

Olfactory System of Highly Trained Dogs Detects Prostate Cancer in Urine Samples

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Abbreviations and Acronyms

DRE = digital rectal examination
LR = likelihood ratio
PC = prostate cancer
PSA = prostate specific antigen
TURP = transurethral prostate resection
VOC = volatile organic compound

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Purpose: We established diagnostic accuracy in terms of the sensitivity and specificity with which a rigorously trained canine olfactory system could recognize specific volatile organic compounds of prostate cancer in urine samples.

Materials and Methods: Two 3-year-old female German Shepherd Explosion Detection Dogs were trained to identify prostate cancer specific volatile organic compounds in urine samples. They were tested on 362 patients with prostate cancer (range low risk to metastatic) and on 540 healthy controls with no non-neoplastic disease or nonprostatic tumor. This cross-sectional design for diagnostic accuracy was performed at a single Italian teaching hospital and at the Italian Ministry of Defense Military Veterinary Center.

Results: For dog 1 sensitivity was 100% (95% CI 99.0–100.0) and specificity was 98.7% (95% CI 97.3–99.5). For dog 2 sensitivity was 98.6% (95% CI 96.8–99.6) and specificity was 97.6% (95% CI 95.9–98.7). When considering only men older than 45 years in the control group, dog 1 achieved 100% sensitivity and 98% specificity (95% CI 96–99.2), and dog 2 achieved 98.6% sensitivity (95% CI 96.8–99.6) and 96.4% specificity (95% CI 93.9–98.1). Analysis of false-positive cases revealed no consistent pattern in participant demographics or tumor characteristics.

Conclusions: A trained canine olfactory system can detect prostate cancer specific volatile organic compounds in urine samples with high estimated sensitivity and specificity. Further studies are needed to investigate the potential predictive value of this procedure to identify prostate cancer.

Key Words: prostatic neoplasms, diagnosis, dogs, olfactory perception, volatile organic compounds

PROSTATE cancer represents the fifth most frequent cancer in the world.¹ Although PSA testing has increased PC detection, the main drawback is its lack of specificity and accuracy. High serum PSA levels can be detected in men with nonmalignant conditions.² Therefore, many men with increased PSA values undergo biopsy

sampling,^{3–5} although this procedure is invasive, offers a low level of accuracy (ie only 30% detection rate at the first biopsy) and is prone to various complications, including sepsis and death.^{6,7} Consequently there is a need for a more sensitive diagnostic method.³ Dogs are used for detecting explosives and drugs through their

olfactory system, which can perceive thresholds as low as parts per trillion.⁸ As outlined in 1971 by Pauling et al⁹ and in 2012 by Lippi and Cervellin¹⁰ VOCs can be identified in human urine samples. Several studies have shown that dogs may be trained to identify patients with cancer by tracing the presence of a unique odor signature.

In 1989 Williams and Pembroke provided the first evidence on sniffer dogs.¹¹ In 2001 Church and Williams reported on a 66-year-old man in whom a patch of eczema developed at which a pet Labrador persistently sniffed.¹² Histopathology revealed basal cell carcinoma. Since 2001, groups have reported the detection of bladder,^{13–15} lung and breast,¹⁶ skin¹⁷ and ovarian^{18,19} cancers, and infectious diseases²⁰ using the canine sense of smell.

Gordon²¹ and Cornu²² et al extended the use of detection dogs to PC. Although Gordon et al did not report positive results, they pointed out procedural errors that needed to be addressed by later researchers.²¹ They concluded that the study was unfortunately not successful but it provided lessons in the form of mistakes, which were presented in the hope that others might benefit from them. On the other hand, Cornu et al noted 91% sensitivity and specificity.²² Cornu et al took a step forward from Gordon et al,²¹ although they acknowledged important biases.²² A limited series of patients was enrolled, only 1 dog was used and the control group included patients older than 50 years with PSA greater than 8 ng/ml who were at high risk for undetected PC.

The opinion of Lippi and Cervellin that the most problematic issue has been the heterogeneity of performance among studies as well as in the same study¹⁰ together with the limited patient cohorts and nonstandardized training methodologies led us to design an accurate procedure to investigate whether dog olfactory detection remains a myth or could become a real clinical opportunity. We assessed diagnostic accuracy in terms of the sensitivity and specificity at which a rigorously trained canine olfactory system could recognize PC specific VOCs in urine in a large series of patients with PC of different stages and grades vs a heterogeneous control group.

MATERIALS AND METHODS

Participants

A total of 902 study participants were recruited between November 2012 and November 2013 at Humanitas Research Hospital. Participants were divided into a PC group and a control group.

The PC group comprised 362 male patients, including 1) 180 who had been treated with open or robotic radical prostatectomy surgery, 2) 120 with increased serum PSA

(greater than 2.5 ng/ml) or abnormal DRE who had undergone prostate biopsy²³ and had a histological diagnosis of PC, 3) 22 in whom PC was detected incidentally at TURP or who had very low or low risk PC on active surveillance, 4) 29 who had metastatic PC or were receiving hormonal therapy for biochemical relapse and 5) 11 with synchronous primary PC and another different tumor. These participants were recruited consecutively. A urine sample was spontaneously collected from each patient before prostate biopsy, radical prostatectomy or TURP.

The control group comprised 540 participants, including 1) 50 healthy, nonpregnant, younger and older female volunteers, 2) 72 female patients with a nonneoplastic condition (ie urinary infection, urolithiasis, neurological or metabolic disorder, obesity, hyperthyroidism or hypertension) or with cancer (bladder, breast, kidney, ovary, vulva, uterus, stomach, colon, liver, skin, blood, tonsil or pancreas), 3) 60 healthy male volunteers 18 to 25 years old with a family history negative for PC, 4) 240 men older than 45 years with a family history negative for PC, negative DRE and serum PSA less than 1 ng/ml or less than 2.5 ng/ml that had been stable with time who had urological and/or systemic disease, 5) 40 men with serum PSA less than 2.5 ng/ml that had been stable with time who had urinary obstruction treated with TURP for benign prostatic hyperplasia and 6) 78 men with serum PSA less than 2.5 ng/ml that had been stable with time who had a family history negative for PC, negative DRE and nonprostatic cancer.

Some participants were on pharmacological treatment (supplementary table 1, <http://jurology.com/>). No study exclusion criteria were assumed in regard to medical history, alcohol consumption, drugs, food, tobacco or other habits. Each participant was informed about the study and provided informed consent. The study was approved by the ethical committee at Humanitas Clinical and Research Center, where patients were treated.

Urine Samples

For each subject a urine sample of approximately 30 ml was spontaneously collected before prostate biopsy or radical prostatectomy in a sterile urine container. It was immediately stored at -20°C in different compartments according to the PC group vs controls to avoid potential contamination. Samples were transported at a controlled temperature to CEMIVET (Italian Ministry of Defense Military Veterinary Center) and stored at -20°C until use (supplementary material, <http://jurology.com/>, and fig. 1).

Canine Training

A professional team trained 2, 3-year old female German Shepherd Explosive Detection Dogs (Zoe and Liu). The CEMIVET team comprised a chief medical veterinary surgeon, a head trainer and 2 handlers. The dogs were trained using the clicker training method (ie operant conditioning). They were taught to sit in front of the urine sample recognized as cancerous (videos 1 and 2, and fig. 1). Training was a full-time job for the team. A total of 200 urine specimens from the PC group and 230 from the control group were analyzed during the training phase from June 2012 to October 2012 and not reused during the evaluation phase.

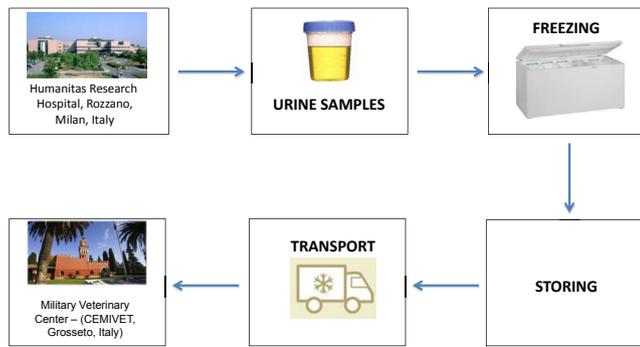


Figure 1. Sample collection, freezing, storage and transport phases.

All team members were blinded to runs except the chief medical veterinary surgeon, who observed the runs from outside the setup room. The team involved in the training and evaluation phase were not informed about the demographic and clinical characteristics of study participants.

The purpose of our training procedure was to teach the 2 dogs to recognize and store a pool of VOCs specifically present in the urine of patients with PC while ignoring urine free from PC specific or other interfering VOCs. The various stages of the training set were done with the 2 dogs in parallel. To define a standardized working methodology the training program and materials were designed specifically for the experimental procedure (supplementary material and supplementary figure, <http://jurology.com/>).

Briefly, after defrosting 2 ml of each urine sample were housed in circular perforated metal containers to allow the passage of odors (fig. 2). The metal containers were placed in thermally sealed plastic packets to avoid operator dependent or other contamination.

Phase 1 of the training procedure was done with urine from patients in the PC group vs that from healthy young female controls. The choice of healthy female participants was dictated by the need to be certain that no specific prostate VOCs could confuse the work of the 2 dogs. After documenting the positive feedback of the 2 dogs we moved to phase 2. This involved urine from the PC group vs that from women in the control group of various ages

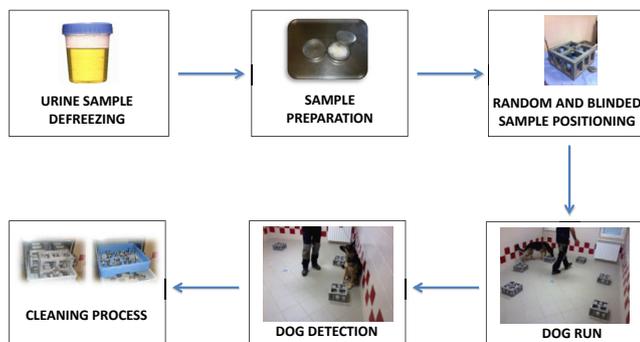


Figure 2. Procedural phases, including dog detection analysis

with nonneoplastic or neoplastic disease who were undergoing various drug therapies. After documenting the positive feedback of the 2 dogs we moved to phase 3. This involved comparing urine from the PC group with that from healthy young male volunteers in the control group. After documenting the positive feedback of the 2 dogs we moved to phase 4. This concentrated on urine from patients in the PC group vs that from men older than 45 years in the control group.

To gradually increase the complexity of the test and exclude conditioned reflexes in the dogs the runs were designed to use 1 positive sample followed by 2 negative samples. The number of negative samples was then increased up to a ratio of 1 positive to 5 negative samples. We evaluated single runs of 6 samples. The number of positive samples was also progressively increased, including 0 of 6, 1 of 6, 2 of 6, 3 of 6, 4 of 6, 5 of 6 and 6 of 6.

We simultaneously ran checks containing no positive samples to test the accuracy of the dogs and exclude the possibility that they might report a nonexistent find simply to earn the reward. The position of positive samples was always random, as determined by ad hoc software. The dogs reported the positive sample only after making the full run twice. The results of the training procedure were not considered for statistical purposes.

Canine Test Evaluation

After completing the training phase we proceeded to the evaluation phase. The run scheme included 6 urine samples. All urine samples from the PC and control groups were analyzed blindly. Each dog tested all 902 samples after random positioning. When the daily runs concluded, the chief medical veterinary surgeon checked the results and collected all data. According to the study by Cornu et al²² after each failure a new run was done 3 times using the same and other random samples. Diagnostic performance was evaluated after excluding repeat runs.

Statistical Analysis

Baseline demographic and clinical characteristics were summarized using descriptive statistics, including the median and range for continuous variables, and the absolute frequency for categorical variables. Diagnostic test performance was determined by sensitivity (the conditional probability of the dogs indicating cancer when the condition existed), specificity (the conditional probability of the dogs ignoring a sample from a healthy donor), and the positive and negative LR. A positive LR indicated the ratio of the conditional probability of the dog indicating cancer when it existed to the conditional probability of the dog indicating cancer when it did not exist. A negative LR indicated the ratio of the conditional probability of the dog ignoring a sample from a donor with PC to the conditional probability of the dog ignoring a sample from a healthy donor. For sensitivity and specificity the simple proportion is shown as a point estimate with the 2-sided 95% Clopper-Pearson confidence limit. For diagnostic LR the ratio is shown as a point estimate with the 2-sided exact 95% CI.

Diagnostic test performance was evaluated in the whole study population, after excluding females and when considering only control men older than 45 years.

An exact logistic regression model was used to test whether baseline demographic and clinical characteristics were statistically associated with the ability of the dogs to recognize PC specific VOCs. All statistical tests were 2-sided with exact $p < 0.05$ considered statistically significant. Statistical analysis was done with Stata®, release 9.

RESULTS

Tables 1 and 2 show the baseline clinical and demographic characteristics of the PC and control groups, respectively. The results of the training procedure were not considered for statistical purposes. Our analysis was descriptive in essence. Analysis focused on estimating sensitivity/specificity and positive/negative LRs in various participant subgroups.

In the evaluation phase the 2 dogs achieved a certain performance in all 902 study participants. For dog 1 sensitivity was 100% (95% CI 99.0–100.0), specificity was 98.7% (95% CI 97.3–99.5) with 7 wrong cases of 540, positive LR was 77.1 (95% CI 37.0–161.0) and negative LR was 0 (95% CI 0–0.02). For dog 2 sensitivity was 98.6% (95% CI 96.8–99.6) with 5 wrong cases of 362, specificity was 97.6% (95% CI 95.9–98.7) with 13 wrong cases of 540, positive LR was 41.0 (95% CI 23.9–70.1) and negative LR was 0.01 (95% CI less than 0.01–0.03).

After excluding all 122 female controls dog 1 achieved 98.3% specificity (95% CI 96.6–99.3) with 7 wrong cases of 418, a positive LR of 59.7 (95% CI 28.6–124.5) and a negative LR of 0 (95% CI 0–0.02). Dog 2 achieved 96.9% specificity (95% CI 94.7–98.3) with 13 wrong cases of 418, a positive LR of 31.7 (95% CI 18.6–54.2) and a negative LR of 0.01 (95% CI less than 0.01–0.03).

When considering only men older than 45 years in the control group, dog 1 achieved 98.0% specificity (95% CI 96.0–99.2) with 7 wrong cases of 358, a positive LR of 51.1 (95% CI 24.6–106.5) and a negative LR of 0 (95% CI 0–0.02). Dog 2 achieved 96.4% specificity (95% CI 93.9–98.1) with 13 wrong cases of 358, a positive LR of 27.5 (95% CI 16.2–47.0) and a negative LR of 0.01 (95% CI less than 0.01–0.03, supplementary table 2, <http://jurology.com/>).

On univariate analysis all PC predictive parameters, including patient age, Gleason score, clinical stage, 7th edition AJCC (American Joint Committee on Cancer) pathological stage, PSA, free-to-total PSA ratio and PHI (Prostate Health Index), showed no statistically significant association with the reporting status of the neoplastic sample (table 3). Neither prostate and tumor volumes nor PC topography in different prostate regions influenced the results. Reporting by the dogs showed no difference in PC clinical or pathological stage. No drug received by patients interfered with the results. Finally, the 2 dogs correctly reported all patients with synchronous PC and other malignancies.

The 7 and 13 false-positive identifications signaled by dogs 1 and 2, respectively, were in controls, specifically in men older than 45 years with a family history negative for PC, negative DRE, stable serum PSA less than 1 or less than 2.5 ng/ml, and urological and/or systemic disease, in men with stable serum PSA less than 2.5 ng/ml who had urinary obstruction and underwent TURP for benign prostatic hyperplasia and in men with stable serum PSA less than 2.5 ng/ml and a family history negative for PC, negative DRE and nonprostatic cancer. The false-negative identifications of dog 2 were made in the PC group, including 3 patients in the subgroup treated with prostatectomy, 1 in the subgroup with increased PSA or abnormal DRE, biopsy and a PC diagnosis, and 1 in the subgroup with incidental PC detected during TURP, or very low or low risk PC on active surveillance (supplementary material and supplementary table 2, <http://jurology.com/>).

DISCUSSION

Today all patients in whom an initial prostate biopsy is negative are followed by consecutive PSA measurements and some undergo further biopsies with time. According to Djavan et al repeat biopsies 1, 2, 3 and 4 have a detection rate of 22%, 10.5%, 5% and 4%, respectively.²⁴ Ploussard et al reported a 16.7%, 16.9% and 12.5% detection rate for repeat biopsies 1,

Table 1. Baseline clinical and histopathological characteristics of 362 patients with PC by subgroup

	Median Subgroup (range)				
	Prostatectomy	Pos Biopsy	Incidental PC or Surveillance	Metastatic PC or Hormonal Therapy	Synchronous PC + Other Ca
No. pts	180	120	22	29	11
Age	65 (46–75)	67 (47–80)	69 (58–78)	72 (60–88)	69 (62–82)
PSA (ng/ml)	7 (0.4–72)	7.5 (1–175)	8 (2–10)	13 (0.1–90)	9 (3–22)
Free/total PSA	14 (1–31)	13 (0.01–41)	18.5 (7–33)	Not done	15 (9–24)
PHI	30.5 (6–97)	26.5 (10–77)	39 (28–55)	Not done	Not done
Vol (ml):					
Prostate	45 (15–150)	43 (6–150)	39 (5–160)	Not done	48 (32–78)
Tumor	15 (1–90)	Not done	Not done	Not done	13 (3–60)

Table 2. Baseline clinical and histopathological characteristics of 540 controls by subgroup

Health Status Subgroup	No. Subjects	Median Age (range)	Median ng/ml PSA (range)	Median Free/Total PSA (range)	Median ml Prostate Vol (range)
Female:				Not defined	
Nonpregnant healthy volunteers	50	34 (18–54)			
Ca or nonCa disease	72	38 (18–61)			
Male:				Not defined	
Healthy, no PC family history	60	22 (18–25)			
NonCa disease, PSA less than 1 ng/ml	120	60 (45–85)	0.6 (0.1–1.1)	33 (0.4–69)	35 (20–98)
NonCa disease, PSA less than 2.5 ng/ml	120	63 (45–87)	1.4 (0.4–2.5)	28 (17–58)	45 (20–120)
Post-TURP nonCa disease	40	72 (54–86)	2.2 (0.6–13)	24 (11–40)	45 (22–100)
Ca	78	63 (53–78)	2.1 (0.3–2.5)	20 (17–32)	37 (15–110)

2 and 3, respectively.²⁵ Prostate biopsy may be associated with complications, including pain, hematuria, infection, urinary retention, erectile dysfunction, rectal bleeding and hemospermia, and more severe complications such as rectal or urethral fistula, Fournier gangrene, sepsis and death.^{26–28}

In 1989 after the first case report dogs were first used to detect cancer.¹¹ In the last 24 years preliminary studies have been published on the subject regarding different methods and organs with interesting results but no conclusive evidence. These considerations led us to design a study to eliminate all of the criticisms to more accurately establish the levels at which a rigorously trained canine olfactory system could recognize PC specific VOCs in urine.

Our study demonstrated certain findings. 1) The 2 dogs achieved sensitivity and specificity of more than 98.6% and 96.4%, respectively. 2) The ability of the dogs to recognize PC specific VOCs was independent of Gleason score, clinical and pathological stage, PSA, free-to-total PSA ratio, PHI, prostate and tumor volume, patient age and tumor topography. In regard to patients on androgen deprivation therapy the dogs always identified their urine samples as positive regardless of PSA or imaging stage. 3) The dogs never reported patients with another neoplasm, suggesting the specificity of VOCs and the selective capacity of canine olfaction. 4) No medication used by participants influenced the results.

The recognition of PC specific VOCs in urine from patients who underwent radical prostatectomy

and in whom PC related distant metastasis subsequently developed suggests that VOCs might depend on a tumor metabolic process. The independence of tumor volume and aggressiveness, and the dog detection rate is surprising. We hypothesize that a minimal quantity of PC specific VOCs can be detected independent of tumor volume or stage.

This study has some limitations. When considering a control group of adult males, even if family history and DRE are negative and PSA is less than 2.5 ng/ml, we should bear in mind that a small fraction may have PC. In the PSA era this limit cannot be eliminated. Therefore, we cannot totally exclude incidental PC in certain control subgroups, including men older than 45 years with a family history negative for PC, negative DRE, stable serum PSA less than 1 or less than 2.5 ng/ml, and urological and/or systemic disease, men with stable serum PSA less than 2.5 ng/ml who had urinary obstruction and underwent TURP for benign prostatic hyperplasia and men with stable serum PSA less than 2.5 ng/ml and a family history negative for PC, negative DRE and nonprostatic cancer.² According to current guidelines it is compulsory to emphasize that it is unethical to biopsy or rebiopsy individuals at no risk for PC.

Unanswered questions remain, such as what the dogs actually smell. Further studies should be done to investigate whether the dogs recognize a single odor or a mixture of PC specific VOCs. At the same time the study of synthetic noses may leap forward,

Table 3. Logistic regression model of probability of dog 2 correct diagnosis by PC predictive parameters and sensitivity

PC Predictive Parameters*	Prostatectomy		Pos Biopsy		Incidental PC or Surveillance	
	OR (95% CI)	p Value	OR (95% CI)	p Value	OR (95% CI)	p Value
PSA	1.07 (0.92–1.49)	0.753	1.19 (0.96–2.87)	0.667	1.20 (0.60–2.76)	0.454
Free/total PSA	0.87 (0.62–1.16)	0.328	1.04 (0.81–1.48)	0.955	0.96 (0.70–1.27)	0.818
PHI	1.01 (0.94–1.10)	0.986	Not defined		1.18 (0.83–2.12)	0.526
Vol:						
Prostate	1.00 (0.96–1.07)	0.943	1.02 (0.94–1.18)	0.950	1.02 (0.96–1.14)	1.000
Tumor	1.01 (0.95–1.12)	0.973	Not defined		Not defined	
Gleason score	1.87 (0.40–23.45)	0.767	1.21 (0.18–not defined)	0.867	1.73 (less than 0.01–702.2)	1.000
Pathological stage (III–IV/ I–II)	1.37 (0.07–82.02)	1.000	Not defined		Not defined	

*Predictive parameters not defined in metastatic PC or hormonal therapy and synchronous PC plus other cancer subgroups.

although dogs will remain a key point in the evolution of this field of research.^{29,30}

Another important question is how a dog that detects PC specific VOCs could be used in daily practice. The potential predictive power of this method is planned to be investigated in the future by studying patients with negative biopsies, increased serum PSA and adequate followup. At the same time proper followup of patients who undergo radical prostatectomy would indicate whether the dogs recognized positive samples before or after biochemical recurrence.

CONCLUSIONS

This study demonstrates that a rigorously trained dog could detect PC specific VOCs with high estimated sensitivity and specificity. Further studies

are needed to investigate the potential predictive value of this procedure to recognize PC.

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REFERENCES

- Siegel R, Naishadham D and Jemal A: Cancer statistics, 2012. *CA Cancer J Clin* 2012; **62**: 10.
- Thompson IM, Pauler DK, Goodman PJ et al: Prevalence of prostate cancer among men with a prostate-specific antigen level < or =4.0 ng per milliliter. *N Engl J Med* 2004; **350**: 2239.
- Schroder FH, Hugosson J, Roobol MJ et al: Screening and prostate-cancer mortality in a randomized European study. *N Engl J Med* 2009; **360**: 1320.
- Catalona WJ, Smith DS and Ornstein DK: Prostate cancer detection in men with serum PSA concentrations of 2.6 to 4.0 ng/mL and benign prostate examination. Enhancement of specificity with free PSA measurements. *JAMA* 1997; **277**: 1452.
- Schroder FH, van der Crujisen-Koeter I, de Koning HJ et al: Prostate cancer detection at low prostate specific antigen. *J Urol* 2000; **163**: 806.
- Anastasiadis A, Zapala L, Cordeiro E et al: Complications of prostate biopsy. *Expert Rev Anticancer Ther* 2013; **13**: 829.
- Presti J Jr: Does the yield of prostate cancer biopsy and repeat biopsy justify the frequency of their use? *Nat Clin Pract Urol* 2008; **5**: 246.
- Quignon P, Rimbault M, Robin S et al: Genetics of canine olfaction and receptor diversity. *Mamm Genome* 2012; **23**: 132.
- Pauling L, Robinson AB, Teranishi R et al: Quantitative analysis of urine vapor and breath by gas-liquid partition chromatography. *Proc Natl Acad Sci U S A* 1971; **68**: 2374.
- Lippi G and Cervellin G: Canine olfactory detection of cancer versus laboratory testing: myth or opportunity? *Clin Chem Lab Med* 2012; **50**: 435.
- Williams H and Pembroke A: Sniffer dogs in the melanoma clinic? *Lancet* 1989; **1**: 734.
- Church J and Williams H: Another sniffer dog for the clinic? *Lancet* 2001; **358**: 930.
- Willis CM, Church SM, Guest CM et al: Olfactory detection of human bladder cancer by dogs: proof of principle study. *BMJ* 2004; **329**: 712.
- Leahy M: Olfactory detection of human bladder cancer by dogs: cause or association? *BMJ* 2004; **329**: 1286.
- Welsh JS: Olfactory detection of human bladder cancer by dogs: another cancer detected by "pet scan". *BMJ* 2004; **329**: 1286.
- McCulloch M, Jezierski T, Broffman M et al: Diagnostic accuracy of canine scent detection in early- and late-stage lung and breast cancers. *Integr Cancer Ther* 2006; **5**: 30.
- Campbell LF, Farmery L, George SM et al: Canine olfactory detection of malignant melanoma. *BMJ Case Rep*, October 14, 2013.
- Horvath G, Jarverud GA, Jarverud S et al: Human ovarian carcinomas detected by specific odor. *Integr Cancer Ther* 2008; **7**: 76.
- Horvath G, Andersson H and Nemes S: Cancer odor in the blood of ovarian cancer patients: a retrospective study of detection by dogs during treatment, 3 and 6 months afterward. *BMC Cancer* 2013; **13**: 396.
- Bomers MK, van Agtmael MA, Luik H et al: Using a dog's superior olfactory sensitivity to identify *Clostridium difficile* in stools and patients: proof of principle study. *BMJ* 2012; **345**: e7396.
- Gordon RT, Schatz CB, Myers LJ et al: The use of canines in the detection of human cancers. *J Altern Complement Med* 2008; **14**: 61.
- Cornu JN, Cancel-Tassin G, Ondet V et al: Olfactory detection of prostate cancer by dogs sniffing urine: a step forward in early diagnosis. *Eur Urol* 2011; **59**: 197.
- Taverna G, Maffezzini M, Benetti A et al: A single injection of lidocaine as local anesthesia for ultrasound guided needle biopsy of the prostate. *J Urol* 2002; **167**: 222.
- Djavan B, Ravery V, Zlotta A et al: Prospective evaluation of prostate cancer detected on biopsies 1, 2, 3 and 4: when should we stop? *J Urol* 2001; **166**: 1679.
- Ploussard G, Nicolaiew N, Marchand C et al: Risk of repeat biopsy and prostate cancer detection after an initial extended negative biopsy: longitudinal follow-up from a prospective trial. *BJU Int* 2013; **111**: 988.
- Mkinen T, Auvinen A, Hakama M et al: Acceptability and complications of prostate biopsy in population-based PSA screening versus routine clinical practice: a prospective, controlled study. *Urology* 2002; **60**: 846.
- Loran OB, Veliev EI, Sokolov EA et al: Rectourethral fistula after repeat transrectal prostate biopsy. *Urology* 2013; **82**: e18.
- Loeb S, Vellekoop A, Ahmed HU et al: Systematic review of complications of prostate biopsy. *Eur Urol* 2013; **64**: 876.
- Roine A, Veskimäe E, Tuokko A et al: Detection of prostate cancer by an electronic nose: a proof of principle study. *J Urol* 2014; **192**: 230.
- Asimakopoulos AD, Del Fabbro D, Miano R et al: Prostate cancer diagnosis through electronic nose in the urine headspace setting: a pilot study. *Prostate Cancer Prostatic Dis* 2014; **17**: 206.