

Accuracy of Canine Scent Detection of Non–Small Cell Lung Cancer in Blood Serum

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Context: Early detection provides the best opportunity for lung cancer survival; however, lung cancer is difficult to detect early because symptoms do not often appear until later stages. Current screening methods such as x-ray and computed tomographic imaging lack the sensitivity and specificity needed for effective early diagnosis. Dogs have highly developed olfactory systems and may be able to detect cancer in its primary stages. Their scent detection could be used to identify biomarkers associated with various types of lung cancer.

Objective: To determine the accuracy of trained beagles’ ability to use their olfactory system to differentiate the odor of the blood serum of patients with lung cancer from the blood serum of healthy controls.

Methods: Over the course of 8 weeks, operant conditioning via clicker training was used to train dogs to use their olfactory system to distinguish blood serum from patients with malignant lung cancer from blood serum from healthy controls in a double-blind study. After training, non–small cell lung cancer and healthy control blood serum samples were presented to the dogs, and the sensitivity and specificity of each dog were analyzed.

Results: Four dogs were trained for the study, but 1 was unmotivated by training and removed from the study. Three dogs were able to correctly identify the cancer samples with a sensitivity of 96.7%, specificity of 97.5%, positive predictive value of 90.6%, and negative predictive value of 99.2%.

Conclusion: Trained dogs were able to identify non–small cell lung cancer samples from healthy controls. The findings of this study provide a starting point for a larger-scale research project designed to explore the use of canine scent detection as a tool for cancer biomarkers.

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According to the American Cancer Society, lung cancer is the leading cause of cancer death worldwide for both women and men.¹ Thirteen percent of new cancers are a form of lung cancer, and more than 200,000 people in the United States receive a diagnosis of lung cancer annually.¹ The 5-year survival rate for stage IA1 non–small cell lung cancer (NSCLC) is 92%, and the 5-year survival rate for stage IIIC NSCLC is about 13%.² After metastasis, the 5-year survival rates range from 10% to less than 1%, depending on the stage.²

Currently, early detection provides the best opportunity for survival; however, symptoms do not often appear until later stages, which makes early detection difficult. Available screening technology, such as chest x-ray or computed tomographic (CT) imaging, can introduce additional health risks and be costly and potentially inaccessible, especially for people who are underprivileged or who live in rural communities. These barriers may deter some patients from seeking screening. Additionally, chest x-ray imaging has a high false-negative rate, whereas CT with computer-aided diagnosis has a high false-positive rate.³ Previous studies have indicated that 90% of missed lung cancers occur when using chest radiographs, and it is difficult to identify small, central, juxtavascular lung cancers on CT images.³

Novel, less invasive, and more cost-effective methods that have greater sensitivity and specificity are needed to reduce mortality and improve quality of life. The major barrier to create more efficient testing methods for lung cancer is the need to discover a testable molecule or molecules definitive for the disease, especially in its early stages. Distinct olfactory patterns for chronic obstructive pulmonary disease were detected by Dragonieri et al⁴ using electronic nose, or *e-nose*, technology. Although the exact biomarkers the dogs detect are currently unknown, other studies^{4,6} have indicated the existence of chemical substances, such as epidermal growth factor receptor, that exhibit distinct olfactory patterns specific to lung cancer. Many variations of *e-nose* technology have been available since the 1980s, but application and effectiveness varies widely. Miniaturization and simplification of *e-nose* technology is improving, but it still does not match the ability of canine scent detection of cancer. Roine et al⁷ used an *e-nose* to differentiate between prostate cancer and prostatic hyperplasia and found that it had 78% sensitivity and 67% specificity. Meanwhile, Horvath et al⁸ found that giant schnauzers were able to detect and indicate ovarian cancer by smelling blood serum with 100% sensitivity and 97.5% specificity.

The olfactory acuity of a dog is at least 10,000 times more sensitive than that of a human, which is likely due to their more expansive olfactory epithelium and olfactory receptors and their ability to retain air in their nasopharynx during exhalation.⁹ Scent hounds are a set of working dogs bred for their scent detection capabilities and have a polymorphism in their olfactory receptor genes that may account for their superior olfactory capabilities, even among the *Canis familiaris* species.^{9,10} Beagles are a medium-sized member of the scent hound family and have 225 million olfactory receptors.¹¹ In comparison, humans have 5 million olfactory receptors.⁹

The purpose of this initial study is to determine whether trained beagles are able to differentiate the odor of the serum of patients with lung cancer from that of healthy controls. This study could provide evidence to support the use of canine scent detection as a tool for biomedical researchers to identify biomarkers associated with various types of cancer.

Methods

This double-blind study was approved by the Ethical and Independent Review Services Internal Review Board (IRB) and an IRB authorization agreement between Lake Erie College of Osteopathic Medicine IRB and the Ethical and Independent Review Services IRB. The study, which consisted of a training phase and a testing phase, took place at BioScent DX in Myakka City, Florida, from April 1, 2017, to May 31, 2017.

Dogs

Four 2-year-old beagles were trained for the study. None of the beagles had been previously trained to detect lung cancer samples. The dogs were selected because of their impressive olfactory system and because they are also relatively small, calm, highly trainable, and highly sociable.¹² One dog, Canine No. 4, was eliminated from the study because she was poorly motivated by food and did not respond well to any training method used.

Samples

The serum samples of patients with newly diagnosed lung cancer were purchased from Boston BioSource. The serum was selected because of its commercial availability and confidence level that the serum was from patients who recently received a new diagnosis of NSCLC but had not started any treatment. The positive samples were collected from patients before surgery and chemotherapy. Control samples were obtained from healthy male and female donors through Boston BioSource. Data regarding sex, age, and race/ethnicity were collected on all donors to minimize confounding factors, but the smoking status of donors was unknown.

Non–small cell lung cancer was chosen for this study because it is more common and easier to treat if detected early, as opposed to small cell lung cancer, which is more aggressive. The samples were identified with an identification number, logged into a computer, and stored in a freezer at -30°C for 1 week before first use. Prior to training, each sample was stored in 2 mL of Cryovial (Thomas Scientific), freestanding polypropylene vials with external threaded caps. Researchers used 250 μL of each blood serum sample for training. During the training phase, the samples were thawed for 15 minutes, and the vials were placed in new 12-mL syringe cases. The syringes with the samples were placed in metal canisters with multiple perforations, and the canisters with the cancer and control samples were evenly placed at the dogs' eye level. The dogs went from sample to sample and were given the opportunity to smell each sample individually (Figure 1).

Dog Training

Blood serum samples from patients with newly diagnosed NSCLC and matching controls were used to train and test each dog. Operant conditioning, specifically the “clicker-training method,” was used to train the dogs to positively identify samples from patients with lung cancer in the presence of control samples. During training and testing, the dogs were followed by a handler as they evaluated 5 evenly spaced canisters. Four canisters contained a control sample and 1 canister contained a



Figure 1.

The blood serum samples were placed in metal canisters with multiple perforations and positioned at the dog's eye level.

positive lung cancer serum sample. Dogs were permitted to take as much time as they needed to evaluate each sample. The dog indicated a correct response by sitting in front of the canister. A correct response to a control sample was given by ignoring the sample and continuing on to the next canister. If the dogs sat in front of a control sample, they were told “no” and encouraged to move on to the next sample. If the dogs correctly indicated a positive serum sample, they were rewarded with the sound of a clicker and a training treat.

Training was done in 3 phases over 8 weeks. Phase 1 took place during the first 2 weeks, and the dogs were trained to correctly identify the presence of a treat in a canister. Phase 2 took place during the second 2 weeks of training, and a positive serum sample containing lung cancer was paired with a treat. As the dogs successfully identified the station with the positive sample and the treat, the amount of treat was weaned down until the dog was able to successfully identify the positive sample in the absence of the treat. Phase 3 took place during the final 4 weeks of training, and no treat was paired with the positive lung cancer serum samples. After successful completion of 3 training phases, the testing phase began.

Testing Design

A double-blind procedure was used to minimize the possibility of experimenter bias. The samples were prepared the same way as the training samples. During the

testing phase, 10 new samples were used that were positive for NSCLC and had not been encountered during training. Additionally, the control samples that were used in the testing phase came from different donors from the control samples in the training phase. Each dog performed 10 test runs, and each test run was set up like the training runs. The dogs were allowed to sniff each canister for 5 to 10 seconds. A positive response was indicated by the dog sitting in front of the canister. A correct response to control samples was to ignore the sample and to move on to the next sample. All of the testing was performed on the same day. A 10-minute rest period occurred each time the samples were changed out, which allowed the odor from the previous test to dissipate and the scent from the new samples to accumulate in the area.

Statistical Analysis

The *P* value was calculated using the Easy Fisher Exact Test calculator (Social Science Statistics) and a 2 × 2 table setting the statistically significant value at *P*=.05. All other statistics, meaning sensitivity/specificity and positive/negative predictive values, were calculated using MedCalc (MedCalc Software).

Results

Samples from 10 donors (6 women and 4 men) were used in the testing phase. Their ages ranged from 26 to 80 years (mean, 58.2 years). The samples from female

donors (mean age, 64 years) came from 3 black women, 2 white women, and 1 Hispanic woman. The samples from male donors (mean age, 49.5 years) came from 2 black men, 1 white man, and 1 man of mixed race.

Canine No. 1 indicated a positive sample on 10 of the 10 cancer samples and 1 of the 40 control samples during his test runs. Canine No. 2 indicated a positive sample on 10 of the 10 cancer samples and 0 of the 40 control samples during her test runs. Canine No. 3 indicated a positive sample on 9 of the 10 cancer samples and 2 of the 40 control samples during her test runs (Table).

Figure 2 shows the sensitivity and specificity of each dog’s scent detection. Canine No. 1 had the second highest accuracy and scored 100% in sensitivity and 97.5% in specificity. Canine No. 2 had the highest accuracy and scored 100% in both sensitivity and specificity. Canine No. 3 scored 90% in sensitivity and 95% in specificity.

The total sensitivity was 96.7%, and the total specificity was 97.5% (*P*<.001). The positive predictive value (the proportion of patients who tested positive for lung cancer who had the disease) was calculated at 90.6%. The negative predictive value (the proportion of patients who tested negative for lung cancer who did not have the disease) was calculated at 99.2%.

Discussion

This study supports the use of scent hounds to detect cancer in humans based on a specific odor and confirms

Table.
Number of True and False Positive and Negative Indications For Each Dog During Testing ^a

Dog	True Positive	False Positive	True Negative	False Negative
Canine No. 1	10	1	39	0
Canine No. 2	10	0	40	0
Canine No. 3	9	2	38	1

^a True-positive results were indicated by a dog sitting in front of a sample containing non-small cell lung cancer blood serum. False-positive results were indicated by a dog sitting in front of a control sample. True-negative results were indicated by a dog ignoring the control sample. False-negative results were indicated by a dog ignoring a sample containing non-small cell lung cancer blood serum.

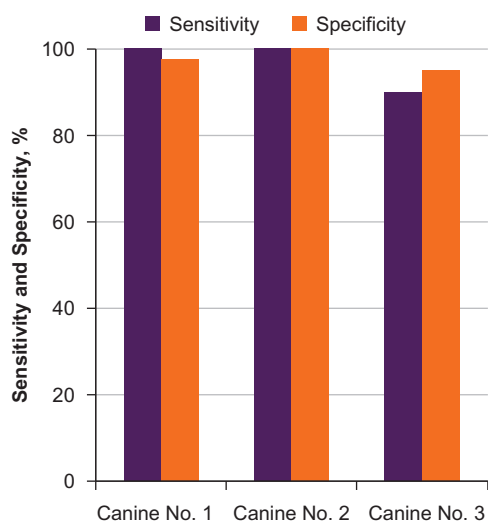


Figure 2.
The sensitivity and specificity for detecting lung cancer samples of each dog in the testing phase.

the results of previous studies that have indicated that dogs can be rapidly trained to detect the pathophysiologic metabolic changes of cancers through olfaction.¹³⁻¹⁹ Current early detection screening modalities have the potential to decrease morbidity and mortality from lung cancer; however, their sensitivity and specificity are low. New, less-invasive, cost-effective methods with greater sensitivity and specificity are needed. Canine scent detection can be used as a tool to develop these new methods because of dogs' acute scent detection abilities that have detection thresholds as low as parts per trillion.¹⁴ The present study, though small, indicates that trained dogs have the potential for high sensitivity and specificity in their detection abilities. If the biomarkers detected by the dogs can be isolated and identified, a clinical screening tool created based on these biomarkers may also have high sensitivity and specificity.

Limitations

This is a pilot study with a relatively low sample size; after 1 dog was eliminated for poor response to training, there were only 30 positive and 120 negative samples used. Although the *P* value of the data was significant,

additional studies are needed to verify the efficacy of the data. Another limitation was the narrow scope of the study that only determined whether the dogs could detect the cancer. Future studies are needed to determine whether dogs can detect cancer at an earlier stage than the current methods of chest x-ray and CT imaging. Studies should also determine whether dogs can detect recurrence of lung cancer while patients are undergoing chemotherapy and whether they can detect cancer in the presence of concurrent diseases such as diabetes. Larger and more focused studies are needed to expand on the preliminary findings of this study.

Future Research

At the time of publication, our team was in the early stages of conducting a larger study that used canine scent detection to isolate and identify the biomarkers in positive NSCLC and breast cancer samples. We plan to fractionate the cancer samples based on chemical and physical properties and then present the fractions to the trained dogs for evaluation. The fractions that the dogs identify as containing cancer biomarkers will be fractionated further and presented to the dogs again. Once sufficiently fractionated, the resulting fractions will be evaluated using liquid chromatography–tandem mass spectrometry to determine the identity of the molecules present. The end goal of this larger study is to identify the minimum number of biomarkers necessary in a sample for a positive identification of cancer. Once identified, biomedical researchers can work toward developing a simplified and cost-effective, non-canine scent detection system.

Canine No. 1 has been bred with a beagle who has been equally impressive in scent detection of breast cancer, and the resulting litter of 6 puppies are now being trained in an effort to produce a genetic line predisposed to scent detection of cancer. At a later time, these same dogs, will be trained to detect lung cancer in exhaled breath condensate and saliva from donors.

Conclusion

This study shows that dogs can differentiate between blood serum samples taken from patients with confirmed NSCLC and healthy controls. Our findings provide a starting point for a larger study that will work to isolate and identify biomarkers present in positive cancer samples. Further investigation into the biochemical molecules detected by dogs could provide a foundation for the development of a highly sensitive, specific, and cost-effective method for early cancer detection. Early detection of cancer is one of the best ways to improve patient outcomes, and current methods of early detection rely on expensive imaging equipment, which can be an insurmountable obstacle for underserved and rural communities.

Author Contributions

All authors provided substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data; all authors drafted the article or revised it critically for important intellectual content; all authors gave final approval of the version of the article to be published; and all authors agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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