ECRONICON OPEN ACCESS

VETERINARY SCIENCE
Research Article

# The Ability of Dogs to Detect Human Prostate Cancer Before and After Radical Prostatectomy

**Gianluigi Taverna[1]*, Lorenzo Tidu[2], Fabio Grizzi[3], Brian Stork[4], Alberto Mandressi[1], Mauro Seveso[1], Giorgio Bozzini[1], Paolo Sardella[2], Giuseppe Latorre[2], Giovanni Lughezzani[5], Nicolo' Buffi[5], Paolo Casale[5], Girolamo Fiorini[5], Massimo Lazzeri[5] and Giorgio Guazzoni[5]**

*[1]Department of Urology, Ospedale Humanitas Mater Domini, Castellanza, Italy*

*[2]Italian Ministry of Defense's, Military Veterinary Center, CEMIVET, Italy*

*[3]Department of Immunology and Inflammation and Humanitas, Clinical and Research Center, Italy*

*[4]West Shore Urology, Muskegon, Michigan, USA*

*[5]Department of Urology, Humanitas Clinical and Research Center, Italy*

***Corresponding Author:** Gianluigi Taverna, Department of Urology, Ospedale Humanitas Mater Domini, Via Gerenzano, 2 - 21053 Castellanza, Varese, Italy.

**Received:** July 12, 2015; **Published:** July 23, 2015

## Abstract

**Evaluation:** The ability of highly-trained dogs' olfactory system to detect prostate cancer specific volatile organic compounds (VOCs) in men after undergoing radical prostatectomy and the eventual biochemical recurrence (BCR) was assessed.

**Materials and methods:** One hundred-fourteen consecutivemen with clinical localized PCa undergoing radical prostatectomy between November 2011 and May 2013 were investigated. For each patient urine and serum samples were collected prior to radical prostatectomy, forty-five days and every six months during the successive follow-up (mean: 28 months; median: 28 months; range 19-37 months). Two dogs were trained to sit when they detected PCa specific volatile organic compounds (VOCs) in the urine samples.

**Results:** Preoperatively, both dogs were able to detect PCa specific VOC's in the urine samples of men with PCa with 100% accuracy. Forty-five days post-radical prostatectomy, 104 (91.2%) patients had a serum prostate-specific antigen (PSA) levels < 0.01 ng/ml, 6 (5,2%) patients had a serum PSA levels > 0.01 but < 0.2 ng/ml and 4 (3,5%) patients had a serum PSA > 1 ng/ml. Forty-five days following surgery, neither dog detected prostate cancer specific VOC's in the urine samples of the 104 men with a serum PSA level < 0.01ng/ml. Both dogs detected PCa specific VOC's in samples collected from 2 out of 6 men with PSA levels > 0.01ng/ml and < 0.2 ng/ml and both dogs were able to detect VOC's in the samples each of the four men with PSA levels > 1ng/ml (i.e. persistent disease). During the successive followup 9 of 110 patients (8.1%) had BCR. Both dogs were able to detect PCa VOC's in the urine samples of 7 of these 9 patients (77.7%).

**Conclusions:** Highlytrained dogs are able to detect BCR in men who have previously undergone radical prostatectomy alone for PCa. Our understanding of the use of the canine olfactory system in PCa detection continues to evolve.

***Keywords:*** *Human prostate cancer; Biomarkers; Canine Olfactory System; Volatile Organic Compounds; Detection; Biochemical recurrence; Radical prostatectomy*

## Background

Prostate cancer (PCa) is the fifth most common cancer in the world and the second most commonly diagnosed non-cutaneous cancer in men in the United States [1]. Prostate-specific antigen (PSA) is a widely used tumour biomarker for PCa [2]. Following radical prostatectomy, the PSA valuerepresents the gold standard for PCa follow-up. It is now accepted that any potential therapeutic strategy following biochemical recurrence (BCR) of PCa is based on rising serum PSA levels over time. Although a high degree of variability in the definition of BCR exists [3], a widely recognized definition of BCR is PSA of 0.2 ng/mL for patients who have previously undergone radical prostatectomy [3,4].

Over the past decade, various urine, blood, and tissue biomarkers have been proposed to replace PSA [5-8]. Among them, volatile organic compounds (VOCs) have been recognizedas a valid alternative diagnostic tool [9]. Therefore, the research to detect and identify some VOCs, which are a source of odours, as a way to develop new diagnostic methods, has been of great importance to medical sciences. VOCs are molecules capable of volatilizing at room temperature and are the product of different metabolic pathways. VOCs can be detected by sophisticated biochemical techniques or using animals that have a highly sensitive sense of smell [9-14]. Since 2001, various studies have reported the detection of bladder, lung, skin, breast, and ovarian cancers and infectious diseases using a dog's sense of smell [15,16]. Gordon., *et al*. [17] and Cornu., *et al*. [18] have extended the use of "detection dogs" to PCa. Recently, we establish the diagnostic accuracy, in term of sensitivity and specificity, in which a rigorously trained canine olfactory system could recognize PCa-specific VOCs in urine samples [15]. We analysed a series of 903 participants and found that two dogs achieved a sensitivity and specificity more than of 98.6% and 96.4%, respectively [15].

It is known that followingradical prostatectomy, serum PSA should decrease to lower levels (roughly, < 0.01 ng/ml) and remain constant. Independent of tumour stage, it is a common practice to follow patients for evidence of recurrence with serial PSA measurements. Here we evaluate the ability of highly-trained dogs' olfactory system to detect PCa recurrence in men who have previously undergone radical prostatectomy for PCa.

## Methods

### Patients

A total of 114 consecutive participants, with different grades and stages of prostate cancer, who underwent radical prostatectomy for PCa, were enrolled between November 2011 and May 2013. All patients were staged pre-operatively with either Choline-PET or abdominal CT scan and bone scintigraphy. No patients enrolled in the study had clinical evidence of metastatic disease. For all patients, the clinical and epidemiological baseline characteristics including age, PSA, pathological stage, surgical margins and tumour volume were known (Table 1). The follow-up of patients consisted the analysis of serum PSA levels forty-five days after radical prostatectomy and then at 6-month intervals. The cut-off value in the definition of BCR was a PSA of 0.2 ng/mL [3,4]. The Ethical Committee of the Center treating the patients (Humanitas Clinical and Research Center, Rozzano, Milan, Italy) approved the study (n. CE ICH - 260/11). Each participant was informed about the study, questions were answered and signed consent was obtained. The entire study was conducted in collaboration with the Italian Ministry of Defence's, Military Veterinary Center (CEMIVET, Grosseto, Italy).

### Urine sample collection

For each subject a urine sample (roughly, 15 ml) was collected prior toradical prostatectomy, as well asduring successive follow-up (45 days and every 6 months) in a sterile urine container and immediately stored at -20°C. These samples were then transported at controlled temperatures to the CEMIVET and stored at -20°C until used.

### Experimental procedure

A professional team trained two three-year old female German Shepherd Explosive Detection Dogs, (Zoe, chip n. 961100000401699 and Liu, chip n. 941000002579688). The team included 4 persons: a chief medical veterinary surgeon (LT), a head trainer and 2 handlers (PS, GL). We proceeded to the "evaluation phase" as previously described [15]. Both dogs tested all urine samples collected from patients before radical prostatectomy and during the follow-up.

**Table 1** Baseline patient (n = 114) characteristics

| | |
|---|---|
| **Age** (years) | 63.5±6.3 (52-75) |
| **PSA** (ng/ml) | 9.8 ±13.8 (3-90) |
| **Tumor volume** (%) | 17,9±15.8 (1-60) |
| **Prostate volume** (ml) | 51.3±24 (15-150) |
| **Gleason score** | |
| 3+3 | 51 |
| 3+4 | 42 |
| 3+5 | 3 |
| 4+3 | 12 |
| 4+5 | 6 |
| **Pathological stage** | |
| pT2aN0 | 9 |
| pT2bN0 | 3 |
| pT2cN0 | 72 |
| pT3aN0 | 21 |
| pT3bN0 | 6 |
| pT3bN1 | 3 |
| **Surgical margins** (yes, %) | 15 (13.1) |

**Statistical analysis**

Baseline clinical and histopathological characteristics were summarized using descriptive statistics (mean and standard deviation for continuous variables, and absolute frequencies for categorical variables, range). The statistical analysis was performed using Stata Statistical Software: Release 9, College Station, TX: Stata Corp LP.

## Results

Both dogs detected PCa specific VOCs in the urine samples of men with PCa, pre operatively (Table 2). Forty-five days post-radical prostatectomy, 104 (91.2%) patients had a serum PSA levels < 0.01 ng/ml, 6 (5.2%) patients had serum PSA levels > 0.01 but < 0.2 ng/ml and 4 (3.5%) patients had a serum PSA levels > 1 ng/ml. Forty-five days following surgery, neither dog detected PCa specific VOCs in the urine samples of the 104 men with a serum PSA level < 0.01 ng/ml. Both dogs detected PCa specific VOCs in samples collected from 2 out of 6 men with PSA levels > 0.01 ng/ml and < 0.2 ng/ml and both dogs were able to detect PCa specific VOCs in the samples of all four men with PSA levels > 1 ng/ml (patients considered with persistent disease). During the successive follow-up (mean: 28 months; median: 28 months; range 19-37 months) 9 of 110 patients (8.1%) had BCR. Both dogs were able to detect PCa specific VOCs in the urine samples of 7 of these 9 patients (77.7%). The patients that were not recognized by both dogs had not similar clinical characteristics. One patient was aged 60 years and had a pre-operatory serum PSA value 6.2 ng/ml, clinical stage T1c, Gleason score 3+4, pathological stage pT2cN0, tumour volume 45%, prostate volume 53 ml and negative surgical margins with a BCR 18 months after radical prostatectomy. The other patient was aged 56 years and had a pre-operatory serum PSA value 13 ng/ml, clinical stage T1c, Gleason score 4+3, pathological stage pT3bN0, tumour volume 17%, prostate volume 40 ml and positive surgical margins with a BCR 12 months after radical prostatectomy. The ability of dogs to anticipate the BCR was found in 2 out 7 patients.

| | n | BCR | Detection rate | |
|---|---|---|---|---|
| | | | DOG 1 | DOG 2 |
| **Before Radical Prostatectomy** | 114 | | 114 (100%) | 114 (100%) |
| **After radical prostatectomy** | | | | |
| PSA < 0.01 ng/ml | 104 | | 0 | 0 |
| PSA > 0.01ng/ml < 0.2 ng/ml | 6 | | 2 (33,3%) | 2 (33,3%) |
| PSA > 1 ng/ml* | 4 | | 4 (100%) | 4 (100%) |
| **Median follow-up (28 months)** | 110 | 9 (8.1%) | 7 (77.7%) | 7 (77.7%) |

**Notes:** *Persistent disease; BCR, Biochemical recurrence.

## Conclusions

Here we have shown for the first time that highly-trained dogs are able to detect PCa BCR following radical-prostatectomy. The use of dogs for cancer detection emerged after the first case report in 1989 [15]. Recently, we demonstrated that a rigorously trained canine olfactory system can recognize PCa specific VOCs in urine samples with a sensitivity and specificity more than of 98.6% and 96.4%, respectively [15]. Additionally, the dogs' ability to recognize PCa specific VOCs was independent of the Gleason score, clinical and pathological stage, PSA, free-to-total PSA ratio, prostate health index, prostate and tumour volume, age of the patient and tumour topography.

The nature and number of PCa specific VOCs the dogs are detecting in the urine samples of men with PCa is currently not known [2]. The recognition of PCa specific VOCs in urine from patients who have undergone radical prostatectomy and gone on to developed PCa metastases suggests that these VOCs depend on an active metabolic tumour process [15]. In the present study we found that both dogs detect each patient with persistent disease following radical prostatectomy.

Forty-five days following surgery, neither dog detected prostate cancer specific VOC's in the urine samples of men with a serum PSA level < 0.01 ng/ml and during the successive follow-up both dogs were able to detect PCa VOCs in the urine samples of 7 out of 9 patients (77.7%) with progressive biochemical relapse.

The present study confirms that if a persistent cancer is present after radical prostatectomy it continues to produce PCa specific VOCs that are recognized by trained dogs. When the serum PSA level is less than or equal to 0.01 ng/ml the dog does not recognize the presence of cancer. An unanswered question remains. Why were the dogs only able to recognize 7 out of 9 patients with BCR? Ahypothesis might be the different PCa metabolism, excessive VOCs dilution, or a simple mistake performed by two dogs. Our study was aimed to investigate whether VOCs were produced after radical prostatectomy and detected by ahighlytrained dogs' olfactory system. The present study adds helpful information for the comprehension of the VOCs metabolism before and after radical prostatectomy.

In conclusion, highly trained dogs were able to detect BCR in men who had previously undergone radical prostatectomy for PCa. Our understanding of the use of the highly trained canine olfactory system in PCa testing continues to evolve. Further studies will be fundamental to comprehend the biochemical nature and genesis of PCa specific VOCs. Accordingly with Robert R. Bahnson this future step encourages all of us to redouble our efforts to improve the detection of prostate cancer [2]. Clearly noninvasive olfactory diagnosis by trained canines would likely be less expensive and also have fewer complications. Furthermore, identifying the abnormal substance would open the door for therapeutic targets and provide an avenue for molecular imaging.

## Bibliography

1. Michaelson MD., *et al*. "Management of complications of prostate cancer treatment". *CA: a cancer journal for clinicians* 58.4 (2008): 196-213.
2. Bahnson RR. "Detection of Prostate Cancer in Urine by Dogs". *The Journal of urology* 193.4 (2015): 1083.
3. Heidenreich A., *et al*. "EAU guidelines on prostate cancer. Part II: Treatment of advanced, relapsing, and castration-resistant prostate cancer". *European urology* 65.2 (2014): 467-479.
4. Heidenreich A., *et al*. "EAU guidelines on prostate cancer. part 1: screening, diagnosis, and local treatment with curative intent-update 2013". *European urology* 65.1 (2014): 124-137.
5. Bratt O and Lilja H. "Serum markers in prostate cancer detection". *Current opinion in urology* 25.1 (2015): 59-64.
6. McDunn JE., *et al*. "Metabolomic signatures of aggressive prostate cancer". *The Prostate* 73.14 (2013): 1547-1560.
7. Sridharan S., *et al*. "Prediction of prostate cancer recurrence using quantitative phase imaging". *Scientific reports* 5 (2015): 9976.
8. Stephan C., *et al*. "Prostate-specific antigen and other serum and urine markers in prostate cancer". *Biochimica et biophysica acta* 1846.1 (2014): 99-112.
9. Elliker KR., *et al*. "Key considerations for the experimental training and evaluation of cancer odour detection dogs: lessons learnt from a double-blind, controlled trial of prostate cancer detection". *BMC urology* 14 (2014): 22.
10. Leitch O., *et al*. "Biological organisms as volatile compound detectors: a review". *Forensic science international* 232.1-3 (2013): 92-103.
11. Concha A., *et al*. "Using sniffing behaviour to differentiate true negative from false negative responses in trained scent-detection dogs". *Chemical senses* 39.9 (2014): 749-754.
12. Phillips R. "Prostate cancer: eNose--man's new best friend?" *Nature reviews Urology* 11.4 (2014): 187.
13. Persaud K and Dodd G. "Analysis of discrimination mechanisms in the mammalian olfactory system using a model nose". *Nature* 299.5881 (1982): 352-355.
14. Lippi G and Cervellin G. "Canine olfactory detection of cancer versus laboratory testing: myth or opportunity?" *Clinical chemistry and laboratory medicine: CCLM/FESCC* 50.3 (2012): 435-459.
15. Taverna G., *et al*. "Highly-Trained Dogs' Olfactory System Detects Prostate Cancer in Urine Samples". *The Journal of urology* 193.4 (2014): 1382-1387.
16. Stone L. "Prostate cancer: sniffing out prostate cancer". Nature reviews Urology 11.12 (2014): 662.
17. Gordon RT., *et al*. "The use of canines in the detection of human cancers". *Journal of alternative and complementary medicine* 14.1 (2008): 61-67.
18. Cornu JN., *et al*. "Olfactory detection of prostate cancer by dogs sniffing urine: a step forward in early diagnosis". *European urology* 59.2 (2011): 197-201.

**Volume 2 Issue 1 July 2015**