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Early Detection of Prostate Cancer: The Role of Scent

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Abstract: Prostate cancer (PCa) represents the cause of the second highest number of cancer-related deaths worldwide, and its clinical presentation can range from slow-growing to rapidly spreading metastatic disease. As the characteristics of most cases of PCa remains incompletely understood, it is crucial to identify new biomarkers that can aid in early detection. Despite the prostate-specific antigen serum (PSA) levels, prostate biopsy, and imaging representing the actual gold-standard for diagnosing PCa, analyzing volatile organic compounds (VOCs) has emerged as a promising new frontier. We and other authors have reported that highly trained dogs can recognize specific VOCs associated with PCa with high accuracy. However, using dogs in clinical practice has several limitations. To exploit the potential of VOCs, an electronic nose (eNose) that mimics the dog olfactory system and can potentially be used in clinical practice was designed. To explore the eNose as an alternative to dogs in diagnosing PCa, we conducted a systematic literature review and meta-analysis of available studies. PRISMA guidelines were used for the identification, screening, eligibility, and selection process. We included six studies that employed trained dogs and found that the pooled diagnostic sensitivity was 0.87 (95% CI 0.86–0.89; I^2, 98.6%), the diagnostic specificity was 0.83 (95% CI 0.80–0.85; I^2, 98.1%), and the area under the summary receiver operating characteristic curve (sROC) was 0.64 (standard error, 0.25). We also analyzed five studies that used an eNose to diagnose PCa and found that the pooled diagnostic sensitivity was 0.84 (95% CI, 0.80–0.88; I^2, 57.1%), the diagnostic specificity was 0.88 (95% CI, 0.84–0.91; I^2, 66%), and the area under the sROC was 0.93 (standard error, 0.03). These pooled results suggest that while highly trained dogs have the potentiality to diagnose PCa, the ability is primarily related to olfactory physiology and training methodology. The adoption of advanced analytical techniques, such as eNose, poses a significant challenge in the field of clinical practice due to their growing effectiveness. Nevertheless, the presence of limitations and the requirement for meticulous study design continue to present challenges when employing eNoses for the diagnosis of PCa.

Keywords: prostate cancer; olfactory system; electronic nose; dog smelling; diagnosis; biomarkers

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1. Introduction

Today, prostate cancer (PCa) is the second most common cancer in the world, with almost 400,000 deaths annually [1]. The development of PCa is a multifactorial process

due to the interaction of various risk factors, including age, ethnicity, family history, lifestyle, environmental factors, and genetic factors. Despite ongoing research efforts, the underlying cause of PCa remains incompletely understood. Recently, screening for PCa using digital rectal examination (DRE) and serum prostate-specific antigen (PSA) has come under considerable criticism due to several trials demonstrating that PSA serum levels often leads to overdiagnosis and overtreatment, as well as the inability to accurately differentiate between low-, intermediate- and high-risk PCa. PCa diagnosis is currently based on the invasive procedure of transrectal ultrasound (TRUS) guided needle biopsy of the prostate [2]. This method is linked to several adverse reactions. Additionally, nearly two-thirds of biopsies administered after detecting increased PSA levels were found to be unnecessary, highlighting the low accuracy of this marker. This can result in the over-diagnosis of PCa and unnecessary treatment. Moreover, various investigations have revealed a lack of consensus among pathologists regarding the Gleason scale, indicating the potential benefit of implementing artificial intelligence algorithms in computational pathology to offer additional support and a secondary evaluation to medical experts [3]. Multiparametric magnetic resonance imaging (MRI) of the prostate has been recommended as an initial diagnostic test for men presenting with suspected PCa, with a negative MRI enabling safe avoidance of biopsy and a positive result enabling MRI-directed sampling of lesions [4]. The main role of prostate MRI is to detect only clinically significant PCa. The prevalence of clinically significant PCa in men referred to urology clinics is approximately 30% [4]. This suggests that a considerable number of patients may undergo invasive biopsy procedures unnecessarily. However, if an MRI scan shows a negative result, up to half of these patients can safely avoid the biopsy. On the other hand, a positive MRI can accurately target tumor lesions and provide reliable tissue sampling [4]. The negative predictive value of MRI is high, around 90%, and shows minimal variability across different centers. In contrast, the positive predictive value is comparatively low, with reported values of 17%, 46% and 75% for lesions with Prostate Imaging-Reporting and Data System (PI-RADS) scores of 3, 4 and 5, respectively [4]. Therefore, MRI is most effective as a rule-out test. However, studies comparing MRI-detected lesions with histopathology on prostatectomy specimens have shown that 8–24% of Grade Group 2 PCa cases might be undetectable by MRI [4]. This can be attributed to technical limitations, the presence of cribriform glands, and/or a sparse pattern of tumor growth. It has been reported that the diagnostic strategy involving PSA's low sensitivity, the invasiveness of prostate biopsy sampling, and the variability in performing and interpreting MRI is constrained by various factors. Successful implementation of this approach necessitates experienced clinicians, optimized equipment, effective interdisciplinary communication, and standardized workflows. Given the above limitations, it is crucial to identify a diagnostic strategy that can aid in identifying patients with clinically significant PCa. In this regard, several biomarker tests have been developed to assess the risk of clinically significant PCa and reduce the need for unnecessary prostate biopsies [5]. Throughout history, medical professionals have sought to employ urine as a means of evaluating disease without the need for invasive procedures [6,7]. As a byproduct of the kidneys, urine facilitates the elimination of bodily waste products from the bloodstream. Urine possesses the potential to provide valuable insights not only into the health of the urinary and renal systems but also into remote organs via plasma acquired through glomerular filtration. Consequently, examining urine could enable the detection of biomarkers for both urogenital and systemic diseases [6,8]. The analysis of volatile organic compounds (VOCs) and volatile organic metabolites (VOMs) has emerged as a new frontier for cancer diagnosis in particular because it is non-invasive and potentially inexpensive (Figure 1) [9–16]. The concept has been demonstrated with different types of cancer [10,16–21]. VOCs typically originate from primary or secondary metabolic processes and are characterized by their lipophilic molecular nature. These compounds are often categorized based on their molecular structure or functional group. It is now accepted that cell modifications lead to peroxidation of membrane components and consequent release of specific VOCs in biological fluids, such as urine, blood, saliva and sweat [13,22–27].

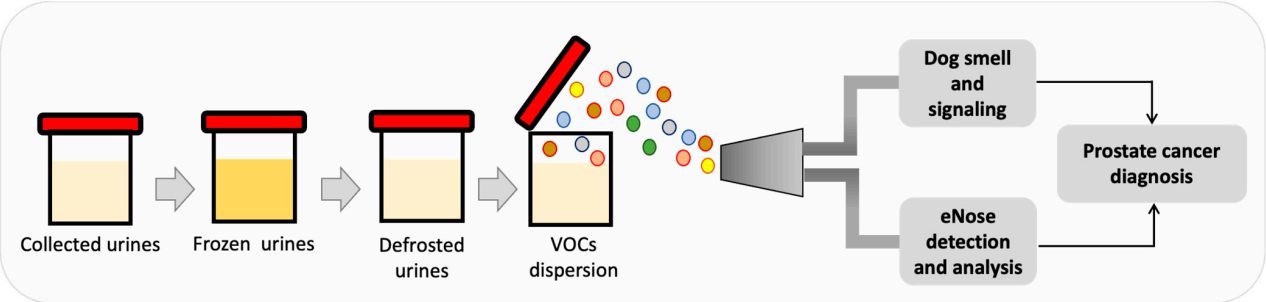


Figure 1. Human urine has been demonstrated as a source of biochemical compounds called volatile organic compounds (VOCs) for their volatility helpful in the diagnosis of neoplastic as well as non-neoplastic, including infectious diseases and detectable by the dog olfactory system or technological devices, such as the eNoses, when opportunely trained.

The metabolic end-products of these cells have been suggested as promising non-invasive biomarkers for various diseases [9,28–30].

In 2015, we demonstrated that highly trained dogs exhibited a high accuracy in detecting PCa-specific VOCs [31–33]. These findings have been recently supported by Guest et al. [34], as well as by previous studies conducted by other authors [35–39]. However, the routine integration of dogs into clinical practice faces several limitations, including the requirement for specialized centers, extensive training for both dogs and handlers, the aging profile of dogs, and challenges in incorporating dogs into clinical protocols [40–42]. Furthermore, the US Food and Drug Administration (FDA) does not recognize dogs as a clinical "device" https://www.fda.gov/medical-devices (Accessed on 7 June 2023).

The advancement of technological devices that mimic dogs' ability to detect VOCs from biological fluids opens up possibilities for utilizing their potential in clinical diagnosis. Among these devices, electronic noses (eNoses) represent a potential tool capable of mimicking the olfaction ability of dogs by combining an array of non-specific gas sensors with a pattern recognition unit based on machine learning algorithms [34,43–45]. While eNoses are not as powerful as mammalian olfaction [46,47], there are promising examples in the literature that demonstrate their potential for diagnostic applications [10,48–50]. As a result, we evaluated the accuracy of an ad hoc eNose to recognize PCa in urine samples from 174 subjects, including 88 (50.6%) men with PCa and 86 (49.4%) healthy subjects [51]. The eNose reached a sensitivity 85.2% (95% CI 76.1–91.9), a specificity 79.1% (95% CI 69.0–87.1), and an accuracy, represented as the area under the receiver operating characteristic (ROC) curve, equal to 0.821 (95% CI 0.764–0.879). Although these findings resulted promising they are susceptible to supplemental improvements.

We conducted a systematic literature review and meta-analysis following PRISMA guidelines to investigate the potential use of the eNose as an alternative to canine sense of smell for diagnosing PCa. Our objective was to gain a comprehensive understanding of the challenges and factors associated with employing eNose technology, by addressing its current limitations and acknowledging its intricacies. This research aimed to provide insights into the issues and considerations that arise when utilizing eNose for the diagnosis of PCa.

2. Materials and Methods

This study followed the Preferred Reporting Items for Systematic Reviews and Meta-analyses (PRISMA) reporting guidelines for the identification, screening, eligibility and selection process http://www.prisma-statement.org/ (Accessed on 3 April 2023) [52–54].

We carried out two electronic searches in PubMed, Scopus and Web of Science with the keywords: (*a*) "dog(s)" AND "sniffer" OR "scent" OR "smell" AND "Volatile Organic Compound(s)" AND "prostate cancer" within all fields, without date or language restrictions (i.e., up to 11 April 2023), and (*b*) "electronic nose(s)" AND "sniffer" OR "scent" OR "smell" AND "Volatile Organic Compound(s)" AND "prostate cancer" within all fields, without any date or language restrictions. We analyzed the title, abstract, and full text

(when available) of all identified items using these search criteria. Our focus was on selecting studies that described the diagnostic performance of dogs or eNose in identifying PCa in urine samples. Additionally, we examined the references of the selected articles to identify any potentially useful documents. For the pooled analysis, we included studies that provided false positive (FP), false negative (FN), true positive (TP), and true negative (TN) values, allowing us to estimate diagnostic sensitivity, specificity, and accuracy using a summary receiver operating characteristic curve (sROC) with a 95% confidence interval (95% CI). Any study found from the electronic search that lacked TN, TP, FN, and FP values was excluded from the pooled analysis. The following information were independently gathered by three reviewers (F.G, C.B, and M.A.A.A.H) and tabulated from the articles: (*a*) For studies involving dogs: author, number of dogs, age of dogs, sex, breed, type of human sample, and number of cases. (*b*) For studies involving eNose: author, type of human sample, number of cases, and type of eNose. The Egger's test for publication bias was performed using the free RStudio (RStudio Team, 2022. RStudio: Integrated Development for R. RStudio, PBC, Boston, MA, USA, URL http://www.rstudio.com/ (accessed on 22 May 2023). A funnel plot was used to visually represent the potential publication biases.

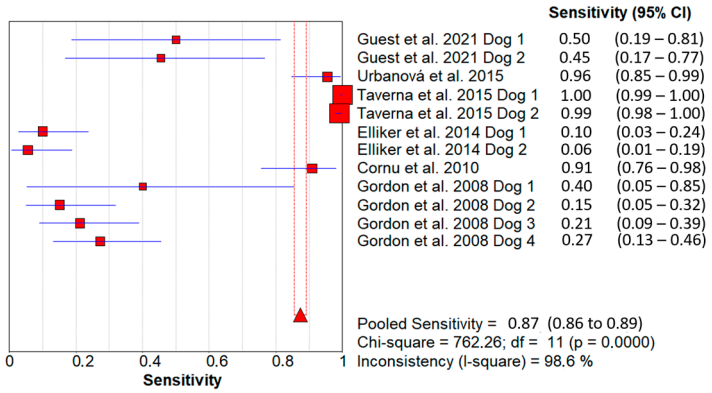
Between-study heterogeneity was calculated using $\chi 2$-test and I^2 statistic. The statistical analysis was performed using Meta-DiSc 1.4 (Unit of Clinical Biostatistics team of the Ramón y Cajal Hospital, Madrid, Spain) [55].

3. Results

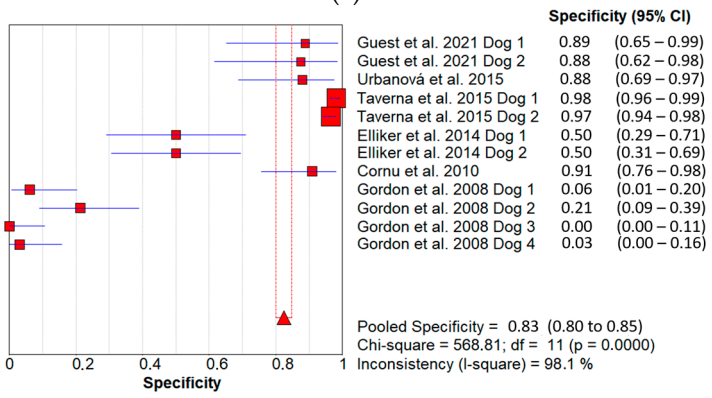
The electronic search led to identify nine documents regarding the use of dogs, following elimination of duplicates across scientific databases. Three of the investigations were disregarded as they did not provide data regarding the number of true/false positive and true/false negative outcomes for detecting the existence of PCa [35,56,57]. Furthermore, a study was omitted as the researchers trained canines to indicate the presence of sarcosine in urine samples, which is a prospective marker for the diagnosis of PCa [36]. The studies included in our pooled analysis (n = 6), totaling 12 dogs (58% females, 17% males, and 25% not indicated) with a mean age of 40 months (range: 24–108 months), and a total of 1391 subjects analyzed, as summarized in Table 1. The biological material presented to the dogs for PCa signaling and detection were human urine samples. The analysis is shown in Figure 2a–c. The pooled diagnostic sensitivity was 0.87 (95% CI, 0.86–0.89; I^2, 98.6%), the diagnostic specificity 0.83 (95% CI, 0.80–0.85; I^2, 98.1%), whilst the sROC was 0.64 (standard error, 0.25) (Figure 2a–c).

Table 1. Summary of studies which explored the performance of dogs trained to identify prostate cancer in human samples.

Authors	Dogs, n	Age, Months	Sex, Female, n (%)	Breed	Human Samples	Cases, n (Diseased %)
Guest et al. [34]	2	48	2 (100%)	Labrador	urine	50 (24%)
		84		Wire Haired Hungarian Vizsla		
Urbanová et al. [58]	1	36	1 (100%)	German Shepherd	urine	70 (64%)
Taverna et al. [31]	2	36	2 (100%)	German Shepherd	urine	902 (40%)
		36		German Shepherd		
Elliker et al. [39]	2	36	NA	Border Collie	urine	117 (43%)
		108		Yellow Labrador		
Cornu et al. [38]	1	NA	NA	Belgian Malinois Shepherd	urine	66 (50%)
Gordon et al. [37]	4	48	4 (100%)	Chihuahua mix	urine	186 (31%)
		30		Miniature goldendoodle		
		72		Pembroke Welsh corgi		
		24		Border collie		



(**a**)



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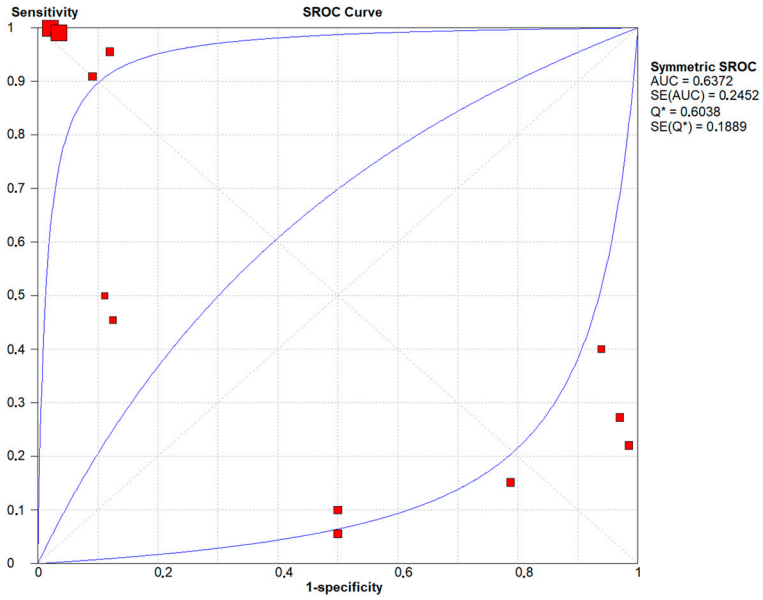


(**c**)

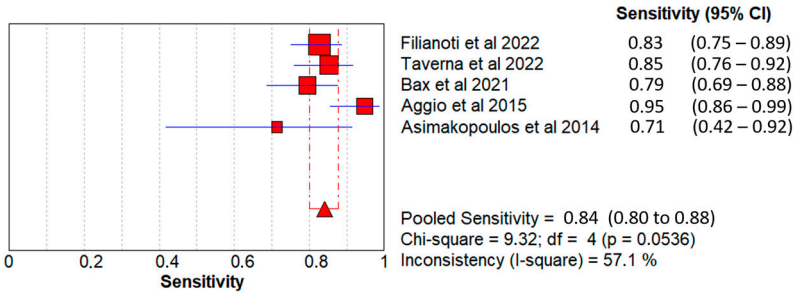
Figure 2. Pooled diagnostic sensitivity (**a**), specificity (**b**), and accuracy (sROC, (**c**)) of dogs trained to identify PCa in human urine samples. In the forest plot illustrating sensitivity and specificity, each study is represented by a red square, with the size proportional to the sample size. The red triangle represents the pooled sensitivity or specificity estimate, accompanied by the confidence interval. The dashed lines extending from the red triangles indicate the range of uncertainty surrounding the estimated pooled sensitivity or specificity. The confidence intervals are displayed in blue. Q* indicates the Q* index as the point in the ROC space which is closest to the ideal top left-hand corner of the ROC curve. [31,34,37–39,58].

We identified 10 documents regarding the use of eNose following elimination of duplicates across scientific databases. Five of these studies were excluded as they did not show information on the number of true/false positive and true/false negative results for diagnosing the presence of PCa [59–63]. Therefore, five studies have been included in our pooled analysis, totaling five different eNoses, and a total of 757 subjects analyzed as summarized in Table 2. The biological material presented to the devices for PCa detection was human urine. The analysis is shown in Figure 3a–c. The pooled diagnostic sensitivity was 0.84 (95% CI, 0.8–0.88; I^2, 57.1%), the diagnostic specificity 0.88 (95% CI, 0.84–0.91; I^2, 66%), whilst the sROC was 0.93 (standard error, 0.03) (Figure 3a–c).

Table 2. Summary of studies which explored the performance of dogs trained to identify PCa in human samples.

Authors	Human Samples	Cases, n (Diseased %)	eNose
Filianoti et al. [64]	urine	272 (49%)	Cyranose C320 (Sensigent LLC, Baldwin Park, CA, USA)
Taverna et al. [51]	urine	174 (88%)	Ad Hoc, project Diag-Nose
Bax et al. [44]	urine	115 (68%)	Ad Hoc, project Diag-Nose
Aggio et al. [65]	urine	155 (58%)	NA
Asimakopoulos et al. [66]	urine	41 (34%)	ENQBE, University of Rome Tor Vergata

The potential publication bias was investigated using the Egger's test [67,68]. For the pooled analysis of dog studies, the test for funnel plot asymmetry was found: z = 1.0064, *p*-value = 0.3142. The limit estimate (as standard error approaches 0) = −4.8018 (CI: −18.9795, 9.3760). The non-statistic significant *p*-value suggests that there is no evidence of funnel plot asymmetry, indicating that any observed asymmetry is likely due to chance rather than publication bias (Figure 4a). For the pooled analysis of eNose studies, the test for funnel plot asymmetry was found: z = 0.9729, *p*-value = 0.3306; The limit estimate (as standard error approaches 0) = 2.4716 (CI: 0.0022, 4.9411). The non-statistic significant *p*-value suggests that there is no evidence of funnel plot asymmetry, indicating that any observed asymmetry is likely due to chance rather than publication bias (Figure 4b).



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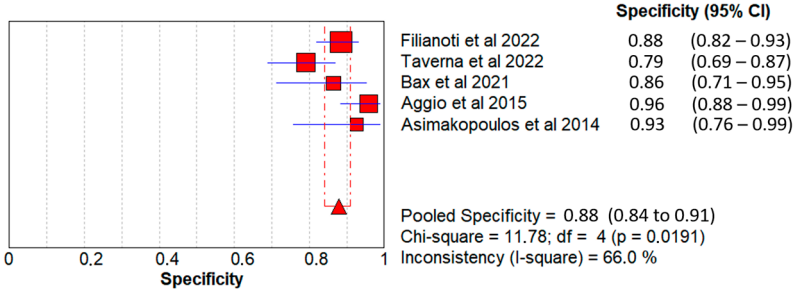

(**b**)

Figure 3. *Cont.*

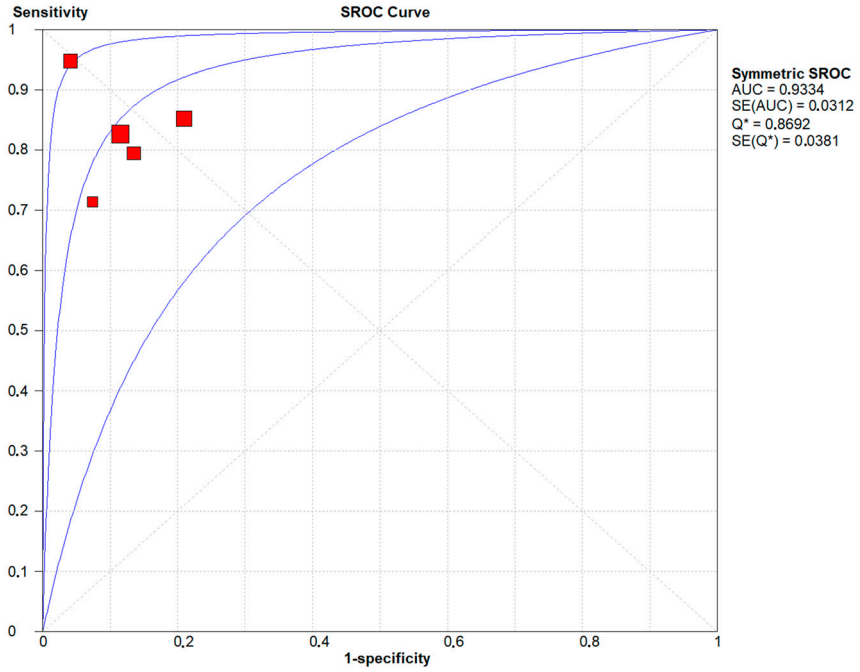

Figure 3. Pooled diagnostic sensitivity (**a**), specificity (**b**), and accuracy (sROC, (**c**)) of eNoses trained to identify PCa in human urine samples. In the forest plot illustrating sensitivity and specificity, each study is represented by a red square, with the size proportional to the sample size. The red triangle represents the pooled sensitivity or specificity estimate, accompanied by the confidence interval. The dashed lines extending from the red triangles indicate the range of uncertainty surrounding the estimated pooled sensitivity or specificity. The confidence intervals are displayed in blue. Q* indicates the Q* index as the point in the ROC space which is closest to the ideal top left-hand corner of the ROC curve. [44,51,64–66].

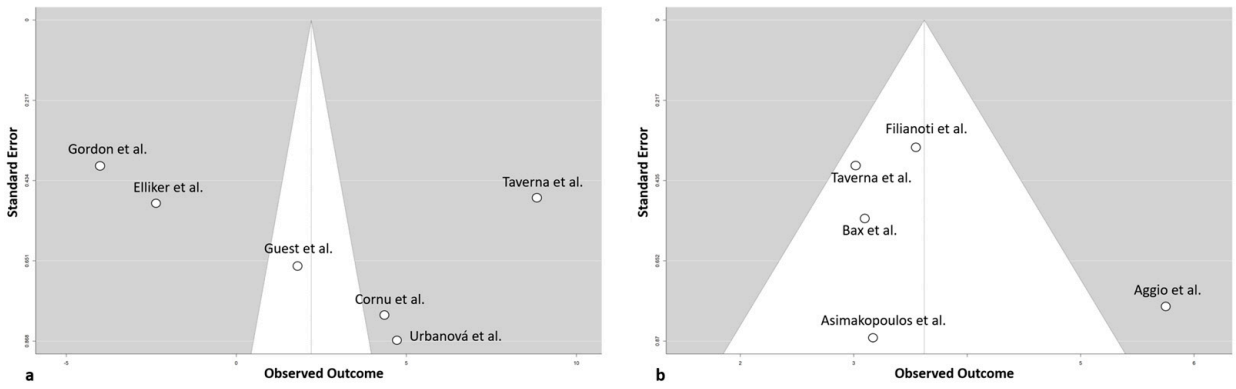

Figure 4. The funnel plot for potential publication bias in the pooled analysis of dogs (**a**) and eNose to diagnose PCa in urine samples (**b**) exhibits symmetry, indicating no publication bias. This observation is consistent with the results of Egger's test for publication bias, where the *p*-value is greater than 0.1. The points on the funnel plot for dog studies are distributed outside the cone, which represents the 95% confidence interval, due to the high heterogeneity (**a**). [31,34,37–39,44,51,58,64–66].

4. Discussion

In 2015, we have shown that highly trained dogs recognized PCa specific VOCs with a high accuracy [31]. These promising results led us also to investigate the ability of highly trained dogs to detect PCa biochemical recurrence (BCR) in men who had previously undergone radical prostatectomy for PCa [69]. We found that highly trained dogs can also detect PCa BCR. The notion that dogs might be able to detect malignant

tumors based on their odor is not a novel concept. It was initially proposed by Williams and Pembroke in 1989 [70]. Since then, various original reports and commentaries have been published detailing the olfactory identification of various types of cancer, including PCa [18,31,34,37,38,71–80]. Dogs possess an incredibly sense of smell, with the ability to detect concentrations as low as one part per trillion (ppt). It is known that humans with a highly developed sense of smell can detect odors diluted as low as 0.01 ppb (parts per billion) [81,82].

The use of dogs in clinical settings faces several limitations, making it difficult to widely implement their abilities [40–42,51]. To harness the potential of VOCs, it is crucial to develop a technological tool that mimics the olfactory system of dogs (Figure 1). This tool should be simple to manufacture, easily accessible, and practical for use in clinical settings. Among the available options, the eNose has garnered increasing attention in recent years for the non-invasive diagnosis of various cancers [10,11,49,83–94]. This technique holds great promise for clinical practice as a real-time non-invasive diagnostic tool and for monitoring disease progression and immunotherapy treatment effects [95]. If compared to dog's sense of smell, the eNose is more feasible due to its relatively low cost, user-friendly nature, and the ability to generate outputs that do not require interpretation by specialized personnel. However, all the studies underline the presence of limitations and the significance of careful study design when using eNose for cancer diagnosis, including PCa. Recently, Arrieta et al. have also reported that one drawback of the eNose is the absence of mathematical models of data analysis that yield easily interpreted findings at point of care [96].

Here we have conducted a systematic literature review and meta-analysis of available studies to explore the eNose as a potential alternative to canine olfaction in the diagnosis of PCa. Our aim was to explore the existing limitations and complexities of eNose technology, allowing us to gain a deeper understanding of the challenges and factors involved in using eNose for PCa diagnosis. By thoroughly examining these issues and considerations, our objective was to provide a comprehension of the potential obstacles and key aspects to consider when implementing eNose in the diagnosis of PCa.

It is known that a meta-analysis offers a more complete overview of the current research available by combining relevant data extracted from multiple independent studies. The primary advantage of a meta-analysis is that it provides a more thorough understanding of a given research issue and enhances statistical validity when compared to that of a single study [97]. Additionally, meta-analysis is a tool that can improve our comprehension of the heterogeneity underlying individual studies and help identify potential differences on the outcome of independent research [98].

The six studies included in our pooled analysis focused on the use of canine olfaction for PCa diagnosis, enrolled 12 dogs with a mean age of 40 months (range: 24–108 months), and a total of 1391 subjects analyzed (Table 1). The biological material presented to the dogs for PCa signaling and detection were human urine samples. The pooled diagnostic sensitivity was 0.87, the diagnostic specificity 0.83, whilst the sROC was 0.64 (standard error, 0.25) (Figure 2a–c).

The term heterogeneity in meta-analysis refers to the situation where multiple studies investigating the same research question have different underlying effect measures [98–100]. The I^2 statistic quantifies the percentage of variation in effect estimates across studies that is due to heterogeneity rather than chance alone [98–100]. The value of I^2 does not depend on the number of studies included. As highlighted by Higgins and Thompson [98], an I^2 value of 0% indicates that all the variability in effect estimates is solely due to sampling error within the included studies, with no contribution from heterogeneity. Conversely, a higher percentage reflects greater between-studies variation. In our systematic review and meta-analysis, we included all the available studies that employed dogs to detect and signal PCa-specific VOCs in urine samples, encompassing a total of 12 dogs and 1391 subjects. The derived I^2 values for sensitivity (98.6%) and specificity (98.1%) point towards considerable heterogeneity across the evaluated studies. However, this does not undermine the fact that highly trained dogs have the capacity to signal and diagnose PCa through the smell of

urine. The meta-analysis, however, emphasizes the significant impact of various inherent variables on the results of the studies, which display non-uniformity. This heterogeneity is not substantially curtailed by pooling. This predicament can be assessed from both statistical and biological perspectives. Biologically speaking, it is broadly recognized that dogs have exceptional smell perception [42,101]. Their remarkable olfaction aids in collecting vital environmental information, recognizing individuals, making decisions, and learning [67]. It is crucial to recognize that the study results are, however, heavily influenced by numerous factors including training techniques, sample size, dog breed, age, individual abilities, and the relationship between the dog and its handler [102]. This is evident in various studies. All of the combined studies validate the possibility of using highly trained dogs for diagnosing PCa. Yet, they all also underscore significant differences mainly due to the previously mentioned factors. The higher the I^2 value, the greater the heterogeneity of the combined study results, signifying noticeable dissimilarities between the studies. This heterogeneity is not conspicuous when examining the individual studies.

Our pooled analysis also showed a statistical significance of the chi-squared statistic for heterogeneity and the non-significant AUC. As previously stated, it is unquestionable that dogs have exceptional olfactory abilities and can be trained to detect PCa by smelling urine [42,103,104]. Nonetheless, the multitude of factors such as training methodologies, dog breeds, age, individual skills, and the relationship between the dog and its handler that can impact the results of these studies leads to the noted heterogeneity, as indicated by the high I^2 value and the non-significant AUC. In our analysis, we incorporated all studies that employed dogs to diagnose PCa via urine odor detection. Gordon et al. reported that two of the four dogs tested performed notably better than would be expected by chance in terms of specificity, while none showed significant sensitivity [37]. They proposed that more careful handling of urine samples and the enforcement of a more rigorous training protocol during their research could potentially shed new light on the feasibility of using dogs for cancer detection. Gordon et al. [37] utilized a variety of breeds and a unique training protocol, leading to discordant results. Breed variations are often presumed in olfactory abilities, with German shepherds, hounds, and labradors frequently chosen for odor-detection tasks, while toy breeds and brachycephalic dogs such as pugs are typically excluded. However, the selection of breeds for scent detection tasks may be more a reflection of "traditional practices" rather than being grounded in "empirical evidence". This element can affect the results of any diagnostic method that involves breed-specific considerations.

Excluding the study by Gordon et al. [37] from our pooled analysis, we found an AUC of the sROC of 0.96 with a standard error of 0.02, thus establishing a statistical significance ($p < 0.00001$). The pooled analysis demonstrates a sensitivity of 0.93 [CI: 0.91–0.94; I2, 98.6%] and a specificity of 0.94 [CI: 0.92–0.95; I^2, 93%]. Hence, based on these findings it can be affirmed that dogs detect PCa in a manner that exceeds random selection. As stated by Gordon et al. [37], their findings were not positive. They highlighted procedural errors that future researchers needed to rectify. They concluded that although their study was not successful, it offered valuable insights in the form of identified mistakes, which they shared in the hope that others might learn from them. In the study carried out by Taverna et al. [31], two German Shepard Explosive Detection Dogs (EDD) were used, and a highly specific training protocol, established and standardized by the Italian Defense Minister, was put into practice. The dogs in the study underwent training using the "clicker training method", which is based on the principles of operant conditioning. This method has proven to be effective in various fields, including tasks related to scent detection. The inclusion of a substantial sample size and the execution of precise and standardized training protocols are crucial elements that enhance the reliability and validity of the results acquired. In 2011, Cornu et al. [38] assessed the effectiveness of trained dogs in detecting PCa from human urine samples. They employed a Belgian Malinois shepherd, trained by a professional and dedicated two-person team throughout the study. The dog was part of the French Army Veterinary Department and was selected from a group of young dogs intended for EDD training. The dog was trained using the clicker training method. The training was a

full-time commitment for the Team, who engaged with the dog five days a week over the study period (October 2007 to June 2010).

Based on these characteristics, it is evident that except from the sample size, both studies utilized highly trained military dogs, a standardized training method, and a lengthy training period overseen by a highly professional Armed Forces Team. Both studies demonstrate superior findings, as also highlighted in the pooled analysis. This further substantiates that the highly varied results (and hence heterogeneity and its I^2 value, pooled specificity, pooled sensitivity, AUC of sROC, and AUC statistical significance) are largely attributed to the study design. When we exclude the studies by Taverna et al. [31] and Gordon et al. [37], which represent the studies with the highest and lowest performance, respectively, the pooled AUC of sROC is statistically significant ($p < 0.00001$). At the same time, when we also exclude the study of Cornu et al. [38], which has the same study design as Taverna et al. [31], the AUC of the sROC continues to maintain statistical significance ($p < 0.00001$).

Alongside to the above considerations, and although, the answer to the question: "What do sniffer dogs really smell in the urine?" could offer valuable insights into the potential of non-invasive biomarkers for laboratory diagnostics of PCa, no study has yet conducted an experiment using a well-defined artificial matrix to identify the targets of olfactory detection [105].

It is now accepted that VOCs are produced by natural or abnormal metabolic processes in the body, as well as by the bacteria residing in or on the body. The analysis of VOCs from clinical samples presents an appealing option for disease diagnosis, as it is non-invasive and swift [106]. It is known that cancer represents a condition in which the VOC profile may vary. This could be due to differences in the expression of various metabolic pathways between cancer cells and normal cells.

Various alternative methods to canine olfaction have been suggested and implemented for the examination of individual VOCs or VOC profiles in a biological sample. These techniques vary from advanced and costly approaches that can analyze breath or headspace samples directly in real-time, such as selected ion flow tube mass spectrometry or proton transfer mass spectrometry. However, no study has identified a specific and accurate marker or pool of markers that can be introduced into the clinical practice, because of partial results achieved in this field up to now [9,107]. An additional gas sensor-based technique that has been proposed as a substitute for urine sniffing dogs to detect patients with prostate cancer is the eNose. While this method cannot identify specific compounds, it offers advantages such as its speed and relative affordability [106]. The eNose was initially developed in the 1980s [108] and it attempts to imitate the biological olfactory system by analyzing the complete chemical profile of a complex mixture of chemical compounds, rather than detecting each compound individually.

Sensor-array (electronic-nose) devices utilize the vast amount of information gathered from numerous sensors, although they have not yet attained the sensitivity of other techniques. The composite output of the array depicts a pattern of changes based on the collection of chemicals that the sensors have detected. These sensors do not identify the specific VOCs and their concentrations; rather, they represent a pattern, or "smellprint," of changes based on the entire volatile mixture present in the sample associated to the disease state. The volatile compound pattern may indicate not only an infecting organism or disease state, but also the host response's metabolism, as well as other associated conditions.

Similar to the human nose, the eNose requires previous exposure or training to a specific odor or pattern in order to recognize it. Typically, an eNose comprises an array of chemical sensors that are broadly tuned to various chemical groups. When a sample is presented to the array, each sensor's unique response to the complex odor is recorded. This pattern can then be learned using a pattern recognition algorithm.

Commercial eNoses that utilize electro-resistive sensing technologies, including metal oxides and conducting polymers, have been employed in the detection of cancer and treatment response [87,88,95,109–113]. One of the most significant drawbacks of eNoses is

sensor drift, which is a well-known limitation that can impact the accuracy of diagnostic algorithms. Bax et al. have recently introduced a data processing algorithm known as orthogonal signal correction to compensate for sensor drift, achieving an improvement in restoring accuracy from 55% to 80% in one-year-old sensors [44].

In our pooled analysis we have included five studies focused on the use of eNose for PCa detection, totaling 5 eNoses and 757 subjects analyzed as summarized in Table 2 demonstrates the diagnostic sensitivity 0.84, the diagnostic specificity 0.88, whilst the sROC was 0.93 (standard error, 0.03) (Figure 3a–c).

One limitation of the pooled analysis to draw conclusive statements is the low number of studies eligible. In line with the Cochrane Consumers and Communication Guidelines https://cccrg.cochrane.org/ (accessed on 22 May 2023), it is accepted that a meta-analysis can be performed with as few as two studies, provided their results can be meaningfully combined and are sufficiently alike. In our literature analysis, we combined the results of five studies that specifically examined the use of eNose in diagnosing prostate cancer through urine samples. We acknowledge that by including various types of tumors or non-neoplastic diseases, and different biological media (e.g., urine, saliva, breath, blood, tumor specimen), more comprehensive pooled findings could potentially be obtained. Yet, it is crucial to say that combining such diverse diseases with unique biological characteristics and odor signatures might introduce biases and hinder accurate interpretations, as also demonstrated in studies by Scheepers et al. [49], Afonso et al. [114], and other authors. Considering the assumptions above, our study has shown an I^2 of 57% for sensitivity and 66% for specificity, indicating a "significant" degree of heterogeneity. However, the statistically significant AUC of 0.93 ($p < 0.001$) suggests that despite the heterogeneity, the eNose shows potential in diagnosing PCa. These findings, however, underline the presence of limitations.

Although sensor drift [44], the lack of mathematical models [96], and environmental variables (e.g., temperature and humidity) [45] still represent drawbacks of eNose technology, its high diagnostic accuracy demonstrated in all studies, coupled with its relatively low cost and rapid measurements, make it a compelling choice for routine testing in clinical settings [86] and with the possibility of reducing the number of invasive procedures such as prostate biopsies in clinical practice [64].

It is indubitable, that both eNoses and dogs offer unique capabilities in detecting cancer. While eNose provides an alternative method to dogs' olfaction for diagnosing PCa through urine sample analysis, it is still limited by technical challenges. Continued research is needed to address issues related to sampling methodology, analysis, reproducibility, external validation, and the high risk of biases that require standardization [45,49,50]. Furthermore, it is crucial to test eNose technology in intention-to-treat populations to determine its practical application. In a clinical setting, eNose may have advantages over dogs as the results are not influenced by factors such as training protocols, breeds, or individual skills that can significantly impact a dog's performance, as also evidenced in our pooled analysis. The quantitative data and analysis provided by eNoses can also assist clinicians in making more informed decisions and tracking patient progress over time. Another advantage of eNose is the ability to analyze numerous samples in a short period, making them suitable for large-scale screening programs, in contrast to dog sessions, which are typically reported to involve one session per day with four acquisition sessions [115]. Similar to dogs, eNoses can be trained to detect various pathologies, assuming that each pathological state is characterized by a specific VOCs fingerprint [50,116–118]. This suggests that eNoses have the potential to expand beyond PCa and be utilized for the detection of different diseases based on their distinct VOCs profiles.

It remains unquestionable that using eNoses eliminates potential ethical concerns associated with using animals for diagnostic purposes, such as the possible stress on the dogs and their handlers or potential infections that the dog can acquire while analyzing hazardous biological media.

It is important to note that certain technological concerns must be resolved in order for the technology to be dependable and adaptable on a larger scale, such as addressing calibration transfer issues [119]. Additional research is needed to establish the validity of utilizing the eNose technology in diagnostic practice, either alone or in conjunction with a nomogram. It is encouraging that engineers, clinicians, biologists, and mathematicians continue to contribute together towards a common quantitative understanding of cancer complexity using technology as a tool to improve the scientific knowledge.

5. Patents

Taverna G., Grizzi F., Capelli L., Sironi S., Bax C., and Eusebio L. METHODS TO ASSESS THE RISK OF BEING AFFECTED BY PROSTATE CANCER. International application number PCT/EP2020/055555; International filling date 03.03.2020. (Humanitas Mirasole S.p.A. 60%, Politecnico di Milano 40%).

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