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Olfactory Detection of Prostate Cancer by Dogs Sniffing Urine: A Step Forward in Early Diagnosis

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Abstract

Background: Volatiles organic compounds (VOCs) in urine have been proposed as cancer biomarkers.

Objective: To evaluate the efficacy of prostate cancer (PCa) detection by trained dogs on human urine samples.

Design, setting, and participants: A Belgian Malinois shepherd was trained by the clicker training method (operant conditioning) to scent and recognize urine of people having PCa. All urine samples were frozen for preservation and heated to the same temperature for all tests. After a learning phase and a training period of 24 mo, the dog's ability to discriminate PCa and control urine was tested in a double-blind procedure. Urine was obtained from 66 patients referred to a urologist for elevated prostate-specific antigen or abnormal digital rectal examination. All patients underwent prostate biopsy and two groups were considered: 33 patients with cancer and 33 controls presenting negative biopsies.

Measurements: During each "run," the dog was asked to signal a cancer urine among six samples containing only one cancer urine and five randomly selected controls. Sensitivity and specificity of the test were assessed.

Results and limitations: The dog completed all the runs and correctly designated the cancer samples in 30 of 33 cases. Of the three cases wrongly classified as cancer, one patient was rebiopsied and a PCa was diagnosed. The sensitivity and specificity were both 91%.

Conclusions: This study shows that dogs can be trained to detect PCa by smelling urine with a significant success rate. It suggests that PCa gives an odor signature to urine. Identification of the VOCs involved could lead to a potentially useful screening tool for PCa.

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1. Introduction

Prostate cancer (PCa) is the most frequent noncutaneous malignancy in men, with an incidence as high as 192 280 cases per year in the United States [1]. Although prostate-specific antigen (PSA) blood testing remains the most widely used tool for PCa detection [2], important efforts have been conducted to determine alternative biomarkers to overcome its lack of specificity [3]. Novel urine or blood biomarkers have been proposed in the last decade, but none of them is currently widely used [4].

Volatile organic compounds (VOCs) in urine have been proposed as alternative biomarkers [5]. In the case of PCa, it can be postulated that specific VOCs may be present in urine that reveal the presence of a malignant tumor. Basic research has provided a recent finding suggesting that sarcosine could be an indicator of the aggressiveness of prostatic malignant disease [6], but no extensive work searching for VOCs in urine related to the presence of PCa has been published. Promising results about other malignant diseases have been presented [7].

VOC detection can be made by sophisticated biochemical techniques or using animals that have a highly sensitive sense of smell [7]. Some previous work suggested that dogs trained to smell urine could recognize lung, bladder, or breast cancer with various success rates, but no positive results have been published concerning PCa [8,9]. To determine if some VOCs in urine could result in a specific odor associated with PCa, we specially trained a dog and conducted a double-blind study to check its ability to detect PCa by sniffing urine.

2. Materials and methods

2.1. Dog training

A Belgian Malinois shepherd was trained by a professional and dedicated team of two people from the beginning to the end of the study. The dog belonged to the French Army veterinary department and was chosen among young dogs destined for explosives detection training. The dog was never trained before.

The first objective was to teach the dog to discriminate between urine from individuals with PCa and urine from controls. The dog was trained by the clicker training method (a kind of operant conditioning). The dog was given his ball as a reward for alerting to a cancer urine. The dog was taught to sit in front of the sample of urine recognized as cancer. Training was a full-time job for the team, who worked with the dog 5 d/wk over the study period (October 2007 to June 2010). During the training phase, runs were not blinded and the dog was always presented cancer urines and full, undiluted control urines. Total duration of the training phase, from the initial phase of learning through the unblinded exercises phase, was 16 mo.

2.2. Patients and samples

Urine samples were obtained from Caucasian patients recruited in our tertiary reference center who had given written consent for analysis of their urine for research programs, including genetic analysis. All men included were referred to a urologist because they had an elevated PSA level or abnormal findings on digital rectal examination (DRE). Data

collected at the visit were age, height, weight, PSA level, and DRE data. Urine was collected during the first consultation after DRE. All patients underwent prostate biopsies according to a standard procedure (12 cores) and were classified as cases or controls after pathologic examination of the specimens. Patients were not selected in case of history of urothelial carcinoma or other malignant disease. There were no exclusion criteria regarding other medical history, alcohol consumption, drugs, food, tobacco consumption, or other habits.

A total of 108 patients supplied urine. Forty-two urine samples (26 cancers and 16 controls) were used for the training phase, and 66 patients were tested in the double-blind phase. Patients studied in the double-blind phase were 33 PCa cases and 33 controls, as determined by prostate biopsy.

2.3. Study design

The global study design is described in Fig. 1. The double-blind testing phase consisted of consecutive runs. For each run, the dog was presented six samples (five controls and one cancer). During each run, the cancer urine was one of the 33 selected cancer samples and the 5 control urines were samples randomly selected among controls. Samples were anonymized and numbered so that people conducting the test were not able to discriminate cancer from control samples. The samples were frozen at -4°C from the time of urine collection to the time of testing. Each urine sample was slowly heated to 37°C with the same material immediately before examination in a dedicated area outside the testing room.

During each run, the dog had to scent successively the six samples that were hidden in boxes. Each box had a hole so that the dog could not access the urine itself, but only its odor. After a mean time of 30 s, the dog had to sit in front of a box to designate the cancer sample. In case of success (dog sitting in front of PCa urine sample), the result was classified as a true positive and the controls as true negatives, and the next cancer sample was tested. In case of mistake (dog sitting in front of control urine sample), the control sample was classified as false positive and the cancer sample as a false negative. The false-positive sample was excluded from the pool of controls used for the future runs, and the cancer sample was retested in association with other controls. A new prostate biopsy was proposed to the patient who provided the false-positive sample.

2.4. Statistical analysis

Descriptive analysis was conducted with XLStat for Windows (Addinsoft, Paris, France).

3. Results

Characteristics of patients who supplied urine for the testing phase are given in Table 1. Thirty-three runs were conducted during the double-blind testing phase. The mean duration of each run was approximately 30 s. In 30 cases, the dog sat in front of the cancer sample. In three runs, the dog sat in front of a control sample. In these three cases, the control samples incorrectly classified were considered false positives, and the three cancer cases were considered false negatives. Consequently, during the testing phase, the dog correctly classified 60 samples out of 66. Results of the test are presented in Table 2. After each failure (three cases), a new run was conducted as described above, implicating the same cancer sample and other control samples. The dog classified cancer samples as true

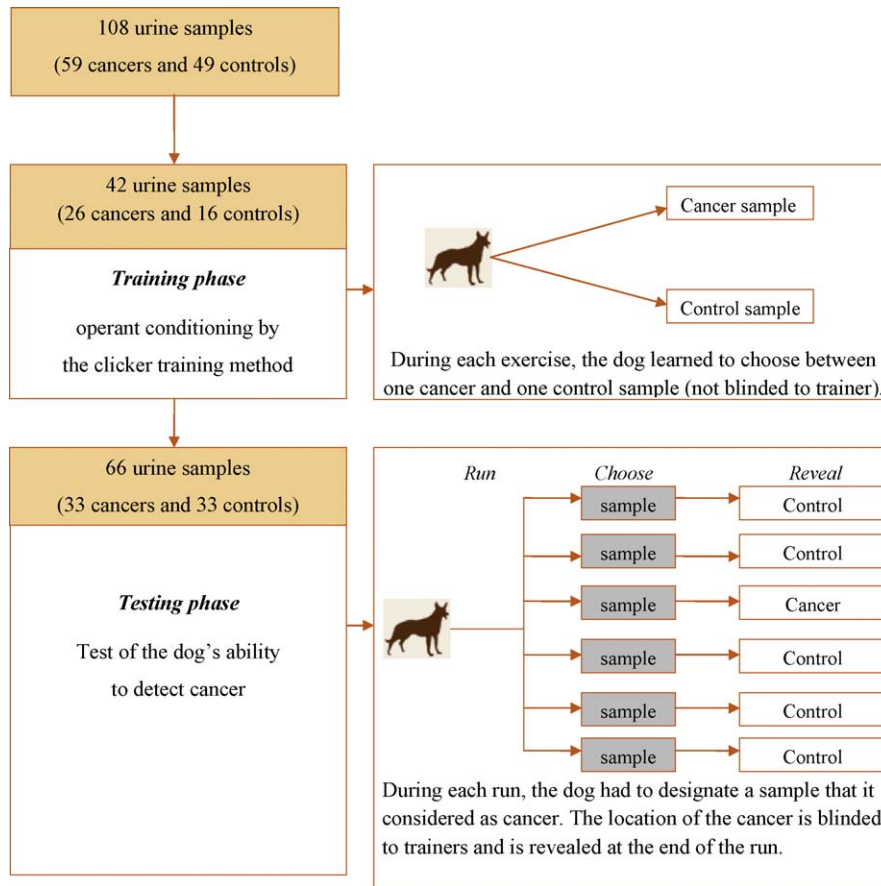


Fig. 1 – Study design.

positives during these runs. The three patients who provided the urine samples that were classified as false positive underwent a new biopsy, and one was diagnosed with PCa.

Table 1 – Characteristics of patients who supplied urine for the testing phase (n = 66)

Characteristics	Cancers (n = 33)	Controls (n = 33)	p value
Age, yr	64.1 ± 7.1 [51–77]	63.2 ± 7.1 [51–79]	0.79
PSA (ng/ml)	11.7 ± 15.1 [2.9–85]	8.3 ± 4.1 [2–16.8]	0.77
DRE			
Nonsuspect	23	25	0.78
Suspect	10	8	
Gleason score			
≤6	16		n/a
7	14	n/a	
≥8	4		

PSA = prostate-specific antigen; DRE = digital rectal examination.

Table 2 – Results of the testing phase

	Positive	Negative
Cancers	30	3
Controls	3	30
Sensitivity, %	91	
Specificity, %	91	

4. Discussion

The use of canines for cancer detection emerged after the first case report in 1989 about a melanoma detected by a dog on his owner's leg [10]. The scientific basis of this ability of dogs to detect the odor signature of cancer is believed to be linked to the VOCs produced by malignant cells [5]. Indeed, basic research studies have established that during tumor growth, protein changes in malignant cells lead to peroxidation of the cell membrane components and produce VOCs that can be detected in the headspace of the cells [11,12]. In particular, VOC analysis of exhaled breath for cancer detection (including PCa) has been recently studied with promising results [13]. Specific detection of lung cancer based on the odor of urine samples has been recently proposed through animal models [7], but no specific investigation has been conducted specifically focusing on urologic tumors.

Previous studies have shown that trained dogs are able to detect bladder, lung, or breast cancer in urine with better than chance probability [8], but no positive result was obtained for PCa [9]. Our experience thus shows for the first time that, regularly trained by a dedicated team, a dog can distinguish a PCa urine sample among controls with powerful results. This difference from previously reported studies can be explained by our professional method of training by a dedicated team and the fact that one dog was completely dedicated to the task, with no previous training.

These preliminary data reflect the existence of a potential odor signature of PCa that may correspond to one or multiple VOCs. These molecules remain unknown for the moment and should be assessed by specific gas chromatography/mass spectrometry analysis. To date, metabolomics studies have only individualized sarcosine as a potential biomarker for PCa [6].

Although our results provide a new insight in the field, the present study is subject to limitations. First, we obtained these powerful results with one dog, and this may not be reproducible with other dogs. Indeed, the type of dog used in the study can influence the results since canine olfactory-receptor polymorphisms have been shown to influence odor detection performance by sniffer dogs [14]. Second, an intrinsic potential limitation of our work is that selected controls were patients age >50 with elevated PSA (to be comparable with cancer patients regarding these characteristics). Control patients had mean PSA value of 8.3 ± 4.1 [range: 2–16.8]. Given these values, it can be considered that 20–30% of these control patients with negative prostate biopsies have PCa, according to previous reports [15]. Consequently, seven patients considered as controls in this study should have PCa, and therefore the dog should have designated seven control samples as cancer (false positives in the study context). In our work, three samples (instead of seven expected) were classified as false positives, and one patient was found to have cancer on a new biopsy. This limited number of false positives could be related to the design of the study since the dog had to choose between cancers and controls. Indeed, patients with positive prostate biopsy may have a greater tumor volume than patients with PCa and negative biopsies. If the dog is detecting a quantitative parameter (odor of the VOC), it may choose the sample with the higher amount of VOC. Moreover, given the small number of patients studied, these results may reflect a lack of power, although they are consistent with the imperfections of prostate biopsy. However, the present work is a proof-of-principle study, and the use of these dogs is not supposed to be generalized. We tested a limited number of subjects in a costly, long study that makes it difficult to conceive of an extended use for this test in clinical practice. Potential biases of odor detection, including associated diseases and food and drink consumption, have not been explored in our protocol. However, despite this lack of information, the findings of our study are powerful and suggest that the potential biomarkers recognized by the dog overcome these potential confounding factors.

Finally, results of the present study should be preliminary to further metabolomic studies focused on VOC evaluation that are currently being performed. Previously unreported, the proof of principle presented here is a step forward and is the beginning, rather than the end, of the story. This conditioned dog should be used in the near future to validate candidate molecules emerging from metabolomic screening.

5. Conclusions

The present study brings the proof that a specially trained dog by a professional team can be conditioned to recognize

PCa among controls only by sniffing urine. This study opens the door of VOC detection for PCa diagnosis. Metabolomic studies should complete this approach by determining the volatile molecular signature of PCa.

Author contributions: Jean-Nicolas Cornu had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Study concept and design: Cussenot.

Acquisition of data: Cancel-Tassin, Girardet, Ondet.

Analysis and interpretation of data: Cancel-Tassin, Cornu.

Drafting of the manuscript: Cornu.

Critical revision of the manuscript for important intellectual content: Cussenot.

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