The Use of Canines in the Detection of Human Cancers

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ABSTRACT

Objectives: To determine whether canines could be trained to identify patients with cancer by sniffing the urine obtained from a patient with breast or prostate cancer from among samples obtained from healthy volunteers.

Design: Dogs of different breeds were trained by their owners to detect the urine sample from a patient with cancer from among 6 other age- and sex-matched healthy volunteers. After the training was completed, using new samples, 2 test runs were used for each patient with breast cancer and three runs for the patients with prostate cancer against the same matched samples. The configuration of the samples was different for each run. A total of 18 and 33 runs were carried out, respectively.

Results: For each cohort, specificity and sensitivity were measured. In the breast cancer tests, of 6 dogs, only 2 performed better than chance in specificity and none were more sensitive than chance. For the prostate sample testing, 4 dogs were used. Two performed significantly better than chance in specificity and none in sensitivity.

Conclusions: Although this study did not produce the outcomes desired, the literature supports a potential to use canines for human cancer detection. Better management of urine samples and a more stringent training protocol during our study may have provided new evidence as to the feasibility of using canines for cancer detection. A comparison of the 3 dog cancer scenting studies is also presented.

INTRODUCTION

A dog’s sense of smell is estimated to be 100,000 times more sensitive than humans and is attributed to their having 20 to 40 times more nasal receptor cells than humans do.1,2 This ability is used in finding bombs, drugs, and people.3 It has been postulated that this capacity could be used to detect human cancers. Recent work has suggested that the volatile organic compounds detected by dogs from patients with cancer are products of the major histocompatibility complex genes.4 A dog’s capability to detect human cancers has been a topic of speculation and source of anecdotes for years. The first published account of a dog’s finding cancer was in 1989 in William and Pembroke’s letter in Lancet.5 They described a woman seeking medical attention because of her pet’s continued interest in a skin lesion. A biopsy demonstrated this to be a malignant melanoma. Subsequent lay articles and a later paper in Lancet continued propagating the “fact” that dogs had detected skin, breast, and other malignancies by smell.6,7 Observations made these accounts plausible. Malignancies have been shown to produce volatile chemicals that can be detected in breath or urine. Formaldehyde has been de-
detected in the headspace of urine from patients with bladder and prostate cancer at significantly higher concentrations than in the urine of normal controls.

Willis et al. provided the first scientific experiment in September 2004 to support the belief that dogs could be trained to identify the unknown biomarkers of a cancer. Six (6) dogs correctly selected urine from patients with bladder cancer on 22 of 54 occasions in blind testing. This aggregate 41% and individual best of 56% success rate was greater than by chance alone.

A study involving 5 dogs distinguishing both breath samples of 55 patients with lung cancer and 31 patients with breast cancer from those of healthy controls was next reported by McCulloch et al. Their results were a 99% sensitivity and specificity among patients with lung cancer and 88% and 98% in patients with breast cancer.

Before the aforementioned studies were published, we initiated a small trial testing the hypothesis that 2 dogs could be trained to detect the odor signature in the urine of patients with prostate cancer. Shortly after the Willis paper was published, our protocol was modified to train 6 dogs for breast cancer and 4 for prostate cancer.

In 2005, invasive breast cancer was diagnosed in over 200,000 women in the United States, with over 35,000 women dying. Studies suggest that 28–65% of the lower mortality seen since the early 1990s is due to the early-stage cancers being diagnosed by screening mammography and improved treatments.

Prostate cancer is the most common form of noncutaneous cancer among men in the United States. An estimated 30,350 men died of the disease in the United States in 2005. Early diagnosis helps the 10-year survival, with localized prostate cancer being 75% compared with 55 and 15%, respectively, among those with regional extension and distant metastases. The digital rectal exam and the prostate-specific antigen (PSA) are the standard screening methods to diagnose this cancer.

African-Americans are less likely to undergo cancer screening for both prostate and breast, which leads to more advanced cancers when discovered.

Cancer screening methods need to be simple to perform, inexpensive, reliable, and reproducible. False-positive results lead to other invasive procedures while cancers are missed with false negatives. Segments of the population are not screened lead to other invasive procedures while cancers are missed with advanced cancers when discovered. We wanted to investigate whether canines, by sniffing urine, could identify 2 common cancers and thus lay the foundation for this to be used as a cancer screening modality.

**MATERIALS AND METHODS**

**Patients and samples**

The Scripps Clinic Institutional Review Board approved the study that took place from late 2004 to early 2006. Men and women at least 40 years of age with untreated, biopsy-proven, breast or prostate cancer were recruited primarily from the Scripps Clinic. Patients with prostate cancer also came from the practices of Kaiser Permanente, University of California at San Diego and Dr. Warren Kessler. There were a total of 62 breast cancers and 188 controls and 57 prostate cancers and 186 controls enrolled. There were no men with breast cancer. Age-matched controls, most with normal PSA tests or mammograms within 6 months, were also recruited. Patients with a history of cancer other than breast or prostate cancer were excluded from the study except for individuals with superficial basal cell skin cancer. Questionnaires about medications, medical history, and food and drink ingested within the prior 24 hours were completed. In the breast cancer cohort, the enrollees were asked about the use of deodorants and perfumes. There was a great deal of overlap in the medical conditions and medications used in both the controls and patients with cancer. The most common chronic diseases were hypertension and hyperlipidemia.

Consenting patients provided a random urine sample at enrollment. The urine sample was divided into 1-mL aliquots in Nunc cryo vials. Early in the study the samples were frozen, stored, and then thawed prior to placing the aliquots into the Nunc vials (Nalge Nunc International, Rochester, NY) and the vials into mason jars. The latter were labeled with a subject number and as breast, prostate, or no cancer. The jars were stored at least to −20°C until they were distributed to the trainers to keep in standard freezers they had at their residences until needed. Samples were thawed, used for training within 30 minutes, and then discarded in a biohazard-approved manner. For the purpose of training the dogs, urine from 53 patients with breast cancer and 134 controls and 46 patients with prostate cancer and 120 controls was used.

**Training**

The study design utilized the most qualified, by credentials, professional trainers in the area. Most were Certified Pet Dog Trainers (CDPT), worked with tracking dogs, and/or had earned titles in various canine competitions. Only R.T.G. was not a professional trainer. The training was at their homes because of the lack of a common central training site. Due to logistics and individual time constraints, trainers worked alone with their dogs. The various breeds used was determined by what the trainers owned (Table 1). LOVE HEELS Canine Partners, a San Diego assistance dog training and placement organization, monitored the animals’ welfare.

The objective was to teach the dogs to discriminate between urine from individuals with breast or prostate cancer and urine from those who were cancer free. Each dog was trained to discriminate only one cancer type. Training was by means of operant conditioning, using the clicker training method with food treats as rewards for alerting to a cancer urine. The trainers agreed upon a general outline to best
achieve our intended outcome. The specifics of the training, however, were left to the individual trainers because we felt that they knew their animals best and that this knowledge would produce the best results. Two (2) trainers trained 2 dogs. The trainers, all volunteers, had full-time jobs and trained as often as their daily routines permitted. This averaged out to between 2 and 7 times per week.

Urine from patients with cancer was progressively presented against empty test tubes, water, diluted control urines, and finally full-strength control urines. Samples new to the dogs or used in prior training sessions were utilized, depending on sample availability. All dogs were provided samples from each of the cancer and control participants. In the latter phases of training, the animals were challenged with 1 positive urine against 6 controls as would be run in the test. Training was considered complete when the dogs could consistently identify a new cancer urine sample among new control samples most of the time. Blinding was introduced late in the training.

Testing

Urine samples from new patients were used for the final testing. As in the Willis study, 1 cancer and 6 control samples were evaluated in each test run. In an effort to have more statistical power and assess specificity, this study introduced replicate runs. A statistician (J.K.) created a schema, with random permutations of the order of the runs and the entry of the various samples in each row. The cancer samples were age matched to within 10 years with the 6 controls. A team, not involved in any of the testing, prepared the test urine samples, none of which had been used in the training. Each Nunc cryo vial of urine was placed in a screw-top 20-mL Fisher (Thermo Fisher Scientific, Waltham, MA) brand scintillation vial. The latter were labeled A–G according to the test template. The test runs were blinded to the trainers and the results were not decoded until all the dogs had completed their testing.

The “breast dogs” were tested on 9 cancers and 54 controls. Two (2) runs were used for the urine of each cancer patient against the same 6-control patient’s urine but in different A–G locations. One (1) of the breast control patients, Neg. #30, was diagnosed with breast cancer after the dogs had been tested. This resulted in test runs 5 and 14 containing samples from 2 patients with cancer.

The “prostate dogs” were tested on 11 patients with prostate cancer among 66 control patients during 33 runs. Each run contained the urine of 6 controls and 1 patient with cancer randomized and placed in cup holders labeled A–G. The urine of each patient with cancer was used in 3 different test runs with the same 6 control patients but in different A–G locations. Prostate control patient Neg. #3 was di-

<table>
<thead>
<tr>
<th>Dog breed/name</th>
<th>M/F (N or $)</th>
<th>Age (yrs)</th>
<th>Working</th>
<th>Dog results</th>
<th>Training</th>
<th>Min/</th>
<th>Times/day</th>
<th>Days/week</th>
<th>Longest</th>
<th>hiatus</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aussie cocker mix</td>
<td>M (N)</td>
<td>2</td>
<td>—</td>
<td>Yes</td>
<td>B</td>
<td>5 of</td>
<td>28%</td>
<td>15</td>
<td>1</td>
<td>3–4</td>
</tr>
<tr>
<td>Collie mix/Betty</td>
<td>F (S)</td>
<td>8</td>
<td>—</td>
<td>Yes</td>
<td>B</td>
<td>5 of</td>
<td>28%</td>
<td>15–30</td>
<td>1</td>
<td>4–5</td>
</tr>
<tr>
<td>German shepherd</td>
<td>F (S)</td>
<td>4</td>
<td>Tracking, agility, obedience</td>
<td>—</td>
<td>B</td>
<td>4 of</td>
<td>22%</td>
<td>30</td>
<td>1</td>
<td>2–3</td>
</tr>
<tr>
<td>Rhodesian ridgeback/Kalina</td>
<td>F (S)</td>
<td>5</td>
<td>Obedience, agility, rally</td>
<td>—</td>
<td>B</td>
<td>5 of</td>
<td>28%</td>
<td>15–30</td>
<td>T1</td>
<td>2–3</td>
</tr>
<tr>
<td>Boxer/Darla</td>
<td>F (S)</td>
<td>6.5</td>
<td>Obedience, agility, rally</td>
<td>Yes</td>
<td>B</td>
<td>3 of</td>
<td>17%</td>
<td>15–30</td>
<td>1</td>
<td>2–3</td>
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<tr>
<td>Italian greyhound</td>
<td>M (N)</td>
<td>5</td>
<td>Agility</td>
<td>Yes</td>
<td>B</td>
<td>2 of</td>
<td>11%</td>
<td>30</td>
<td>1</td>
<td>3–5</td>
</tr>
<tr>
<td>Chihuahua mix/ Ginger</td>
<td>F (S)</td>
<td>4</td>
<td>Service; therapy</td>
<td>Yes</td>
<td>P</td>
<td>2 of</td>
<td>6%</td>
<td>20–30</td>
<td>2x for 3 mos then 1x day</td>
<td>6–7</td>
</tr>
<tr>
<td>Miniature goldendoodle/ Josie</td>
<td>F (S)</td>
<td>2.5</td>
<td>Service; therapy</td>
<td>—</td>
<td>P</td>
<td>5 of</td>
<td>15%</td>
<td>20–30</td>
<td>2x for 3 mos then 1x day</td>
<td>6–7</td>
</tr>
<tr>
<td>Pembroke Welsh corgi/Kyra</td>
<td>F (S)</td>
<td>6</td>
<td>Therapy; obedience</td>
<td>—</td>
<td>P</td>
<td>7 of</td>
<td>22%</td>
<td>15</td>
<td>1</td>
<td>3–4</td>
</tr>
<tr>
<td>Border collie/ Kelly</td>
<td>F</td>
<td>2</td>
<td>Therapy; obedience</td>
<td>—</td>
<td>P</td>
<td>9 of</td>
<td>28%</td>
<td>15</td>
<td>1</td>
<td>3–4</td>
</tr>
</tbody>
</table>

M, male; F, female; N, neutered; S, spayed; Age, age at start of study.
agnosed with prostate cancer shortly after the testing resulting in runs 1, 10, and 19 having samples from 2 patients with prostate cancer.

**Statistical analysis**

The experimental design allowed an assessment of 2 separate issues with each dog: specificity and sensitivity. Simple binomial or multinomial probability calculations were utilized for comparison of each dog’s observed performance with what might be expected under the null hypotheses that dogs demonstrate neither specificity nor sensitivity.\(^{23}\)

**Breast and prostate cancer specificity**

Regarding specificity of the breast cancer dogs, it was noted that there are 9 sets of “replicate” runs; specificity constitutes selection of the same specimen regardless of cancer status in the runs. Under the null hypothesis of “randomness,” the number of “successes” out of 9 would follow a binomial distribution with \(p = 1/7\).

We have 11 “triplicate” runs with the prostate specimens. Under the null hypothesis of “randomness” in the replicate runs, the probabilities of a perfect, 1, and no matches are 0.0204, 0.3673, and 0.6122.

**Breast and prostate cancer sensitivity**

The 18 runs were treated as “independent” in a statistical sense and sensitivity could be summarized as the times the dog selected the cancer specimen. Under the null hypothesis of randomness, the selection of the cancer specimen is basically a chance event. The probability of selection of the cancer specimen is 1/7 in 16 of the runs, 2/7 in 2 of the runs (because of the post hoc determination that 1 “control” specimen was in actuality a cancer specimen).

The 33 prostate cancer runs were also treated as independent. The sensitivity could then be summarized as the number of times the dog selected the cancer specimen in the 33 runs. Under the null hypothesis of randomness, the probability of cancer selection is 1/7 in 30 of the runs, and 2/7 in 3 of the runs. Again, 1 “control” sample was in actuality a “cancer” specimen.

**RESULTS**

**Breast cancer specificity**

Of the dogs evaluated for breast cancer, 2 animals had no matches; 1 animal had 1 match, one animal had 2 matches, and 2 animals had 4 matches (Fig. 1). Only Betty and Diamond had specificity somewhat better than chance: the probability of observing 4 or greater matches out of 9 trials under randomness is 0.0288.

**Prostate cancer specificity**

Four (4) dogs were evaluated with the prostate cancer-testing schema. Two (2) of the dogs performed significantly better than chance: Ginger (4 occurrences of no match, 5 of 1 match, and 2 of 3 perfect matches; chi-square goodness-of-fit statistic = 32.6, \(p = 0.0006\)), and Josie (2 of no match, 2 of 1 match, and 7 of perfect matches; chi-square goodness-of-fit statistic = 208.9, \(p < 10^{-8}\)) (Fig. 2).
Breast cancer sensitivity

Figure 3 shows the number of successes from the 18 runs for the 6 dogs. None of the dogs performed significantly better than chance.

Prostate cancer sensitivity

Figure 4 shows the number of successes of cancer selections from the 33 runs for the 4 dogs. One would expect 5.14 successes out of 33 (standard deviation 2.07) by chance alone; again, no dog performed significantly better than chance.

DISCUSSION

Unlike the 2 previously published studies\textsuperscript{9,10} using canines to detect human cancer, the current study did not demonstrate this ability at levels better than chance. The methodologies of the 3 studies varied considerably (Table 2). Although disappointed, critical observations made during this latest work, especially the protocol deficiencies, may facilitate better designs of future studies.

All of the dogs used freshly thawed aliquots of liquid urine. There were a number of samples that were initially frozen, then thawed, prepared into aliquots, and then re-frozen at no warmer than $-20^\circ$C. Storage time range was from a week to about 5 months. It was not possible to quantify the level of degradation of the yet unknown cancer biomarkers with repeated freeze–thaw cycles, but it is possible that there must be some loss of the volatile components. Would these results have been better if the samples were immediately divided into aliquots and then frozen so that there was only 1 freeze–thaw cycle?

Willis’s group also used urine; however, they trained and tested 2 of the 6 dogs with dried samples stored on filter paper. The 4 dogs trained on wet specimens performed better than the other 2 (50% to 22% correct). The researchers decided to abandon the use of dried urine specimens in their next study, but it is hoped that this was not permanent because dried samples are much easier to store and handle. Perhaps dogs could be trained with liquids and have their proficiency maintained with the dried samples. McCulloch’s group used exhaled breath from patients with lung and breast cancer.

It has been an ongoing theory that certain breeds are better at scent detection than others.\textsuperscript{24} Studies, however, have shown that there is a greater difference in scenting ability between dogs within a breed than between breeds.\textsuperscript{25} The pets the trainers in the current study owned determined the breeds used and resulted in no duplication of a breed. A number were mixed breed, shelter rescued, and/or had specialized skills. The use of shelter dogs for cancer detection should be explored further so that the expense of obtaining dogs for extensive clinical trials would be minimized.\textsuperscript{26}

McCulloch’ group, unlike the other 2 groups and unusual for scent training, trained the dogs to alert to 2 cancers rather than for single cancer discrimination. They reported statistical success with this. This could mean that there is a general biochemical marker common to all cancers, with individual specific cancers having additional markers.

Early recruiting of patients for the current study was sporadic and resulted in having, at times, only a limited number of urine samples to train with. It also took longer than anticipated to obtain enough samples to prepare for the fi-
nal testing. This resulted in the training to be spread over an extended period of time, 12–14 months. Possibly, the animals were periodically memorizing individual patients rather than recognizing an “odor signature” for cancer despite utilizing a large number of training samples. In the future, all urine samples should be available in adequate numbers to complete the study prior to initiating the training. An ongoing system of recruitment of patients with cancer and control patients needs to be established so the dogs have adequate numbers of new samples to maintain their proficiency even after the conclusion of the study. This would also facilitate longitudinal studies.

Willis’ group trained over 7 months, McCullock’s within 2–3 weeks. It would be extremely significant if the method of training dogs in 2–3 weeks could be duplicated.

This study could not have been executed without the time and effort donated by dedicated volunteer professional trainers. However, strict training protocols should be used. The dogs should be trained by a set team of trainers, on a strict schedule over a brief period of time. This would eliminate the irregularities in the training in the current study. The use of blinding during the training should be initiated early to preclude unintended clues by the trainers that may contaminate the process. It was also very difficult for individual trainers to prepare, set up the samples, and then train the dogs. It was time consuming and laborious, producing conflicts with the trainer’s work and family obligations. Using a common central training venue would minimize the mishandling of samples and enhance efficiency.

This study was the only one to incorporate replicates for assessing specificity. There were 3 and 2 replicates (33 and 18 runs) for the prostate and breast cancer arms, respectively. Any study, ultimately attempting to prove canine superiority over conventional cancer screening, must include replicates and in the future go head to head with standard screening methods.

### Table 2. Comparison of Dog Scenting Studies

<table>
<thead>
<tr>
<th></th>
<th>Willis</th>
<th>McCullock</th>
<th>Gordon</th>
</tr>
</thead>
<tbody>
<tr>
<td>CA tested</td>
<td>Bladder</td>
<td>Lung</td>
<td>Prostate</td>
</tr>
<tr>
<td>Source</td>
<td>Urine</td>
<td>Breath</td>
<td>Urine</td>
</tr>
<tr>
<td></td>
<td>dry &amp; liquid</td>
<td>liquid</td>
<td></td>
</tr>
<tr>
<td>Storage time</td>
<td>Max 5 mos</td>
<td>Max 60 days</td>
<td>Max 5 mos</td>
</tr>
<tr>
<td>Dogs</td>
<td>Number</td>
<td>5 each trained</td>
<td>Prostate 4</td>
</tr>
<tr>
<td></td>
<td>Dry 2</td>
<td>Liquid 4</td>
<td>Breast 6</td>
</tr>
<tr>
<td></td>
<td>Varied</td>
<td>Varied</td>
<td>Varied</td>
</tr>
<tr>
<td>Ages</td>
<td>1.5–8 yrs</td>
<td>1.5–7 yrs</td>
<td>2–8 yrs</td>
</tr>
<tr>
<td>CA tested for</td>
<td>One type</td>
<td>Both types</td>
<td>One type</td>
</tr>
<tr>
<td>Training</td>
<td>Site</td>
<td>One-central</td>
<td>Trainer’s home</td>
</tr>
<tr>
<td></td>
<td>Trainers</td>
<td>Dedicated team</td>
<td>Owner</td>
</tr>
<tr>
<td></td>
<td>Frequency</td>
<td>20×/day</td>
<td>15’–30’/day</td>
</tr>
<tr>
<td></td>
<td>Duration</td>
<td>2–3 weeks</td>
<td>12–14 months</td>
</tr>
<tr>
<td>Blinding</td>
<td>Early</td>
<td>Early</td>
<td>Late</td>
</tr>
<tr>
<td>Testing</td>
<td>No. CAs tested</td>
<td>5—all tested</td>
<td>11 Prostate</td>
</tr>
<tr>
<td></td>
<td>Site</td>
<td>Where trained</td>
<td>Where trained</td>
</tr>
<tr>
<td></td>
<td>CA/run</td>
<td>1 of 6</td>
<td>1 of 5</td>
</tr>
<tr>
<td></td>
<td>Runs/CA</td>
<td>1</td>
<td>3 Prostate</td>
</tr>
<tr>
<td></td>
<td>No. of runs per dog</td>
<td>9</td>
<td>10</td>
</tr>
<tr>
<td>Results</td>
<td>Combined</td>
<td>41%</td>
<td>99% Lung</td>
</tr>
<tr>
<td></td>
<td>Best individual</td>
<td>56%</td>
<td>88% Breast</td>
</tr>
<tr>
<td></td>
<td>Stat. signif.</td>
<td>Yes</td>
<td>Not reported</td>
</tr>
<tr>
<td></td>
<td>Replicate runs</td>
<td>No</td>
<td>No</td>
</tr>
</tbody>
</table>

*aPost hoc* determination that one “control” was actually a cancer in 3 of the prostate runs and 2 of the breast cancer runs.

*bSensitivity.*
USING CANINES TO DETECT HUMAN CANCERS

CONCLUSION

It had been shown by prior investigators that there are biochemical markers produced by human bladder, breast, and lung cancers that dogs can identify by scenting. The current study tried to prove that a urine screening method utilizing this scenting ability was feasible for breast and prostate cancer. Unfortunately, the study did not yield success but offered valuable lessons in the form of mistakes, presented here with the hope other people will benefit from them. The use of canines in the detection of bombs, drugs, and other tasks evolved over time. With further studies, there is no reason why this should not be the case with dogs screening for human cancer.

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