitivity and specificity.11,12 In breast cancer, evidence is limited by a lack of assay standardization and by studies that are too small and are not easily comparable, with heterogeneous patient and tumor characteristics.13

Canine scent detection may possibly help overcome some of these drawbacks due to the extraordinary scenting ability of the dog's nose, which has detection thresholds as low as parts per trillion.14,15 In addition, the canine olfactory system appears to go beyond simply low detection thresholds to the capability of discriminating between complex chemical mixtures, such as would be found in exhaled human breath.

Volatile organic compounds potentially diagnostic of cancer, such as alkanes, methylated alkanes, aromatic compounds, and benzene derivatives, have been identified using gas chromatography/mass spectroscopy (GCMS) in the exhaled breath of patients with lung and breast cancers.16-22 In both lung19,22 and breast cancers,18 relative concentrations of these exhaled compounds differ between patients and healthy volunteers. Nevertheless, GCMS cannot detect all or even nearly all chemicals present, potentially missing the most important diagnostic markers. Furthermore, it is not yet standard practice in cancer diagnosis.

Case reports of patient-dog interactions leading to cancer diagnoses first appeared in 198923 and then in 2001.24 This was followed by intriguing evidence that melanoma25

and bladder cancers26 could be detected by dogs through their use of their scenting abilities.

In the present study, we set out to explore 3 questions. Our first objective was to see whether ordinary dogs, with no prior scent discrimination training, could be rapidly trained to identify lung and breast cancer patients by smelling samples of their breath when compared to blank unused sample tubes. Our second objective was to investigate whether dogs could distinguish breath samples of lung and breast cancer patients from those of healthy controls. Finally, we sought to examine whether the dog's diagnostic performance would be affected by disease stage of cancer patients and age, smoking, or most recently eaten meal among either cancer patients or controls.

Methods

Patients and Control Breath Donors

Eligible patients were men and women older than 18 years with a very recent biopsy-confirmed conventional diagnosis of lung or breast cancer. We specifically requested that recruitment centers refer patients as soon as possible following definitive diagnosis so that breath sampling would not interfere with or delay planned conventional treatment. As we suspected that chemotherapy treatment would change the exhaled chemicals in cancer patients, we sought patients who had not yet undergone chemotherapy treatment. As we also suspected that patients with more advanced disease, and thus larger tumors, might be exhaling higher concentrations of the chemicals associated with cancer cells and would therefore be more easily identified by the dogs, we sought patients with any stage disease.

We recruited both patients and controls through local medical centers (Pine Street Clinic, San Anselmo, Calif; Golden Gate Center for Integrative Cancer Care, San Francisco, Calif; California Cancer Care, Greenbrae, Calif). All subjects, both patients and controls, completed a questionnaire about factors we thought could affect exhaled chemicals in the breath: age, current or past smoking, diabetes, dental infection, and most recently eaten meal (described below; see "Analysis"). We recruited 55 patients (35 men and 20

women) with lung cancer and 31 patients (1 man and 30 women) with breast cancer (Table 1). We also recruited as controls 83 volunteers with no prior cancer history. Excluded patients were those who responded to our study recruitment brochure after having begun standard therapy or for whom a positive biopsy report was not available.

All subjects provided written informed consent. Our protocol and patient recruitment materials were approved by an institutional review board (Independent Review Consulting, Corte Madera, Calif). In addition, our animal-training and -handling methods were designed in consultation with 2 veterinarians, an independent dog trainer, and the dog owners, all of whom approved our methods. We did not provide any compensation to subjects for providing breath samples or to dog owners for volunteering use of their dogs in the study.

Equipment and Breath Sampling

For breath sampling, we obtained a cylindrical polypropylene organic vapor sampling tube (Defencetek, Pretoria, South Africa). Each tube is open at either end, is 6 inches long, has an outer diameter of 1 inch, has an inner diameter of 0.75 inches, and has removable end caps. A removable 2-inch-long insert of silicone oil-coated polypropylene "wool" captures volatile organic compounds in exhaled breath as breath passes through the tube.

Table 1. Subjects

Diagnosis and Histology	Stage	Number	Gender		Median Age (Range)
			M	F	
Non-small-cell lung cancer					
Adenocarcinoma	I	4	4		
	II	9	7	2	
	III	10	4	6	
	IV	16	8	8	
Squamous	II	6	3	3	
	III	4	3	1	
	IV	6	6		

All		55	35	20	59 (43-87)
Breast cancer					
Adenocarcinoma	I	8		8	
	II	6	1	5	
	III	12		12	
	IV	4		4	
Lobular	III	1		1	
All		31	1	30	55 (39-73)
Healthy donors		83	41	42	50 (22-79)

To collect breath samples, we asked donors to exhale 3 to 5 times through the tube. We then fitted the tubes with their end caps and sealed them in ordinary grocery store Ziplock-style bags at room temperature between the time of breath sampling and presentation to the dogs.

We believed that breath from deep in the lungs, where oxygen exchange takes place, would contain a higher concentration of exhaled chemical compounds potentially diagnostic of cancer. Therefore, we asked subjects to make their exhalations through the tube as long and deep as comfortably possible. We stopped sampling if subjects became breathless, began to cough, or did not want to continue; thus, the number of breath sample tubes we were able to collect per subject ranged from 4 to 18.

For each person sampled, all of the breath samples were collected during 1 visit. The researcher gathering breath samples was asked to subjectively note whether the forcefulness of subjects' breathing through the tube was "mild" or "strong." Because of variations in recruitment rates and the limited window of time between date of diagnosis and beginning of chemotherapy, sample storage time varied between 1 and 60 days.

Dogs

Five dogs, aged 7 to 18 months, were chosen from a total of 13: 3 Labrador retrievers (2 males and 1 female) and 2 Portuguese water dogs (1 male and 1 female). Dogs were provided by local dog owners and by Guide Dogs for the Blind (San Rafael, Calif). Our selection criteria called for dogs older than 6 months with basic obedience training

typically given to household pets, as defined by the American Kennel Club. The experimenters screened recruited dogs for their level of eagerness to sniff objects and respond to commands. Our training method was a reward-based approach in which the correct behavior is rewarded by simultaneously activating a clicker device and presenting a food snack. Between training sessions, dogs were housed in clean, well-ventilated kennel crates appropriate for the size of each dog. Water was freely available, and high-value treats were provided during each morning's training and testing session. Between each trial, which lasted approximately 10 minutes, each dog was allowed time for free play with dog handlers and was given access to a large fenced play yard. Veterinary care was made available to each dog; however, it was not needed as all dogs completed training and testing without any adverse events and no dogs experienced any injury or illness during the course of our study. In Table 2, we illustrate the sequential training and testing process we used to first train dogs to detect cancer samples, then distinguish them from controls, and finally test their discrimination ability in a double- blind design.

Experimental Setup

Training room. We conducted the training and testing of the dogs in a 3.0 m × 7.3 m room with vinyl tiling and overhead fluorescent and natural window lighting. The room was not climate controlled, and average ambient temperatures during our study (March through August) ranged from 68°F to 75°F. At the end of each working day, the floor was cleaned with Murphy's Oil Soap and water.

Table 2. Sequential Phases of Dog Training and Testing

Sequence of Events at the Station	Location of Cancer Sample Among 5 Stations	Contents of Station With Location of Target Cancer Sample Stimuli Known by:	Contents of Other 4 Stations
Phase			
Training			
I 1. Sniffing	Randomly With Cancer Sample	Cancer patient breath Experimenter and	Blank tubes

	chosen	sample and food	
2. Command (sit/down)		handler	
3. Indication by dog			
4. After dog sits, then:			
Clicker			
Food reward			
Praise			
II	Randomly	Cancer patient breath	Blank tubes
1. Sniffing		Experimenter only	
	chosen	sample and food	
2. If dog indicates correctly:			
Clicker			
Food reward			
Praise			
III	Randomly	Cancer patient breath	Blank tubes
1. Sniffing		Experimenter only	
	chosen	sample	
2. If dog indicates correctly:			
Clicker			
Food reward			
Praise			
Testing			
Single-blinded	Randomly	Cancer patient breath	Control breath
1. Sniffing		Experimenter only	
trials	chosen	sample	sample
2. If dog indicates correctly:			
Clicker			
Food reward			
Praise			
Double-blinded	Randomly	Cancer patient breath	Control breath
1. Sniffing		Experimenter knew	
trials	chosen	sample	sample
2. Possible indication by dog		only location but no	
		or control	
No clicker		identity of test	

	breath samplea,b
No food reward	sample
No praise	

a.Random order among trials, not known to the handler.
b.Sample status not known either to the experimenter or to the handler.

Personnel. During training phases, dog handlers led the dogs on a leash one at a time into the room and, with praise, encouraged the dogs to sniff the stations with a simple command: “Go to work!” Two investigators observed from behind a curtain, concealed from the dogs and handlers.

Breath sample stations. We positioned 5 sample stations on the floor of the room in a single straight line spaced 1 yard apart. Each station consisted of a polypropylene plastic storage container measuring 15 in. long, 12 in. wide, and 10 in. tall. Each station also contained a well 8 in. deep within which we placed the breath sample tubes. To prevent the dog’s nose from touching the breath sample tubes, we placed the breath sample tubes in clear half-pint polypropylene containers measuring 4.5-in. wide by 1.5-in. tall and used new container covers for each trial. To allow

exchange of air and exhaled breath chemicals, 7 holes measuring a quarter inch in diameter were drilled into the canister lids.

Breath sample locations. A single trial consisted of a dog walking past and sniffing each of the 5 stations (1 cancer patient breath sample and 4 control subject breath samples). After each of the 5 dogs in succession had sniffed the lineup of sample stations, both patient and control breath samples were replaced with new samples. To prevent dogs from predicting the location of the cancer patient breath samples, the experimenter rotated the location of the cancer breath sample within the 5 stations using a random number table. One day’s training consisted of 5 trials performed by all 5 dogs, 4 times within a day (total of 100 trials per day).

Classification of dogs' response. Correct responses were (1) indicating by a sitting or lying down response directly in front of a sample station containing a cancer sample (a true positive in sensitivity calculations) and (2) sniffing but not indicating on a control sample (true negative). Incorrect responses were (1) indicating on a control sample (false positive), (2) sniffing but not indicating on a cancer sample (false negative), and (3) hesitation, an incomplete reaction either toward cancer or control samples (either false-positive or false-negative depending on whether hesitation was on a cancer or control sample).

Training

Training consisted of 3 phases (Table 2). For each dog, each training phase was considered completed when he or she could correctly distinguish, for at least 30 consecutive trials, the cancer patient's breath sample from among those of 4 controls, in the experimental lineup of 5 breath samples.

During phase 1 of training, the location of the cancer breath sample was known by both experimenter and trainer (Table 2). One station contained a cancer breath sample, and the remaining 4 stations contained blank sample tubes that had not been used in any breath sampling. To encourage the dogs to seek out the exhaled chemicals associated with cancer, we placed a piece of dog food in the station with the cancer breath sample and covered the container with a piece of paper so the food would not be visible. Dogs walked unleashed past the stations. We did not assign any time limit on the amount of time the dogs spent sniffing samples or the number of times a dog could sniff any sample. To train the dogs to indicate on a cancer sample, when the dog sniffed the station containing the cancer breath sample, an investigator would trigger the clicker device, signaling the dog handler to issue the "sit" command, praise the dog, and offer the dog a food reward before leading him or her out of the room. A trial was considered complete when the clicker was activated, whereupon the dog handler would lead the dog out of the room.

During phase 2 of training, only the experimenter was aware of the location of the cancer breath sample and apart from encouraging the dog with encouraging phrases such as "go to work," gave no "sit" or other verbal commands to the dog. Clicker signal by the experimenter and subsequent food reward and praise by the trainer were given only after the dog correctly indicated on the cancer breath sample. When the dog indicated incorrectly on a control, the experimenter would not signal with the clicker and the handler would remain silent, not give the dog any praise reward, and mildly rebuke the dog by saying "no." Samples used in phases 1 and 2 (contaminated with food scent) were not used again.

Phase 3 of training was identical to phase 2, except that food was no longer placed with the cancer breath samples (Table 2). In summary, this training phase was to train dogs to detect cancer patient breath samples.

Testing: Single-Blinded Experiment

During the single-blinded canine scent-testing experiment, using samples previously used in phase 3 of training, the level of challenge to the dogs was increased by placing a cancer breath sample in 1 station and control subject breath samples in the remaining 4 stations. Thus, dogs now had to distinguish cancer patient breath samples from those of healthy controls. Furthermore, the handler was blinded to the location and status of patient and control breath samples. Although the experimenter did not know the location and status of patient and control breath samples during the single-blinded experiments, the possibility of the experimenter giving the dogs cues was minimized by positioning the experimenter in an adjacent room, behind an opaque curtain that almost completely covered the doorway between the training and observation rooms.

Testing: Double-Blinded Experiment

We designed our double-blinded experiment so that each dog would have the opportunity to sniff breath samples from each subject and each control. During the entire double-blinded testing phase, all breath samples sniffed by dogs, for both cases and controls, were from completely different subjects not previously encountered by the dogs during

training or single- blinded testing. Furthermore, all of these breath samples used during double-blinded testing, for both cases and controls, contribute to the overall results reported in Table 3. For each trial, we used a random- number table to determine the location of the sample being tested in the lineup.

All other methods were identical to the single- blinded testing phase, except that we now (1) placed the target breath sample of interest, whether from patient or control, within the lineup along with 4 other controls and (2) blinded both the experimenters and dog handlers to the status of that target sample in the lineup. Whereas in the single-blinded experiments only the dog handler was blinded to knowledge of the target sample, in the double-blinded experiments, both handler and experimenter were blinded to ensure that neither experimenters nor handlers could be giving any clues to the dogs. Since the experimenters now no longer knew the status of the target breath sample, they did not activate the clicker device after a sitting indication by the dog, and therefore the handler did not reward the dog with any food. After being given the opportunity to sniff and indicate on samples, the dog was simply led out of the room. Only after leaving the training room was the dog acknowledged with the phrase “good work!” During double-blinded testing, each tube was used a median of 20 times (x = 32.35, SD = 24.46; range, 4-99).

Table 3. Sensitivity and Specificity of Double-Blinded Canine Scent Detection Trials

All Samples Analyzed First Instance Only of Each Dog-Donor Combination

Breath Samplea	Breath Sample a			Sensitivity (95% CI)b	Specificity (95% CI)b
	Control	Cancer	Total		
Control	Cancer	Total	Sensitivity	Specificit	
Lung cancer					
Stage I					
No	137	0	137		
13	0	13			
Yes	2	81	83		
1	20	21			

Total	139	81	220	1.00	0.99	14	20	34	1.00	0.93				
Stage II														
No	168	4	172			6	1	7						
Yes	0	135	135			0	34	34						
Total	168	139	307	0.97	1.00	6	35	41	0.97	1.00				
Stage III														
No	275	2	277			19	1	20						
Yes	2	160	162			0	39	39						
Total	277	162	439	0.99	0.99	19	40	59	0.98	1.00				
Stage IV														
No	128	4	132			15	0	15						
Yes	0	188	188			0	43	43						
Total	128	192	320	0.98	1.00	15	43	58	1.00	1.00				
All														
No	708	10	718			53	2	55						
Yes	4	564	568			1	136	137						
Total	712	574	1286	0.99(0.99,1.00)c		0.99(0.96,1.00)c	54	138	192	0.99	0.98			
Breast cancer														
Stage I														
No	99	3	102			1	0	1						
Yes	6	45	51			0	10	10						
Total	105	48	153	0.94	0.94	1	10	11	1.00	1.00				
Stage II														
No	37	1	38											5
Yes	3	19	22											0
Total	40	20	60	0.95	0.93									5
Stage III														
No	70	1	71											1
Yes	4	27	31			0	10	10						
Total	74	28	102	0.96	0.95									1
Stage IV														
No	54	1	55											12

Yes	2	19	21			0
Total	56	20	76	0.95	0.96	12
All						
No	260	6	266			14
Yes	15	110	125			
0	29	29				
Total	275	116	391	0.88(0.75,1.00)		
0.98(0.90,0.99)	14	30	44	0.97	1.00	

ND = not defined.
a. Dog indicates.
b. Sensitivity and specificity, general estimating equations regression adjusted.
c. Adjusted for confounding by current smoking.

Data Management and Analysis

During both the training and testing phases, experimenters monitored each trial, recording observations both on paper and videotape. We audited the entire data set of dog performance for accuracy, comparing paper to videotape records.

Diagnostic accuracy of the double-blinded testing phase was calculated as sensitivity and specificity of the dogs' indication of breath samples compared to biopsy-confirmed conventional diagnosis. Confidence intervals for sensitivity and specificity were estimated using general estimating equations (GEE) random effects linear regression, with standard errors adjusted for clustering on donor.27 The dogs' response to each of the 5 samples sniffed was included in our analysis; dogs were allowed the opportunity to visit each sample station and thus could have potentially indicated every one of the samples in a trial, although in our experiments, this never occurred. Dog handlers did not try to prevent dogs from visiting any individual station. Therefore, since each individual sample station was considered as a unit of analysis, the use of 4 control subject breath samples along with a cancer patient sample in each experimental trial would not change sensitivity or specificity.

During each double-blinded experimental trial, since neither experimenter nor handler knew the location of the cancer sample, there were no clicker activation or food rewards

offered to the dogs. Nevertheless, to examine whether there was systematic change in the dogs' ability to detect a subject's breath sample after sniffing it more than once during the series of double-blinded experimental trials, we estimated sensitivity and specificity at 2 points: (1) for the all double-blinded trials combined and (2) only the first occurrence of each dog-subject combination.

We used the Fisher 2-sided exact test to test for differences between patients and controls in the following characteristics: sampling (time from breath sampling to dog sniffing as well as forcefulness of breath during sampling) and donor characteristics from questionnaire (age, smoking, diabetes, dental infection, and most recent eating of foods that may affect breath, such as fish, spicy foods, alcohol, lamb, pork, coffee, tea, and garlic).

Results

Effectiveness of Training

Each of our 5 dogs, who entered our study with only basic behavioral puppy training, completed our scent detection training within 2 to 3 weeks' time. During the 3 phases of training, we used the breath samples from 27 lung cancer patients, 25 breast cancer patients, and 66 controls.

Table 4. Comparison of Overall Accuracy Between Individual Dogs

Dog *a*	P
2	0.39
3	0.85
4	0.16
5	0.29

X^2 P = .21.

a. Each dog was compared to dog 1, by general estimating equations regression, for overall accuracy, lung and breast cancer combined.

Diagnostic Accuracy: Sensitivity and Specificity in Double-Blind Testing

During double-blinded testing, we used the breath samples from 28 lung cancer patients, 6 breast cancer patients, and 17 controls. To calculate sensitivity and specificity, we counted the dogs' response to each sample sniffed as the unit of analysis (defined above, "Classification of Dogs' Response").

Among lung cancer patients and controls, as compared to biopsy-confirmed conventional diagnosis, overall sensitivity of canine scent detection was 0.99 (95% confidence interval [CI], 0.99, 1.00) and overall specificity was 0.99 (95% CI, 0.96, 1.00; Table 3). These results were GEE adjusted for confounding by the presence of current smoking among 4 lung cancer patients (Table 3).

Among breast cancer patients and controls, overall sensitivity was 0.88 (95% CI, 0.75, 1.00) and overall specificity 0.98 (95% CI, 0.90, 0.99). For both cancers, sensitivity and specificity were remarkably similar across all 4 stages of disease (Table 3).

Sensitivity and specificity were virtually identical at the 2 points analyzed: (1) for all double-blinded trials combined and (2) for only the first occurrence of each dog-donor combination, suggesting that there was no systematic increase in learning by the dogs as the double-blinded testing process continued.

To compare dogs, we counted each trial of 5 breath samples as the unit of analysis, in which the possible responses were simply categorized as "located cancer" or "failed to locate cancer"; the only correct response in this analysis was when the dogs performed perfectly (correctly locating the cancer sample and ignoring all 4 control samples). There was no statistically significant difference in accuracy between the 5 different dogs (X^2 P = .21; Table 4).

Protocol Exceptions

Several recruitment protocol exceptions occurred during our study. The first protocol exception was 3 patients who had been previously treated for cancer and were in

remission at the time of our study. For 1 patient in remission from breast cancer, in 24 of 25 scent trials, the dogs indicated on her breath samples, thus considering her to be a "patient." She continued diagnostic surveillance, including 1 negative magnetic resonance imaging scan 12 months later; 18 months after being identified by the dogs as a cancer case, she was found to have a local recurrence of her breast cancer in the surgical margins. For the other 2 patients in remission, one with lung cancer and the other breast cancer, the dogs did not indicate, thus considering them to be "controls" (90 of 90 trials and 46 of 47 trials). The second protocol exception was 2 patients with lung cancer whose chemotherapy had already begun. In 20 trials, the dogs indicated on these 2 patients with markedly lower sensitivity (40.4%).

Table 5. Comparison Between Cancer Patients and Controls of Potential Confounders a

	P
Study design factor	
Time from breath sampling to testing	.52
Subject factors	
Age	
All ages	<.01
Restricted to age >43	1.00
Current smoking	
Lung cancer	.01
Breast cancer	No current smokers
Past smoking	
Lung cancer	.57
Breast cancer	1.00
Forcefulness of breath during sampling	.37
Diabetes	.38
Dental infection	.55
Most recently eaten meal	
Garlic	.26
Alcohol	.42
Coffee	.58
Tea	1.00
Pork	1.00
Lamb	1.00
Fish	.17
Spicy foods	.41

a. Fisher exact test.

Analysis for Potential Confounders

Time from sampling to testing. There was no statistically significant difference between patients and controls in the time from breath sampling to testing (P = .52, Fisher 2-sided exact test; Table 5).

Age. There was a statistically significant difference in age distribution between patients and controls (P < .001). Since there were no cancer patients younger than 44 years, we dropped from the analysis the 15 controls that were younger than 44. In this restricted analysis, no association between age and cancer status remained between patients and controls (P = 1.00). Furthermore, sensitivity and specificity remained virtually unchanged.

Breast cancer. There was no statistically significant difference between patients and controls in either current smoking (no current smokers among patients) or past smoking (P = 1.00).

Lung cancer. There was an association by current smoking (P = .01) but not for past smoking (P = .57). We therefore adjusted our analysis of overall sensitivity and specificity in lung cancer patients by current smoking.

Interpretation

Using a simple and inexpensive training method, we trained dogs to distinguish breath samples of lung and breast cancer patients from those of healthy controls in only 2 to 3 weeks' time. Among lung cancer patients and controls, overall sensitivity of canine scent detection compared to biopsy-confirmed conventional diagnosis was 0.99 (95% CI, 0.99, 1.00) and overall specificity 0.99 (95% CI, 0.96, 1.00). Among breast cancer patients and controls, sensitivity was 0.88 (95% CI, 0.75, 1.00) and specificity 0.98 (95% CI, 0.90, 0.99). The lack of any statistically significant evidence of differences in accuracy

between dogs suggests that our training method can be applied to different dogs with similar results. However, our article is subject to limitations.

Our high sensitivity compares favorably with that of chest x-ray in detecting early-stage lung cancer, and our high specificity compares favorably with that of CT scan in ruling out lung cancer. In addition, our high sensitivity compares favorably with that of mammography in detecting breast cancer, and our high specificity compares favorably with that of mammography in ruling out breast cancer. However, our specificity may be overestimated because we used only healthy controls (rather than a broad spectrum of subjects that included, for example, those with bronchitis or emphysema as controls for lung cancer or those with fibrocystic breast disease or mastitis as controls for breast cancer). These questions could be better understood by further study in a prospective cohort design that included both cases and controls representing the full spectrum of disease severity seen in the general population.

Although we measured for potential confounding by other odors, our dogs may nevertheless have detected and responded to odors associated with cancer, such as inflammation, infection, or necrosis rather than to cancer specifically. That question could be answered in follow-up research that includes both healthy controls and those with nonmalignant inflammatory conditions and in whom we specifically examine the chemistry of exhaled breath.

Current smoking varied between lung cancer patients and controls. Although after adjusting for confounding by smoking high sensitivity and specificity remained, the design of future trials should include specific recruitment strategies to avoid differences in smoking status between patients and controls.

Because there was no statistically significant difference between patients and controls in the most recently eaten meal, we do not yet know whether significant differences between patients and controls with respect to odors on the breath associated with dietary factors could interfere with training and scenting accuracy of dogs. A larger study with appropriately sequenced training methods may help resolve this uncertainty. In addition,

future work should use specific recruitment strategies to minimize confounding between patients and controls and a prospective cohort design that includes both cases and controls, representing the full spectrum of disease severity seen in the general population. Future research should combine and compare canine scent detection and analytical chemistry methods to identify the optimal diagnostic applications for each method. Breath analysis may provide a substantial reduction in the uncertainty currently seen in cancer diagnosis.

Declarations

There are no conflicts of interest for any study authors. All dogs were treated in a humane and safe manner, consistent with professional dog clicker training.28 None of the dogs were pets of any of the investigators, trainers, or handlers.

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References

1 Fossella FV, Komaki R, Putnam JB. Lung Cancer. New York, NY: Springer-Verlag; 2003.

2 Dammas S, Patz EF, Jr, Goodman PC. Identification of small lung nodules at autopsy: implications for lung cancer screening and overdiagnosis bias. Lung Cancer. 2001;33(1):11-16.

3. Shim, SS, Lee, KS, et al. Non-small cell lung cancer: prospective comparison of integrated FDG PET/CT and CT alone for preoperative staging. Radiology. 2005;236(3):1011-1019.

4. Olsen AH, Njor SH, Vejborg I, et al. Breast cancer mortality in Copenhagen after introduction of mammography screening: cohort study. BMJ. 2005;330:220.

5. Gill PG, Farshid G, Luke CG, Roder DM. Detection by screening mammography is a powerful independent predictor of survival in women diagnosed with breast cancer. Breast. 2004;13:15-22.

6. Barth RJ, Jr, Gibson GR, Carney PA, Mott LA, Becher RD, Poplack SP. Detection of breast cancer on screening mammography allows patients to be treated with less-toxic therapy. Am J Roentgenol. 2005;184:324-329.

7. Gotzsche PC, Olsen O. Is screening for breast cancer with mammography justifiable? Lancet. 2000;355:129-134.

8. Freedman DA, Petitti DB, Robins JM. On the efficacy of screening for breast cancer. Int J Epidemiol. 2004;33:43-55.

9. Aro AR, Pilvikki Absetz S, van Elderen TM, van der Ploeg E, van der Kamp LJ. False-positive findings in mammography screening induces short-term distress: breast cancer-specific concern prevails longer. Eur J Cancer. 2000;36:1089-1097.

10. Buist DS, Porter PL, Lehman C, Taplin SH, White E. Factors contributing to mammography failure in women aged 40-49 years. J Natl Cancer Inst. 2004;96:1432-1440.

11. Kennedy TC, Hirsch FR. Using molecular markers in sputum for the early detection of lung cancer: a review. Lung Cancer. 2004;45(suppl 2):S21-27.

12. Hilbe W, Dirnhofer S, Greil R, Woll E. Biomarkers in non-small cell lung cancer prevention. Eur J Cancer Prev. 2004;13:425-436.

13. Coradini D, Daidone MG. Biomolecular prognostic factors in breast cancer. Curr Opin Obstet Gynecol. 2004;16:49-55.

14. Walker DB, Cavnar P, Taylor J, et al. Canine olfactory thresholds to N-amyl acetate measured naturalistically. Paper presented at: Meeting of the Association for Chemoreception Sciences; April 2003; Sarasota, Fla.

15. Walker DB, Walker JC, Cavnar P, et al. Naturalistic Quantification of Canine Olfactory Sensitivity. Forthcoming.

16. 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;34:1613-1618.

17. Preti G, Labows JN, Kostelc JG, Aldinger S, Daniele R. Analysis of lung air from patients with bronchogenic carcinoma and controls using gas chromatography-mass spectrometry. J Chromatogr. 1988;432:1-11.

18. Phillips M, Cataneo RN, Ditkoff BA, et al. Volatile markers of breast cancer in the breath. Breast J. 2003;9:184-191.

19. Gordon SM, Szidon JP, Krotoszynski BK, Gibbons RD, O'Neill HJ. Volatile organic compounds in exhaled air from patients with lung cancer. Clin Chem. 1985;31:1278-1282.

20. Phillips M, Greenberg J. Method for the collection and analysis of volatile compounds in the breath. J Chromatogr. 1991;564:242-249.

21. Phillips M. Breath tests in medicine. Sci Am. 1992;267(1):7479.

22. Phillips M, Gleeson K, Hughes JM, et al. Volatile organic compounds in breath as markers of lung cancer: a cross-sectional study. Lancet. 1999;353:1930-1933.

23. Williams H, Pembroke A. Sniffer dogs in the melanoma clinic? Lancet. 1989;1:734.

24. Church J, Williams H. Another sniffer dog for the clinic? Lancet. 2001;358:930.

25. Pickel DP, Manucy GP, Walker DB, Hall SB, Walker JC. Evidence for canine olfactory detection of melanoma. Appl Anim Behav Sci. 2004;89:107-116.

26. Willis CM, Church SM, Guest CM, et al. Olfactory detection of human bladder cancer by dogs: proof of principle study. BMJ.

27. Liang KY, Zeger SL. Longitudinal data analysis using generalized linear models. Biometrika. 1986;73:13-22. 2004;329:712.

28. Pryor K. Don't Shoot the Dog! The New Art of Teaching and Training. Rev ed. New York, NY: Bantam; 1999.