Olfactory detection of cancer by trained sniffer dogs: A systematic review of the literature

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Abstract

Early diagnosis of cancer using effective screening methods is crucial for successful treatment. Recently, much attention has been given to the use of odors emitted in the form of volatile organic compounds as diagnostic biomarkers. Studies on special training of dogs to detect different cancers using various odor samples (breath, urine, cancer tissue) have provided promising results. This systematic review highlights the scientific reports testing canine olfaction to detect cancer, dividing them according to the cancer's primary site. Several lines of evidence suggest that dogs may play a critical role in cancer research and diagnosis, eventually be major contributors to a reduction in mortality for certain cancers. Future directions that this field of research should take include efforts to overcome some methodological weaknesses and a certain heterogeneity of performance found across the different studies. Finding adequate responses to the challenges that lie ahead requires also a clear disclosure of what chemical compounds dogs respond to and the quantity of these compounds. Finally, the welfare of dogs involved in these practices should be considered.

Introduction

Cancer is the second leading cause of mortality in EU member states after diseases of the circulatory system, accounting for 25.8% of all deaths in 2012 (Eurostat, 2016). Lung cancer is still by far the most common cause of death from cancer among men (26.0%), followed by colorectal cancer (11.5%) and prostate cancer (10.2%). Breast cancer is the leading cause of death among women (16.3%), followed by lung cancer (14.2%) and colorectal cancer (12.3%) (OECD/European Union, 2014). Ovarian cancer is the sixth most common cause of cancer death in Europe for women, and the 12th most common cause of cancer death overall (Ferlay et al., 2013). Estimated incidence and mortality from malignant melanoma of skin in both sexes are 82,075 and 15,724, respectively (Ferlay et al., 2013).
For many cancer types, early diagnosis could reduce mortality (Yu et al., 2012). Although cancer screening offers the promise of early detection, substantial controversy exists concerning the benefits of common screening procedures, such as mammography, colonoscopy, and prostate-specific antigen (PSA) measurements

(Olsen and Gotzsche, 2001; Etzioni et al., 2003; Woolf, 2001; Lam et al., 2014). In fact, most conventional diagnostic techniques, including X-ray, blood tests, ultrasonography, and magnetic resonance imaging, give only limited information about the presence, size, and location of the lesions (Haick et al., 2014), and some inconsistencies have been reported between the tests (Yu et al., 2012; Sun et al., 2016). Therefore, to finally determine the cancer, a biopsy is often necessary which is invasive, expensive, and at risk of potential morbidity, for example, due to bleeding (Wu et al., 2011). By contrast, a desirable screening method should be noninvasive, painless, inexpensive, and easily accessible to a large number of patients. In addition to all, it should be reliable and allow diagnosis of an early-stage cancer (Buszewski et al., 2012). Currently, intensive studies are being carried out to identify compounds that could be markers of cancer (Libardoni et al., 2006, Miekisch et al., 2004) and could eventually support, or even replace, traditional screening methods. Based on gas chromatography and mass spectrometry (GC-MS) analyses, 1,840 volatile organic compounds (VOCs), so called the "human volatilome," were recently identified as candidate cancer-specific substances in breath, urine, tissue, human breast milk, and/or blood samples from cancer patients (Shirasu and Touhara, 2011; de Lacy Costello et al., 2014). The principle behind the VOC test lies in the fact that vola- tiles reflect the condition of the cells at the locations of disease (Sun et al., 2016). Cancer pathophysiology causes metabolic changes that lead to the alteration of VOC compositions and concentrations (Haick et al., 2014), ultimately generating a cancer-VOC profile. Once produced, the cancer-specific VOCs diffuse throughout the tissue and can be further released into the bloodstream and then excreted into the body fluids and exchanged into the breath (Haick et al., 2014). VOCs are usually present in biological samples at very low concentrations, so it is necessary to enrich them before they can be analyzed using the aforementioned methods. For this reason, among other promising techniques of screening based on emitted odors, such as selected ion flow tube MS (Abbott et al., 2003; Spanel et al., 1996), proton-transfer MS (Warneke et al., 1996; Hansel et al., 1995), and sensor technology (an electronic nose, e-nose) (Gasparri et al., 2016; D'Amico et al., 2010; Cho et al., 2006), the use of trained sniffer dogs for diagnostic purposes is marching on as an unconventional method for VOC biodetection (Elliker et al., 2014). Olfaction is a dog's primary special sense, being a thousand times more sensitive than that of humans (Flanders, 2011). Dogs are able to sniff out about half-a-million odorous compounds at trace concentrations, which are imperceptible to a human nose (Buszewski et al., 2012). This is largely due to anatomical, physiological, and genetic characteristics of these macrosmatic animals, such as a uniquely extended olfactory cortex, the area of the olfactory epithelium in the nasal

cavity, that is particularly extended due to the presence of the turbinate bones inside the nasal cavity, the shape of canine nostrils, which ensures that sufficient odor molecules in the air flow enter the nasal cavity, the extremely high number of olfactory receptors, as well as the proportion of active/ inactive genes of the olfactory receptor proteins (Buszewski et al., 2012). The acuity of the sense of smell, combined with the ability to learn by operant conditioning, makes dogs excellent biodetectors for different kinds of purposes, including tracking (Hepper and Wells, 2005), detection of drugs (Maejima et al., 2007) or explosives (Gazit and Terkel, 2003), finding human victims of disasters (Lit and Crawford, 2006), or searching for human remains (Fenton, 1992).

The ability of dogs to detect cancer in humans based on a specific odor was hypothesized for the first time by Williams and Pembroke (1989), followed by Church and Williams (2001), more than 1 decade later. These 2 reports stimulated further investigations on the use of canine's olfactory prowess to detect the presence of chemical markers of cancer, which showed how these animals, after appropriate training, may be able to discriminate breath, urine, feces of tumor tissue samples of patients with cancer (e.g., lung, breast, prostate, skin, and ovarian cancers) from respective samples taken from healthy volunteers achieving very good accuracy (>80%). Nevertheless, biological samples may contain hundreds of VOCs, with low concentrations, and therefore, it is challenging to elucidate which VOCs are cancer markers that dogs perceive and respond to. Finally, to ensure the highest ethical standards, attention should be given to issues related to the welfare of sniffer dogs, including physiological and behavioral signs of stress, which could also have an effect on canine outputs both in terms of motivation to work and accuracy.

To understand the evidence base of cancer detection using sniffer dogs, this study reviews key studies that have been published in scientific journals to date on this topic, grouping them together according to the cancer's primary site of occurrence. We screened titles and retained those that were described as a study testing a canine's ability to detect human cancer. As only very few studies have been published, this was the only eligibility requirement applied. We conducted a systematic search of electronic databases, including PubMed and Embase, abstract proceedings of major scientific meetings, and bibliographies of all eligible studies. We selected 17 full-text articles, 1 involving melanoma, 6 involving urologic cancers (4 on prostate cancer and 2 on bladder cancer), 2 involving breast cancer, 3 involving ovarian cancer, and 4 involving lung cancer. One study on lung cancer is in progress. Anecdotal and case reports are described only briefly. The appealing perspectives and the potential drawbacks are also highlighted.

Disease-specific studies

Skin cancer

Melanoma is the first type of cancer from which investigation on canine olfactory detection of human malignancy was initiated, following the brief note written by Williams and Pembroke in 1989 (Table 1). They reported a woman who was encouraged to get a skin lesion on her leg examined by her dog constantly sniffing at it. The lesion was excised and diagnosed as a malignant melanoma by histological examination. Chemical markers for melanoma were subsequently found in body fluids (Wakamatsu and Ito, 1990; Kelley et al., 1998; Kelley et al., 2001), suggesting that volatile compounds might be released from melanoma cells on the skin surface in amounts sufficient to allow lesion localization by the canine olfactory system (Pickel et al., 2004). However, despite the potential role of well-trained dogs, little exploration of this area has occurred. In 2001, anecdotal observations similar to those of Williams and Pembroke (1989) were reported by Church and Williams (2001), who described the case of a men who developed a patch of eczema on the outer side of his left thigh and had the lesion excised after his pet dog began to persistently show interest in it. The histological assessment revealed a basal cell carcinoma. In 2004, Pickel et al. carried out more systematic research on melanoma patients. Two dogs were used, both highly trained AKC champions and having titles that are involved with olfactory performance, including utility dog excellent (UDX) and master hunter (MH). One was also certified by the State of Florida as an explosives detection dog. These dogs were purpose-trained to identify the cancer odor using human melanoma tissues either in area trials, box testing, healthy volunteers, or in a small number of actual patients with known or suspected melanoma. The 2 dogs were trained in 11 to 200 area trials, during which they were asked to either retrieve or search for a target tube containing a mixture of basal, squamous, and melanoma tissue samples. Given the good ability showed by the dogs in this phase, the scientists went through the box testing phase. The box data (6 trials completed) demonstrated that the dogs could be trained to localize malignant samples placed in 1 of 10 wells, with the remaining wells being filled with distractor odors likely to be encountered in a medical setting (e.g., adhesive bandages, gauze, latex gloves, rolls of tape). The handler was blind as to melanoma tissue sample location. Five blind test trials were conducted with each dog to assess the ability of the animals to locate melanoma tissue samples “planted” on healthy volunteers. These trials were interspersed with, respectively for dogs A and B, 64 and 68 non-

blind training trials and 26 and 17 blind blank trials. On each trial, the melanoma target was at 1 location and either 9 or 10 distractor stimuli were present. For blind blank trials, varying numbers of empty bandages were attached to different parts of the body. The dogs were also able to locate melanoma, despite a mix of distractor chemicals given off by the living human body. Finally, in actual double-blind patient trials (n 7), 8 to 30 adhesive bandages were placed in several locations on the patient's body, including a bandage over the target lesion. Both dogs missed 1 patient: 1 dog was correct in 6/7 and the other in 3/4 patients. The likelihood of the dogs selecting melanoma by chance alone was reported by Pickel et al. (2004) to be only 10-7.1 to 10-7.3. Moreover, no false-positive responding was recorded by both dogs in blind blank trials in which only empty bandages were planted on healthy volunteers. This study provided the first evidence that there are volatile cues released from melanoma tissue that allow lesion localization by the canine olfactory system.

Nine years after this prospective study, a case report was published (Campbell et al., 2013) of a 75-year-old man who presented after his pet dog licked persistently at an asymptomatic lesion behind his right ear. Examination revealed a nodular lesion in the postauricular sulcus, which was confirmed by histology to be a malignant melanoma. To our knowledge, no other literature exists on a dog's use to detect this type of cancer, and there are no data from rigorously controlled experiments. Having said this, a recent study using SPME GC-MS coupled with a single-stranded DNA-coated nanotube sensor revealed some VOC alterations in melanoma cells, compared to healthy melanocytes and identified 2 compounds (dimethyldisulfide and dimethyltrisulfide) that are unique to melanoma cells (Kwak et al., 2013; Wang et al., 2016). This information could be used for future training practices of dogs in this field.

Urologic cancer

Serum PSA test (at a cutoff of 4 ng/mL), in conjunction with a digital rectal examination, is currently the standard method for prostate cancer screening. Although PSA testing has increased prostate cancer detection, it lacks specificity and accuracy (Catalona et al., 1991). The reason is that PSA levels increase in response to both cancer and noncancerous conditions, such as prostatitis and benign prostatic hyperplasia. Many patients with increased PSA values undergo biopsy sampling (Schroder et al., 2009) although this procedure is invasive, offers a low level of accuracy (e.g., only 30% detection rate at the first biopsy), and is prone to various complications, including sepsis and death (Anastadiasis et al., 2006; Presti, 2007). Urine or blood biomarkers other than PSA have thus been proposed in the

last decade, but none of them is currently widely used, mostly because they still need to be validated in bigger trials and rigorously tested (Tuma, 2010). In 2011, Cornu et al. checked the ability of a specially trained dog to detect prostate cancer by sniffing urine in a double-blind study (Table 2). After a 16-month training phase, during which the trainer worked with the dog 5 days/week using a total of 42 samples (26 cancerous and 16 healthy), the dog underwent a double-blind testing phase, consisting of 33 consecutive runs in which it was presented with 6 samples (5 controls and 1 cancer). During each run, the cancer urine was 1 of 33 selected cancer samples, as determined by prostate biopsy, and the 5 control urines were randomly selected among 33 controls. The olfactory test reported sensitivity and specificity rates of 0.91.

Three years later, Elliker et al. (2014) published a study describing training of 10 dogs of different breeds (4 females and 6 males, age 1-11 years old) to detect prostate cancer in urine samples. In total, 50 prostate cancer samples and 67 control samples from different age-matched individuals were collected and used during the dog training period. Controls were 57 men with benign prostatic hyperplasia and 10 healthy men without clinical symptoms. Fifty-two controls had PSA levels <0.5 ng/mL, 2 had PSA < 1.5 ng/mL, and 7 had PSA between 2.2 and 11.6 ng/mL. Thirteen of these controls, including all with PSA >2.2 ng/mL, had previously undergone prostate biopsy with negative results. A total of 31 prostate cancer and 93 control samples were used in 3 rigorous double-blind tests conducted with the 2 best performing dogs in the training phase. The Labrador dog was involved in tests 1 and 2, whereas the border collie was involved only in test 3. Fifteen arrays each containing 1 CaP sample and 3 controls were presented, 16 in test 3 following the same protocol. During test 1, the dog correctly indicated the prostate cancer sample for 2/15 arrays, indicating that the dog was not discriminating samples based on a cancer odor. In test 2, the same dog correctly identified the position of the cancer sample in 2/16 arrays. In each of these tests, the sensitivity for this dog was 13% and specificity 71%. In test 3, the dog correctly detected the cancer sample in 4/16 arrays, indicating that dog was also not discriminating the samples based on a cancer odor. The sensitivity and specificity for this dog B were also extremely low: 25% and 75%, respectively. Moreover, the 2 dogs did not make similar choices of urine samples in tests 2 and 3, in which samples from the same urine donors were presented in different orders. This could suggest that each dog was using different odor cues to select the samples. A large series of patients with prostate cancer of different stages and grades (n 362) versus a heterogeneous control group with nonneoplastic disease or nonprostatic tumor (n 540) was enrolled in the study of

Taverna et al. (2015). Two explosion detection dogs were trained to identify prostate cancer in urine samples under double-blind conditions. A total of 200 urine specimens from the prostate cancer group and 230 from the control group were analyzed during the training phase. Eleven subjects in the case group had synchronous primary prostate cancer and another different tumor. The control group comprised 122 females, 50 of whom were healthy, nonpregnant volunteers and 72 were patients with a nonneoplastic disease (i.e., 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). For dog 1, sensitivity was 100% (95% confidence interval [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). Because prostate cancer generally progresses slowly, older people are at elevated risk for undetected prostate cancer (Carter, 2011). This demographic pattern could have contributed to worsen the specificity of both dogs because we cannot completely exclude that these participants were not tumor free. It is worth mentioning that the dogs always identified the urine samples from patients on androgen deprivation therapy as positive regardless of PSA or imaging stage. Moreover, the dogs never indicated a finding for patients with another neoplasm, supporting the specificity of VOCs and the selective capacity of canine olfaction. In a further double-blind study (Taverna et al., 2015), the same 2 highly trained dogs were able to detect biochemical recurrence among 114 men after undergoing radical prostatectomy for prostate cancer. Preoperatively, both dogs recognized positive urine samples with 100% detection rate. Interestingly, 45 days after radical prostatectomy, canine olfactory performance varied with the men's levels of serum PSA. In fact, neither dogs signaled the urine samples of the 104 men with a serum PSA level <0.01 ng/mL, while they both identified correctly the samples from 2 of 6 men with PSA levels >0.01 ng/mL and <0.2 ng/mL (detection rate 33.3%) and each of the 4 men with persistent disease (PSA levels >1 ng/mL, detection rate 100%). During the successive postoperative follow-up, 9 of 110 patients (8.1%) had recurrence and both dogs were able to detect prostate cancer's VOCs in the urine samples of 7 of these 9 patients with progressive biochemical relapse (detection rate 77.7%). This study indicates that if cancer persists after radical prostatectomy, it continues to produce specific VOCs, that may be recognized by trained dogs. Unfortunately, to date, the literature on prostate cancer- specific VOCs is still scarce

and provides controversial results (Smith et al., 2010; Peng et al, 2010; Sreekumar et al., 2009; Jentzmik et al., 2010; Wu et al., 2011; Khalid et al., 2015).
Cystoscopy with biopsy is the "gold standard" for bladder cancer detection, but it is expensive, inconvenient, and invasive. Urine cytology is the most widely applied of the noninvasive alternatives available. However, despite its high specificity (90%-98%), the sensitivity is low (20%-50%). Various mass screening options have been considered, including the hematuria dipstick, NMP22, or UroVysion (Babjuk et al., 2011). However, these biomarkers, as well as urine cytology, lack sensitivity (Abogunrin et al., 2012). Currently, there is some evidence to support the hypothesis that bladder cancer is associated with the presence of specific VOCs in the gas emitted from urine samples, which could be detected by dogs (Table 2). Willis et al. (2004) designed an experimental study to determine whether dogs can be trained to recognize an urine odor, or a combination of odors, peculiar of bladder cancer, and distinct from those associated with the secondary effects of neoplasia (e.g., bleeding, inflammation, infection, and necrosis) which may also be present in a variety of nonmalignant conditions of the urinary tract or elsewhere in the body. Six dogs of different (unspecified) breeds and ages, none of which had been previously trained for search or scent discrimination tasks, completed a 7-month period of training to discriminate between urine from 36 bladder cancer patients and those from 108 controls, either healthy or with benign disease. Both air-dried and liquid urine (previously frozen at 400C for up to 5 months and thawed) were tested separately. Dogs, that had to detect bladder cancer by choosing 1 positive urine placed randomly among 6 controls on single-blind experiments, provided evidence that they had this ability (mean success rate of 41% for dried and liquid urine tests combined compared with 14% expected by chance alone). Moreover, logistic regression analysis suggested that this ability was independent of other chemical characteristics of the urine, such as blood, leukocytes, protein, ketones, bilirubin, nitrites, and urobilinogen, which were not significant confounders.

More recently, the same group demonstrated that volatile organic compounds specific to bladder cancer are present in urine headspace, subsequently showing that up to 70% and 73% of tumors could be correctly identified using an electronic (Weber et al., 2011) or a dog's (Willis et al., 2011) nose, respectively. The canine olfactory ability study (Willis et al., 2011) was conducted with 4 purpose-trained dogs, again of unspecified breeds and ages, in a double-blind test. Previously frozen urine samples from 210 participants were divided into a case group (n 30, people with confirmed transitional cell carcinoma) and the following 3 control groups: (1) n 61, healthy people younger than 33 years; (2) n 65, people with altered urine due

to noncancerous and non- urological disease; (3) n 54, people of different ages with benign urological diseases, for example, benign prostatic hyperplasia. The 4 dogs obtained a sensitivity of 64%, with a maximum of 73% given by the best performing dog. Specificity was 92% when the dogs had to distinguish between urines from the case group and those from control group 1, whereas it decreased down to 56% with control urine taken from elder subjects with benign urological diseases. It is conceivable that, as a consequence of age and noncancerous urological conditions, odor patterns originated that may have confounded the dogs, thus reducing their efficiency as cancer bio- detectors. This possibility further strengthens the notion that clear identification of VOCs associated with cancer is warranted. Unfortunately, the identity of the VOCs that contribute to the bladder cancer biomarker profile has yet to be determined (Khalid et al., 2013).

Colorectal cancer

Despite rapid progress in examination technology and therapy, colorectal cancer is a worldwide leading cause of cancer death (Boyle, 2008). Early detection and early treatment are critical for the successful treatment of cancer and are excellent means for reducing both the economic burden and mortality (Sonoda et al., 2011). Although the fecal occult blood test is currently the most economic and noninvasive screening method for colorectal cancer, its positive predictive value is approximately 10%. To our knowledge, there is only 1 study investigating the canine scent detection of colorectal cancer (Sonoda et al., 2011). A specially trained dog from the St Sugar Cancer Sniffing Dog Training Center (Chiba, Japan) participated in this study. The experiments were conducted on exhaled breath and watery stool samples using 5 stations positioned on the floor, 1 station contained a cancer sample and 4 stations contained control samples from volunteers without cancer. In the preliminary training steps, the dog learned to detect different types of cancer in patient breath samples: esophageal cancer, breast cancer, lung cancer, gastric cancer, pancreatic cancer, hepatocellular carcinoma, cholangiocarcinoma, colorectal cancer, prostate cancer, uterine cancer, ovarian cancer, and bladder cancer. During testing, only samples obtained from patients with colorectal cancer were included. Thirty-three cancer samples and 132 control samples were used for breath tests, whereas 37 cancer samples and 148 control samples were used for watery stool tests. Each sample was only used once during testing. We do not know whether and what type of blinding was used. The sensitivity of canine scent detection of breath samples was 0.91 and the specificity was 0.99. The sensitivity using stool samples was 0.97 and the specificity was 0.99. Moreover, the accuracy of

canine scent detection was high even for early cancer, and this might suggest that disease-specific VOCs occur early in the pathogenesis of colorectal cancer. Fifteen VOCs have been identified as candidate substances as potential biomarkers of colorectal cancer, including decanal, nonanal, cyclo- hexane, 4-methyloctane, which are all hydrocarbons and aldehydes that could be due to oxidative stress.

Breast cancer

As for most tumors, regular screening to detect breast cancer early is recommended. However, this disease has a one to several years long period of growth before reaching a size when a woman experiences symptom or when it is detected during a physical examination, such as mammography. In fact, although mammography screening might reduce breast cancer mortality (Gill et al., 2004) and allow treatment with lower toxicity (Barth et al. 2005), results across studies are still controversial (Freedman et al., 2004). Limited evidence exists on identification of biomarkers in blood samples, as an alternate diagnostic approach for breast cancer, due to a lack of assay standardization and poor quality of the scientific literature (Corradini and Daidone, 2004). To date, only 2 studies (McCulloch et al., 2006; Gordon et al., 2008) have evaluated canine scent detection of breast cancer (Table 3), and they provide controversial results. McCulloch and colleagues (2006) trained 5 dogs to investigate whether they could discriminate between either lung or breast cancer patients from healthy controls by smelling their breath samples. Five dogs, chosen from a total of 13, were provided by local owners. For both training and testing, 5 sample stations were positioned on the floor, containing 1 cancer patient breath sample and 4 control breath samples. Overall, 31 patients (1 man and 30 women) with breast cancer and 83 volunteers with no prior cancer history were recruited. During the testing phase, all breath samples sniffed by dogs were from new subjects, not previously encountered during training. Each dog had the opportunity to sniff breath samples from each subject and each control. Sensitivity was 88% and specificity was 98% across the different stages of disease, with no statistically significant difference between the 5 dogs. In the study of Gordon et al. (2008), 6 dogs of unspecified characteristics were trained by their owners to detect breast cancer in urine samples. In the testing phase, a total of 18 runs were carried out using different configurations of the samples and each dog had to discriminate the urine from 1 patient with breast cancer from among 6 other age- and sex-matched healthy volunteers. The authors obtained disappointing results, as only 2 of 6 dogs performed better than chance in specificity and none were more sensitive than chance. Some studies have reported VOCs in the breath of patients bearing

breast cancer lesions, apparently resulting from increased oxidative stress and cytochrome p450 induction (He et al., 2014; Lavra et al., 2015). However, the biological mechanism of produc- tion of volatile biomarkers of breast cancer remains speculative.

Ovarian cancer

Most women with ovarian cancer are diagnosed at an advanced stage, compared with other cancers associated with women (e.g., endometrial cancers, breast cancers, cervical cancers) which are more frequently diagnosed with stage-I disease (Menon and Jacobs, 2000). Potential screening tests for ovarian cancer have not yet been shown to reduce mortality, which is particularly high among advanced-stage ovarian cancer patients (only 21% survive 5 years after initial diagnosis) (Berek et al., 2003). The best prospects for further improvement in ovarian cancer survival reside in early diagnosis. Results obtained by Horvath et al. (2008, 2010, 2013) suggest that the specific odor of ovarian carcinoma can be detected by trained dogs for screening, early diagnosis, and differential diagnosis of different malignant disease (Table 4). In 1 study (2008), the authors trained a dog (Hanna) twice a week for 12 months to discriminate 31 different histopathological types and grades of ovarian carcinomas from healthy control tissues and from other gynecological tumor samples, such as cervical, vulvar, and endometrial carcinomas, which were taken from the same tumor bank and treated identically to the ovarian carcinomas. Samples of small bowel, muscle, fat, and 2 pieces of healthy postmenopausal ovary were set out as controls. In a double-blind test, targets were ovarian carcinoma samples from 20 different individuals, which had not been exposed to the canine in the training tests. Each test consisted of 10 runs, where each run included 2 target samples and 8 controls. In this double-blind test series, sensitivity was 100% and specificity was 97.5%. The dog was able to distinguish between healthy ovarian tissues and ovarian carcinomas, reinforcing the hypothesis that a shift from organ-specific to carcinoma-specific odor occurs due to biological changes during carcinogenesis. In a second study, Horvath et al. (2010) examined whether this cancer-specific odor could also be found in the blood. In this instance, 2 dogs were used: Hanna and Lotti. Hanna was trained over a 9-month period to detect ovarian cancer in blood samples from selected patients, whereas the newly involved dog was trained during the same time period to detect ovarian carcinoma samples consisting of different histopathological types of various grades and stages. Abdominal fat and myomas and healthy postmenopausal ovarium samples were used as controls. Control plasma samples were collected from young, healthy

females and from patients with cervical, vulvar, and endometrial carcinomas for discrimination. Tissue and blood samples used during the training period were not used in the tests. The testing phase was conducted according to the double-blind principle, as both test leader and handler were blinded to the location of the target sample. The testing consisted of 4 sections per dog, 2 on day 1 and 2 on day 2, according to the following schedule: day 1dsection 1, Lotti sniffed tissues; session 2, Hanna sniffed blood; day 2dsection 3, Lotti sniffed blood; section 4, Hanna sniffed tissues. Each section was composed of 10 runs with 6 boxes, 5 containing control materials and 1 containing the target material. For
Lotti, the sensitivity was 100% and specificity was 96% with both tissue and plasma samples. This means that the dog showed a similar level of accuracy even when trained using only ovarian carcinoma samples. For Hanna, the sensitivity and specificity were 100 % in the blood samples' section. In section 4, the sensitivity was again 100%, whereas specificity decreased to 94% compared to section 3. Thus, her performance with tissue from carcinomas decreased slightly after ceasing training with carcinoma tissue, although it still remained high. This study confirms results from previous work (Horvath et al., 2008) and suggested that dogs might be able to successfully detect ovarian carcinomas in the blood and discriminate the odor of ovarian cancer from that of other gynecological malignancies. Unfortunately, it is not clear whether the samples sniffed by the 2 dogs within each section came from the same patients. This information would have strengthened the results. However, this result suggests that it may be possible to use blood samples for early diagnosis and differential diagnosis of ovarian carcinoma instead of tissue biopsies, which are invasive and unsuitable for routine screening (Gould et al., 2015). In a further study, Horvath et al (2013) involved the dogs Hanna and Lotti to investigate whether and how the odor of ovarian carcinoma in the blood might be changed by primary surgery and chemotherapy treatment, through the influence on tumor status. Again, the blood samples used during the training period were not used in the tests. Patients with different life expectancies, based on their initial diagnosis, were recruited considering the reported clinical complete remission before the sixth (final) scheduled chemotherapy course. Blood samples taken before the sixth course of chemo- therapy were used as test material in series I, whereas samples taken 3 to 6 months after the final treatment were used in series 2. Patients in series I were divided into 3 groups: group A, with 3 years of relapse-free survival (CA-125<35 U/mL); group B, who had relapsed within 6 months; and group C, patients who had relapsed between 1 and 2 years after treatment. Samples in series II were all taken from the same patients selected in series I, except for group A because their blood was not present in the blood bank used in the study. Control samples were collected

from female volunteers, who felt healthy, were not pregnant, and were free of gynecological disease. Blood samples from different individuals with CA-125 values > 200 U/mL (CA-125 > 500 U/mL is a poor prognostic indicator for ovarian carcinoma survival, Nguyen et al., 2013), independently of the clinicopathological status, were randomly selected from the biobank as reference material in both series. Tests were conducted in a double-blind fashion. Series I covered 4 days, whereas series II covered 2 days. Ten runs were performed on each day (11 runs on 1 day in series I). In this case, each run included 7 boxes, 5 of which had control materials, 1 a target sample, and 1 a reference sample. Thus, the probability of the dog finding the target sample and ignoring the controls by chance only was 1/6. In series I, Hanna's tests had a sensitivity of 97% and a specificity of 100%, whereas Lotti's tests had a sensitivity of 97% and a specificity of 99%. The dogs' accuracy was lower compared to that observed in the previous studies (Horvath et al., 2008, 2010). The authors suggest that it is likely that surgery combined with 5 chemotherapy courses reduced the number of cancer cells, therefore decreasing the odorant molecules in blood. However, the dogs still had a generally high sensitivity and specificity. This result could provide useful indications to doctors who often do not know how many patients residual cancer cells after complete clinical remission have is declared, and it is unknown whether the final treatment after this will kill any remaining cells (Horvath et al., 2013). For series II, where dogs sniffed blood samples taken 3 months after the final treatment, Lotti's tests had a sensitivity of 60% and a specificity of 96%, whereas Hanna's tests had a sensitivity of 80% and a specificity of 94%. In 6-month test samples, Lotti's sensitivity was again 60% while specificity was 90%, whereas Hanna's tests had a sensitivity of 100% and a specificity of 94%. The dogs were still able to indicate small numbers of living cancer cells with high accuracy in a large group of ovarian cancer patients, and despite the very low limit of detection, they were able to signal probable recurrences that would not be diagnosed by other methods for another 2-3 years (Horvath et al., 2013).

Preliminary results obtained by conducting chemical analysis using GC-MS and nanoarray (Amal et al., 2015) reported higher levels of decanal, nonanal, styrene, 2-butanone, and hexadecane in the exhaled breath of patients with ovarian cancer.

Lung cancer

The 5-year survival rate for lung is 54% for cases diagnosed when the disease is still localized, but only 15% of lung cancers are identified at this early stage (American Cancer Society, 2014). Despite early diagnosis is essential for increasing the survival

rates, it remains a challenge (McCulloch et al, 2012). In the past decade, the analysis of exhaled breath has been suggested as a promising option for the early detection of lung cancer (Gasparri et al, 2016). The exhaled breath may be analyzed using GC-MS (Preti et al., 1998; Phillips et al., 2003), and gas sensor arrays (also known as electronic nose), which produces a characteristic fingerprint which can differentiate healthy controls from individuals affected by lung cancer and/or respiratory diseases such as asthma and chronic obstructive pulmonary disease (COPD) (Di Natale et al., 2014). In parallel, researchers worldwide are currently working to assess validity and reliability of canine olfaction to detect lung cancer odor (Table 5). In 2 studies (McCulloch et al, 2006; Ehmann et al., 2012), dogs were able to distinguish lung cancer patient breath samples from controls. Over all articles published so far on canine detection of lung cancer in humans on the basis of breath odor, the mean sensitivity was 78%, whereas the mean specificity was 71.5% (Jezierski et al., 2015). McCulloch et al. (2006) reported that 5 ordinary household dogs were trained to distinguish by scent exhaled breath samples of 55 non-small-cell lung cancer (NSCLC) patients from those of 83 healthy controls in double-blind tests. Cancer samples were classified into stages 1-4 of adenocarcinoma and stages 2-4 of squamous type. The 5 dogs gave overall sensitivity and specificity of 99%, and no significant statistical difference was found in accuracy among them as well as across all 4 stages of the disease. This last result seems particularly relevant and should be deepened, as it suggests that dogs might discriminate early preclinical stages of cancer, that is the ultimate goal of using dogs for cancer screening. Another contribution to the clinical appraisal of breath analysis as a diagnostic approach to identify lung cancer comes from Ehmann et al. (2012), who published a study in which 4 trained family dogs detected lung cancer with sensitivity of 90% and specificity of 72% in double-blind experiments. The participants were classified into the following 3 groups: group A (n 1/4 110), healthy; group B (n 1/4 60), lung cancer; group C (n 1/4 50), COPD. COPD is a chronic inflammatory condition, which is often associated with the development of lung cancer, particularly in smoking patients (Yao and Rahman, 2009). It has been shown that the level of exhaled biomarkers is altered in patients with COPD compared with healthy control subjects (D'amico et al., 2010; Dragonieri et al., 2009). Dogs were able to identify lung cancer among 4 healthy controls (test I), from COPD when tested among 4 patients with COPD (test II), and from 4 representatives of a mixed study population of COPD patients and healthy controls (test III). The overall sensitivity of the test was 71% and the specificity was 93%. The accuracy of the dog's indication was 100% for Union for International Cancer Control (UICC) stage I, 75% for UICC stages IIa and IIb, 94% for UICC stage IIIa, 75% for UICC stage IIIb, and 63% for UICC stage IV. The

accuracy of sniffer dogs did not favor advanced tumor stages, as it was lower with advanced tumor UICC stage IV. This pattern may be due to the presence of secondary lung tissue reactions (e.g., inflammation or necrosis). To shed light into this aspect, Mazzola et al. (2016) are developing a method for canine olfactory detection of human lung cancer in urine samples including a variety of nonmalignant respiratory disease controls (e.g., bronchitis, asthma, etc.) (Figure). Early findings from the study in progress suggest that dogs are likely to discriminate these benign organ-specific diseases from cancer correctly, as they tend to ignore novel samples from this group of patients, similar to when they smell samples from healthy controls. If this is confirmed, definite evidence would be provided that a specific lung cancer scent exists. As indicated by Ehmann et al. (2012), the interrater variability of the 4 dogs was moderate (k 1/4 0.436), with the best results obtained in test III (mixed population), and the worst in test I (lung cancer vs. healthy controls). In the attempt to improve estimation of accuracy, they also carried out a corporate dog decision analysis, where the "corporate dog decision" was defined by at least 3 dogs making the same decision. However, this arrangement did not significantly raise test scores, resulting in a sensitivity of 72% and a specificity of 94%. In the follow-up single-blind study of Walczak et al. (2012), the training was conducted with 6 dogs. Samples were collected, before any chemotherapy treatment, from 118 patients with lung cancer confirmed histopathologically, and from 305 healthy volunteers who provided a self-declaration of good health. The overall detection sensitivity and specificity of cancer samples in the working phase were 79% and 78%, respectively, at the probability of correct response by chance of 50%. The sensitivity decreased to approximately 50% when the probability of a correct response by chance in 1 single trial was 20%. In addition, when a new set of samples was tested for the first time (first trial), the detection sensitivity fell further (68% and 37% at 50% and 20% probability levels, respectively) although it was still significantly better than by chance alone. In a recent study, Amundsen et al. (2014) used the olfactory test in double-blind conditions to detect lung cancer in unselected patients with suspected lung cancer, achieving even lower sensitivity and specificity. They recruited 93 consecutive patients with suspected lung cancer, all benign or malignant lung disease; none were healthy at the time of inclusion. Patients testing negative for malignant lung disease were followed up for 3 years to detect any future occurrence of cancer. Confirmed diagnoses were categorized into 4 groups: (1) noncancer; (2) small-cell lung cancer (SCLC); (3) NSCLC; and (4) nonlung cancer (pulmonary carcinoid, mesothelioma, or lung metastasis from other primary neoplasms). Both exhaled breath and urine were collected from the subjects for the olfactory examination and tested separately. During testing, 4 dogs were presented

with both exhaled breath and urine samples from patients with either cancer or other lung diseases, including COPD and asthma. The number of cancer samples varied from 0 to 6 samples. In an interim analysis of the first 46 patients, which evaluated the olfactory breath test, sensitivity was 70% and 55.6% for NSCLC and for SCLC, respectively, whereas the specificity for both lung cancer types was only 8.3%. After intensive training of the dogs, breath samples from other patients were tested. The sensitivity for detection of NSCLC decreased to 60% and specificity increased to 33.3%, whereas for SCLC, the sensitivity increased to 100% and specificity increased to 33.3%. In other words, improvement was observed only for the SCLC group. Urine testing was performed after all the exhaled breath samples were tested and in the same manner as with the exhaled breath test. In a first urine test, overall sensitivity and specificity for NSCLC were 65.7% and 25%, respectively, and the overall sensitivity and specificity for SCLC were 90% and 25%, respectively. After the dogs underwent intensive training, a second urine test was performed. Sensitivity and specificity for NSCLC were 60% and 29.2%, respectively, and sensitivity and specificity for SCLC were 80% and 29.2%, respectively.

Over the last 40 years, there have been many studies aiming to characterize organic volatile compounds in exhaled breath from lung cancer patients (Dent et al., 2013). Among the VOCs detected, caprolactam and propanoic acid seem to be the most promising exhaled breath biomarkers for lung cancer (Wang et al., 2014).

Discussion

In this study, we present a comprehensive up-to-date overview of studies on cancer-sniffing dogs.

Our review identified 17 prospective studies focused on com- mon malignancies, such as skin, breast, prostate, bladder, ovarian, colorectal, and lung cancer. Despite the overall good diagnostic performance of dogs, we have identified major shortcomings in at least 13 studies reviewed that might have affected the results and conclusions. Pickel et al (2004), Cornu et al. (2011), Willis et al. (2004), Willis et al. (2011), and McCulloch et al. (2006) used a very small sample size during either training or testing which may have affected outcomes (Elliker et al., 2014) and resulted in low statistical power and overestimates of the dogs' observed ability (Button et al., 2013), respectively. In particular, Cornu et al. (2011) involved only 1 dog and 1 trainer. The results could have been different for another dog or trainer (Bomers et al., 2012).

In all studies reviewed, target and control samples used for testing was new to the dogs. This aspect is of particular relevance because dogs are able to detect, identify,

and memorize the odor of a particular person with high specificity (Marchal et al., 2016). However, the studies differed significantly with respect to sample types and storage methods. In the study by Cornu et al. (2011), the liquid urine samples were stored for an unspecified time period at 40C versus the 200C used by Gordon et al. (2008), Amundsen et al. (2014), and Elliker et al. (2014), respectively, for 5 months, 2- 4 weeks, and 1 day to 6 months, and the 400C for 5 months re- ported in the study of Willis et al. (2004). We cannot exclude the possibility that temperature- and time-dependent variations have occurred in VOC concentrations (Mochalski et al., 2015). Willis et al. (2004) used both dried and wet urine samples, and dried urine sample testing only achieved a 22% success rate compared to the 50% for liquid urine. This decline in performance by the dogs could be due to loss of volatile compounds during the drying process (Moser and McCulloch, 2010). In addition, there is no indication that the same canine was used for both types of samples. This would have helped in providing useful information on the handling and storage of urine samples for VOC analysis (Gould et al., 2015). Different techniques for storage of breath samples were also found among the studies. Breath samples were stored at room temperature for 2-4 weeks in the studies by Amundsen et al. (2014) and Ehmann et al. (2012) (in this last case for an unspecified time period). In the Willis et al. (2004) study, breath samples were instead stored under refrigeration at 40C until for an unspecified period of time before they were analyzed, whereas Gordon and colleagues (2008) stored this type of samples at -200C for 5 months, and then at 180C for an unspecified duration of time. Finally, in the study by McCulloch et al (2006), the breath samples were frozen at 400C for 1 year. In addition, the different storage conditions used, such as the type of container - glass or plastic or other storage container - and storage duration may have been very influential (Moser and McCulloch, 2010). Overall, the possible introduction of storage artifacts is an important potential confounding factor in the development of standard methodologies for sampling and analysis (Kang and Thomas, 2016), that might ultimately affect a dog's detection skills. It is therefore mandatory to find an optimal storing method and standardize the procedure, to preserve the samples' quality and permit more reliable comparison of outcomes across all studies.

Some of the studies reviewed showed a poor selection of case/ control groups, which were either insufficient, too variable, or not well matched. Pickel et al. (2004), for example, included only patients with known or suspected melanoma, who had been pre- selected by a dermatologist. This is a narrowly defined population which may have affected results and conclusions, which may not be generalizable. Moreover, this study lacked the appropriate tissue controls: the authors were training the dogs on human tissue and they never clearly documented the specificity

for cancer in any of their preclinical tests. In the study by McCulloch et al. (2006), only healthy volunteers were used as controls, whereas subjects with noncancerous conditions such as fibrocystic breast disease or mastitis were excluded. Dogs may have detected and responded to odors associated with cancer, such as inflammation, infection, or necrosis, rather than to cancer specifically. All these aspects may have resulted in overestimating the performance of the test. In the Walczak et al. (2012) study, all cancer and control sample donors were 25- to 70-year-old adults, but whether the 2 groups were given the same age distribution structure is not known. If older participants were underrepresented relative to younger volunteers, sampling would be biased, because lung cancer is highly more likely to affect older people. Moreover, although self-description as "healthy" is a common method for identifying healthy controls in case-control studies, a mismatch between individual's actual and perceived health conditions has been reported, which could seriously bias results (Ghorbani et al, 2015). A clear definition of healthy control should be provided, preferably with validation. This was not the case here. The ability to evaluate disease is heavily dependent on the accepted definition of "control subject." Without the proper definitions, the quality of data comes into question and may lead to misinterpretation of results (Boyton et al., 2004). When performing case-control studies in cancer, as in the case of other diseases, the healthy status should be objectively measured, not just self- reported, to provide true and accurate data (Ghorbani et al., 2015). However, we are aware that measuring health can be demanding and challenging due to the high costs, analytic complexity, and lack of good cancer screening tests. The lack of properly age-matched controls in the study of Willis et al. (2004) could have affected the results. In their experiment, 1 of 2 samples provided by the 2 oldest patients was always the target sample used in a testing run. As already noticed by Moser and McCulloch (2010), if during training the dogs learned to indicate the sample from the oldest subject, instead of the cancerous sample, they could still have completed successful testing runs >50% of the time without ever detecting the cancerous sample. Potential hormonal bias may have affected test results in some studies. Taverna et al. (2015) used female controls in the initial phases of the training procedure to exclude that no specific prostate VOCs could confuse the 2 dogs. In the study by Horvath et al. (2010), some blood samples from male individuals were also included. Although the authors of both studies reported no observable sex influence on the dogs' target identification, hormonal differences deriving from the use of controls of the opposite sex could have helped the dogs to differentiate males from females, possibly increasing the probability of success (Willis et al., 2004). In the study by Amundsen et al. (2014), the dogs did not sufficiently discriminate

between organ-specific malignant and benign conditions, and the rather low sensitivity was consistent with the 70% reported by Ehmann et al., who also recruited COPD patients. Currently, research efforts should be aimed at filling this performance gap in the olfactory test's ability to distinguish between malignant and benign conditions. This could definitely legitimize the use of dog olfaction in the detection of lung cancer at an early stage.

In great contrast to related previous studies, the work by Ehmann et al. (2012) holds merit in excluding some potential confounders other than tobacco smoke, such as food odors and drug metabolites. This approach is important because confounding factors may create bias in estimates of test accuracy. In detail, using logistic regression, 9 drugs emerged to act as potential confounders, namely metoclopramide, enoxaparin, dihydrocodeine, tiotropium bromide, clopidogrel, ezetimibe, marcumar, verapamil, and metoprolol. Influence of some other confounding factors in breath samples were investigated by Walczak (2009, 2012). In 1 study Walczak, 2009, smoking negatively influenced detection accuracy as the odds ratio for detection of breath samples from smokers was significantly lower. This is in contrast to data reported by Ehmann et al. (2012), who found no significant influence of tobacco smoking on detection accuracy by canines. Walczak (2009) found that collecting breath samples from cancer patients outside hospital rooms significantly decreased the odds ratio for indication by dogs and significantly increased the odds ratio for false indications of healthy donors. The characteristic "hospital odor" associated with disinfectants is a common component of all samples taken from donors (the vast majority) who are inside a hospital and is likely to be another potential confounder. The potential confounding effect of "hospital odor" was further confirmed in a follow-up study (Walczak et al., 2012), in which no significant differences were found in detection rate for samples taken in a variety of hospitals. Taken together, these data strongly suggest that all subjects should have the same exposure, so breath samples from both patients and controls should be collected in a similar setting, either inside or outside the hospital.

It should be noticed that articles on screening trials have not yet been published (Moser and McCulloch, 2010; Lippi and Cervellin, 2012). This means that the animals were tested to distinguish be- tween normal and cancer samples (either being cancer tissue, blood, or urine), but they have not been tested so far to differentiate cancer patients from patients at risk, who might have confounding or even overlapping biochemical signals that might confuse the dog, decreasing its detecting performance (Salmi, 2016).

Almost all teams of scientists used clicker training with a food reward, either in a

line-up or carousel. This is the most common operant conditioning method used in the training of scent detection dogs (Porritt et al., 2015). However, detection-dog performance was not equally satisfying across all the studies examined. Studies showed that the accuracy of scent line-up identification results depends directly on the quality of a dog's training (Pinc et al., 2011; Harvey et al., 2006). The lack of professional and stringent dog training, carried out systematically and rigorously by a person with considerable education and experience with canine behavior, may have contributed to the poor outcome observed in the study by Gordon et al. (2008). Elliker et al. (2014) were among those who obtained the worst results. In their study, both cancer and control samples from new donors became available in batches of 5 to 10 at intervals over the training period, and it was sometimes necessary to present urine from the same donors several times during training. By contrast, only new samples were used during the 3 tests. It is conceivable that the dogs memorized the odor of each individual donor's urine during training rather than generalize on a common prostate cancer odor. As indicated by the authors, this finding highlights the importance of using extremely carefully controlled double-blind tests, involving the presentation of only new, entirely unfamiliar odor samples. Differences in dog performance might have also been due to inconsistent training schedules (length, frequency of sessions). Meyer and Ladewig (2008) reported that dogs trained once a week learned a given shaping exercise in significantly fewer training

sessions than dogs trained 5 times a week. In addition, the weekly trained dogs tended to have higher success rates than dogs trained 5 times a week. Accordingly, factors which may have influenced the results of the present study include the time that elapses between training sessions, the amount of activity between training sessions, the degree of habituation of the dogs to the training environment, procedures, and the level of arousal in the training situation. Un- fortunately, most of the studies reviewed provided only vague information about these parameters. Inappropriate training may place excessive pressure on dogs, causing them come distressed which influences their ability to learn. This might explain why Horvath et al. (2013) found that the dogs' ability was less pronounced in accuracy in series II days. In fact, both dogs were reported to show restlessness, avoidance behaviors, and unusual barking during these tests. As suspected by the authors, the low concentration of odorant molecules in the test samples might have been frustrating to the dogs, leading to their poorer performance.

Amundsen et al. (2014) trained the dogs intensively, but no information was given as to what "intensive training" was like. To be effective, an appropriate training

program must be designed to achieve consistency in the attitudes, behavior, and practices of working dog trainers and handlers.

Conclusion and future directions

Considering the recent widespread upturn in the trend of malignancies, there is compelling need to improve the screening strategies and discover novel diagnostic approaches for early detection (Lippi and Cervellin, 2012). Early detection is mainly based on clinical examination and medical imaging, which are both hampered by relatively low sensitivity and specificity (Lavra et al., 2015). These techniques may therefore expose patients to over- diagnosis and overtreatment of benign lesions, or, by contrast, to missed diagnosis and failure to treat neoplastic lesions. As specific VOC signatures are associated with the presence of cancer and the related molecular alterations, the analysis of volatile organic com- pounds in biological samples using artificial olfactory systems emerged as a new noninvasive method for early identification of tumors. However, the practical use of dogs is still limited by a lack of validated cancer-derived metabolites and by a lack of sensing technologies optimized to their detection (Lavra et al., 2015). Some detection equipment requires skilled individuals, and the machines are expensive and beneath the detection threshold of dogs. E-noses have concentration thresholds up to 0.1 ppb (Hun Lee and Hyun Park, 2010) versus canine olfaction having a concentration threshold of 10-6 ppb, or lower (Vidic, 2010).

The first 2 anecdotal reports on the potential for canine olfactory detection of cancer in 1989 opened a new avenue of research in cancer early diagnosis. Actually, there is now mounting evidence that dogs may be trained, rapidly and cost-effectively, to recognize the characteristic odor signature of various forms of cancers in body samples from cancer patients. Dogs are already widely used in health care as guide dogs, hearing dogs, and medical alert dogs. In terms of financial viability, the training of scent detection dogs could be adopted from current training regimes used in other industries. Canine detection may offer a cost-effective adjunct to diagnosis, particularly in resource poor settings where investigations are limited (Campbell et al., 2013). Yet in a systematic review (Moser and McCulloch, 2010), only 11 full-text articles were selected for inclusion, and only 5 additional empirical trials have been published since. The importance of sniffer dogs as bio- detectors of cancer is perhaps not given the attention it deserves. This is a potential oversight for scientists and physicians at a time when there is a need for more sensitive, specific, and cost-effective approaches to cancer diagnosis.

Future research should seek to develop optimal canine olfactory testing protocols,

based on internationally standardized training methods, canine selection criteria, and the inclusion of a greater number of dogs. Researchers conducting studies on the ability of dogs to sniff out cancer-related VOCs need to take into account specific aspects of the design that relate to the clinical context and study size to eliminate common biases (Table 6). For example, larger samples of subgroups of case patients and control subjects should be included, based on disease stage and both benign disease and normal healthy organ tissue, respectively. A problem associated with olfactory test research is that organ- and/or cancer-specific signatures may exist but may be masked by confounding and overlapping biochemical signals, including diet and pharmaceuticals (Amundsen, et al., 2014). Studies should control for a greater number of potential confounding variables. Future research should focus on identifying what compounds may signal a cancer diagnosis and are effectively discriminated by dogs, to assess if these elements have the potential of being translated into an electronic nose (Pomerantz, et al., 2015). Finally, there is a need to know more about the behavior and physiological status of dogs during training and work in this particular activity. Dogs could experience stress (both acute and chronic) that might affect their willingness to work in a medical detection setting, thus affecting their performance and excluding them from work. Monitoring of the well-being of the dogs should be associated with a longer and more successful work life.

In conclusion, well-designed studies that address the broad range of weaknesses identified are needed to contribute to the debate on early screening based on emitted odors by purpose- trained dogs. If these studies are planned carefully, to maximize learning and interest in working, while minimizing stress in dogs, they would make a real difference in this field of research, gaining strategic relevance in the One Health perspective.

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Ethical considerations

This study did not require ethical approval.

Conflict of interest

F.P. and M.A. declare no conflicts of interest.

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Table 1
Summary of the mentioned articles on canine olfactory detection of human skin cancer

Reference	Type	Dogs	Professional training	Sample	Disease control group	Probability	Blank trials	Result
Williams and Pembroke, 1989	Anecdotal	1 Border collie/Dobermann mix	No	Skin lesion	No	n/s	-	Histological diagnosis of melanoma
Church and Williams, 2001	Anecdotal	1 Labrador retriever (5 years old, male)	No	Skin lesion	No	n/s	-	Histological diagnosis of basal cell carcinoma
Pickel et al., 2004	Prospective	1 standard schnauzer (4 years old, male), 1 golden retriever (6 years old, female)	Yes	Tissue	No	1/10	Yes	Standard schnauzer: 86% success rate; golden retriever: 75% success rate
Campbell et al., 2013	Case report	1 Alsatian shepherd (unknown age, female)	No	Skin lesion	No	n/s	-	Histological diagnosis of melanoma

n/s, not specified in article.

Table 2
Summary of published evidence of canine ability in detecting urologic cancer–related VOCs

Primary site	Reference	Type of study	Dogs	Professional training	Sample	Disease control group	Probability	Blank trials	Confounders	Result
Prostate	Cornu et al., 2011	Prospective	1 Belgian malinois (unknown age and sex)	Yes	Liquid urine	No	1/6	No	No	91% specificity and sensitivity
	Taverna et al., 2015a	Prospective	2 German shepherds (3 years old, females)	Yes	Liquid urine	Another neoplasm; non-neoplastic disease	1/6	No	No	Dog 1 sensitivity: 100%; specificity: 98.7% (98%)[a] Dog 2 sensitivity: 98.6%; specificity: 97.6% (96.4%)[a]
	Taverna et al., 2015b	Prospective	2 German shepherds (3 years old, females)	Yes	Liquid urine	Another neoplasm; non-neoplastic disease	1/6	No	No	Preoperative mean sensitivity: 100% 45 days postoperative sensitivity: 33% with PSA levels >0.01 ng/mL<0.2 ng/mL; sensitivity: 100% with PSA levels >1 ng/mL 28 months postoperative mean sensitivity: 77.7%
	Elliker et al., 2014	Prospective	1 Labrador retriever (9 years old, unknown sex) 1 Border collie (3 years old, unknown sex)	Yes	Liquid urine	Benign prostatic hyperplasia	1/4	No	No	Labrador retriever sensitivity: 13%; specificity: 71% Border collie sensitivity: 25%; specificity: 75%
Bladder	Willis et al., 2004	Prospective	6 dogs (unspecified)	Yes	Air-dried and liquid urine	Benign urologic and non-urologic disease	1/7	No	Dipstick parameters	Mean success rate: 41% (22% for dried urine and 50% for liquid urine)
	Willis et al., 2011	Prospective	2 cocker spaniels (8 and 6 years old, males), 1 springer spaniel (5 years old, male), 1 Labrador retriever (6 years old, female)	Yes	Liquid urine	Benign urologic and non-urologic disease	1/7	No	Sex, tobacco smoking, dipstick parameters	Mean sensitivity: 64%; mean specificity: 92% (vs. healthy young controls) Mean specificity: 56% (vs. elder patients with benign urologic diseases)

PSA, prostate-specific antigen; VOCs, volatile organic compounds.
[a] Specificity achieved by the dog when considering only men older than 45 years in the control group.

Table 3
Summary of the mentioned articles on canine olfactory detection of breast cancer

Reference	Type of study	Dogs	Professional training	Sample	Disease control group	Probability	Blank trials	Confounders	Result
McCulloch et al., 2006	Prospective	Labrador retrievers (2 males and 1 female) and 2 Portuguese water dogs (1 male and 1 female)	Yes	Breath	No	1/5	Yes	Age, tobacco smoking	88% mean sensitivity, 98% mean specificity
Gordon et al., 2008	Prospective	6 dogs (unspecified characteristics)	No	Urine	No	1/7	No	n/s	Sensitivity no better than chance, 33.3% mean specificity

n/s, not specified in article.

Table 4
Summary of the mentioned articles on canine olfactory detection of ovarian cancer

Reference	Type of study	Dogs	Professional training	Sample	Disease control group	Blank trials	Probability	Result
Horvath et al., 2008	Prospective 1	black giant schnauzer (Hanna, 4 years old, female)	Yes	Tissue	Nonovarian gynecological tumors	No	2/10	Sensitivity: 100% specificity: 97.5%
Horvath et al., 2010	Prospective 2	black giant schnauzers (Hanna and Lotti, 4 and 3 years old, females)	No	Blood, tissue	Nonovarian gynecological tumors	No	1/6	Lotti[a] sensitivity: 100%; specificity: 96% (blood and tissue) Hanna[b] sensitivity: 100%; specificity: 100% (blood), sensitivity/ specificity: 94% (tissue)
Horvath et al., 2013	Prospective 2	black giant schnauzers (Hanna and Lotti, 4 and 3 years old, females)	Yes	Blood[c]	Nonovarian gynecological tumors	No	1/6	*Series I*[d] Hanna sensitivity: 97%; specificity: 100% Lotti sensitivity: 97%; specificity: 99% *Series IIa* Hanna sensitivity: 80%; specificity: 94% Lotti sensitivity: 60%; specificity: 96% *Series IIb* Hanna sensitivity: 100%; specificity: 94% Lotti sensitivity: 60%; specificity: 90%

[a] In this study, Lotti was trained using only ovarian carcinoma samples.
[b] In this study, Hanna was trained only with blood samples from selected patients.
[c] Samples taken after primary surgery and chemotherapy treatment.
[d] Series I: blood samples taken before the sixth course of chemotherapy; series II: blood samples taken 3 (a) to 6 months (b) after the final treatment.

Table 5
Summary of published evidence of canine ability in detecting human lung cancer-related VOCs

Reference	Type of study	Dogs	Professional training	Sample	Disease control group	Probability	Blank trials	Confounders	Result
McCulloch et al., 2006	Prospective	Labrador retrievers (2 males and 1 female) and 2 Portuguese water dogs (1 male and 1 female)	Yes	Breath	No	1/5	Yes	Age, tobacco smoking	Mean sensitivity/specificity: 99%
Ehmann et al., 2012	Prospective	2 German shepherds, 1 Australian shepherd and 1 Labrador retriever, (both sexes, aged 2.5-3 years old)	Yes	Breath	Chronic obstructive pulmonary disease (COPD)	1/6 up to 6/6	No	Tobacco smoking, food odors, drug metabolites	Mean sensitivity: 71%; mean specificity: 93%; specificity: 100% for UICC stage I, 75% for UICC stage II, 94% for UICC stage IIIa, 75% for UICC stage IIIb, 63% for UICC stage IV
Walczak et al., 2012	Prospective	5 German shepherds (males) and 1 Labrador retriever (male), 3 dogs aged between 20 and 22 months, and 3 dogs aged 6 months	Yes	Breath	n/s	1/2, 1/5	No	Tobacco smoke, hospital odor	At 50% probability—mean sensitivity: 79% (68%)[a]; mean specificity: 78% At 20% probability—mean sensitivity: 50% (37%)[a]
Amundsen et al., 2014	Prospective	1 Belgian shepherd (8 years old, male) 1 border collie (4 years old, male) 1 Hard hair dachshund (8 years old, male) 1 Rottweiler (3 years old, female)	Yes	Breath and liquid urine	Nonneoplastic lung diseases	1/6 up to 6/6	No	n/s	*Breath samples* NSCLC—mean sensitivity: 60%; mean specificity: 33.3% SCLC—sensitivity: 100%; specificity: 33.3% *Urine samples* NSCLC—sensitivity: 60%; specificity: 29.2% SCLC—sensitivity: 80%; specificity: 29%

n/s, not specified in article; NSCLC, non−small-cell lung cancer; SCLC, small-cell lung cancer; UICC, Union for International Cancer Control; VOCs, volatile organic compounds.
[a] Sensitivity given by the dogs when a new set of samples was tested for the first time (first trial).

Table 6
A proposed checklist for variables that should be included in future studies

Category	Variable
Dog training	A dog's prior experience with scent work Type of training (professional trainers, owners) Training schedule A dog's stress-related behaviors (frequency) Use of novel samples
Testing	Type of blinding Use of novel samples
Sample collection	Collecting subject (study subject, health care professional) Setting (home, hospital) Storage conditions (temperature, type of container) Storage duration
Participants	Source of cases (hospital based, population based) Selection of controls (nondiseased vs. diseased, noncase individuals) Patient-control matching (age, gender, time)
Other confounders	Medication Diet Last food and fluid intake Smoking habit Alcohol use Deodorants and perfumes Age of menopause if applicable

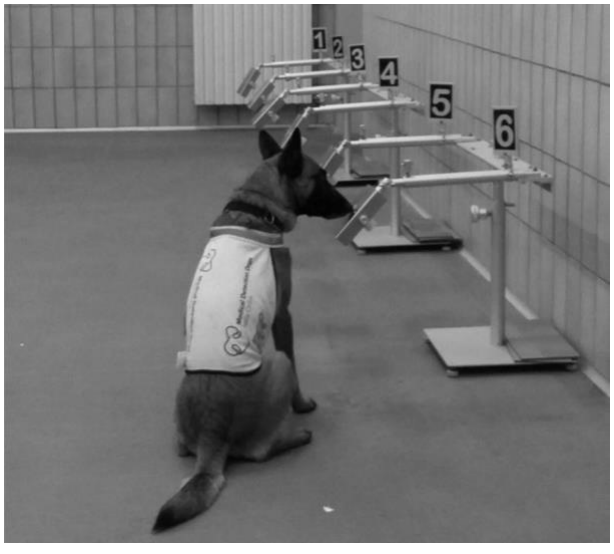

Figure. Dixie, a 8-year-old Belgian malinois, sitting in front of the human urine sample positive to lung cancer in our Veterinary Ethology and Physiology Lab in the Department of Veterinary Medicine, University of Milan, Polo Universitario di Lodi. (Credit: Medical Detection Dogs Italy. Professional trainer: Marco Sincovich).