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Chapter

Canine Detection of the Volatile Organic Compounds Related to Cervical Cancer Cells

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Abstract

The use of trained dogs for the detection of volatile biomarkers in biological samples has great potential to be used for non-invasive diagnosis and monitoring of several diseases such as cancer. It offers early, highly accurate detection with fast response times, non-invasive to patients and allows for repeated sampling. The aforementioned methods are useful as a portable technology to increase detection, screening, and monitoring coverage in populations at risk. In this sense, Cervical Cancer (CC) has become a public health concern of alarming proportions in many developing countries, particularly in low-income sectors and marginalized regions due to different factors that limit the coverage of screening methods and the acceptance rates of women attending their routine gynecological examination. As such, early detection is a crucial medical factor in improving not only their population’s quality of life but also its life expectancy. For the above, the great odor detection threshold exhibited by dogs is not unheard of and represents a potential opportunity to develop an affordable, accessible, and non-invasive method for detection of CC with high sensitivity and specificity values.

Keywords: cervical cancer, dog detection, volatile organic compounds

1. Introduction

There is significant potential to reduce the suffering from cancer and to alleviate the economic burden to individuals, families, and societies. It is known that prevention campaigns and early detection interventions can avert cancer cases and deaths in high- and low-resource settings. Although many countries and communities have limited resources for screening, several common cancers among females such as Cervical Cancer (CC) have known means of prevention and/or early detection that can be applied in resource-appropriate settings [1].

Cervical cancer is one of the female reproductive system cancers, and it is a fundamental cause of cancer morbidity and mortality worldwide. This complex disease
is relatively common with estimates of more than half a million new cases in 2018, and it accounts for 13% of all cancers in women in developed regions [2, 3]. The highest incidence rates (greater than 20 per 100,000 women) are found in Eastern, Western, and Southern Africa, South-Central Asia, South America, Melanesia, and Central Africa [3].

There are different tools to achieve CC elimination. The World Health Organization (WHO) has identified three critical targets to the elimination of this cancer type mainly in the increased coverage of: 1) Human Papillomavirus (HPV) vaccination, 2) Screening for premalignant disease with an HPV test, and appropriate management of women who screen positive, and 3) Reducing mortality from cervical cancer by providing appropriate treatment [4].

In recent years HPV vaccination in high-income countries has resulted in dramatic decreases in HPV infection and associated cervical disease as a primary prevention strategy for CC. Unfortunately, this has not happened in low- and middle-income countries where the access to the vaccination is limited mainly by the high cost, and therefore most women and girls at most risk cannot be protected. As a secondary prevention strategy, progress has been made in cervical precancer screening and treatment, but we must accelerate this momentum to reduce incidence and mortality worldwide to the meager rates found in wealthier countries [5]. In this sense, given that the access of the different CC prevention strategies is not equitable between countries or even inside of each country, due to the differences in infrastructure and access to health care systems so marked that we could find, it is necessary to search new tools and screening strategies. One of these strategies could be constituted by the markers present in the scent of CC cells.

The analysis of odors or volatile biomarkers emitted by cancer cells is of great value in the development of new diagnostic tests as low-risk methods for the early cancer diagnosis and a regular screening for all women, including the marginalized or disadvantaged. These volatile signatures are present in different biofluids and show a physiological status. In cancer, the analysis of these molecules has been demonstrated as a rapid and noninvasive alternative by analytical and biological ways as the use of trained dogs for the detection of several cancers.

Dogs can smell a trace of volatile odorous molecules or biomarkers (parts per trillions) emitted in different biofluids [6]. They have an extraordinary ability to recognizing odorous biochemical signature expressed only in ailing individuals but not in healthy individuals, in much earlier and better ways and with an accuracy comparable or superior to readily available sophisticated diagnostic instruments of the present time [6, 7]. The extraordinary canine sense of smell could avoid the unnecessary painful procedures on patients and minimize the time and expenditure on the diagnosis made through the biopsy and other tests having compromised sensitivity, specificity and predictive values resulting into inadequate accuracy. In CC, it could be an effective promissory weapon in fighting this disease and saving women's lives [7].

2. Cervical cancer as an important public health concern related to a virus infection

Among of the gynecological cancer types, CC must be the most detectable cancer due to access to the anatomical target. Unfortunately, this cancer type is the fourth most frequent cancer in the female population worldwide. More than 85% of cases occur in developing countries in which this malignancy is a public health concern due to its high mortality rates, provoked at least by late detection and lack of coverage for screening procedures [8]. Prior to the appearance of CC, women
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…develop a precancer period that spans approximately two decades. During this extensive period, cervical epithelium cells present morphological and molecular changes that could not be typical of a healthy state neither of cancer; they are in transition state, as in the “limbo”. Thus, these abnormal or precancer cells are known as squamous intraepithelial lesions (SIL) classified as low grade (LSIL) if they only affect the first third of the cervical epithelium, or high grade (HSIL) if they affect more than 50% of cervical epithelium layer [9].

Like other cancers, CC is a multifactorial disease, although there are different risk factors that could be controlled to prevent the development and progression of precursor lesions to invasive cancer. Among these risk factors are the onset of active sexual during the teen period, multiple sexual partners, overuse and uncontrolled of hormonal contraceptives, drug abuse, inadequate and overuse of antibiotic regimens, lack of protection during sexual intercourse and absence of routine gynecological inspections, among others; however, the main etiological factor associated with this type of cancer is the persistent HPV infection considered as sexually transmitted infection [10, 11].

The HPV is an infectious agent that is transmitted through sexual contact and affects the anus-genital and oropharyngeal tracts. This involves the transmission of one or more viral genotypes that infect the epithelia, which ones are classified as low risk (LR-HPV) and high risk (HR-HPV) according to their carcinogenic potential. Persistent infection by any of the HR-HPV genotypes is the cause of the vast majority of SIL [12]. It should be noted that the process of cellular transformation of normal cells to precancerous cells and invasive carcinoma involves a long period of time, as already mentioned, which makes the prevention of this neoplasm 100% feasible through the SIL screening.

3. CC and SIL screening

There are two approaches for CC screening and its precursor lesions. The first one is a cellular level approach (micro) involving cervical Pap smear cytology, and the second one is a tissue level approach (macro) through visual inspection iodine Lugol (VILI) or visual acid acetic inspection (VIA) and colposcopy [9].

A conventional Pap smear, which involves removing epithelial cells from the surface of the cervix with a brush (cytobrush or cervix brush) or spatula and then transferring them to a glass slide where they are prepared with Pap stain to be examined by a cytotecnologist or pathologist and discriminate between normal and abnormal cells by using conventional light microscopy. Colposcopy involves the inspection of the cervical tissue through a coloscope which allows magnification of the cervix up to 40X. The solutions already mentioned can be used to reveal changes in maturation, differentiation or abnormal epithelium vasculature that indicate the presence of SIL or CC [13]. It is worth mentioning that in the last years HPV testing is replacing cytology as the preferred cervical screening method; however, the interpretation of the HPV testing must be carried out with reservation [14].

HPV infection is one of the most prevalent sexually transmitted infections, generally symptomatic, with a worldwide prevalence in women with normal cytology of 11.4% and 99% in CC cases. Nevertheless, HPV infection is a necessary but not a sufficient cause of CC. Therefore, the positivity rate of HPV test is higher than cytology and that most positive test results do not indicate a high absolute risk of CC [15, 16]. These results could interpret as “false positive” CC screening results according to the use of this test as a screening tool for this cancer. This does not mean that HPV is not present; instead, we are referring to the detection of only an HPV infection that is not destined to cause CC [14].
4. Why is there CC?

As we know, CC is a preventable disease, and it has been shown that cervical cytology or Pap smear has decreased the mortality rate of CC in developed countries. Unfortunately, this has not happened in low- and middle-income countries in which almost 9 out of 10 cervical cancer deaths occur, continuing as a priority health problem due to different social and technical factors involved [2].

CC screening program needs to be sufficiently accurate and acceptable for the target population by way of allowing the early detection of the disease and the triage of screen-positive women who requires colposcopy or treatment. To ensure the effectiveness of the screening, it is necessary a coverage rate of at least 80% of the population [2, 17]. Nevertheless, average Pap smear coverage is approximately 18.5% in developing countries, 63% in developed countries and 39.6% across the globe. These percentages could differ in each country but are clear that in any case, the coverage of Pap smear needs to be improved [18].

Pap smear and colposcopy are highly invasive methodologies since they require the introduction of a vaginal speculum to gain access to the cervix, thereby compromising the intimacy of the woman. Most of the screened women suffer shame, pain, inconvenience, or nervousness during the screening procedure, or can experience lower abdominal pain or vaginal bleeding in the days following the test. Women have reported a lack of information before or during gynecological inspections, and sometimes, they have referred a disrespectful attitude and a lack of engagement from the medical staff, resulting on women delaying their gynecological inspection or avoiding it altogether [19].

There are other social aspects interfering with the coverage of Pap screening in the risk population. Inadequate knowledge about the purpose and benefits of Pap smears, the fact that many screened and non-screened women do not know the meaning of an abnormal result. The fear and anxiety of having cervical abnormalities which affect the future decision to have a Pap test, social and health inequalities between women as a lack of health insurance, faults of organization in health-care programs involving in the appointment scheduling and the long waiting times to get a result, religious beliefs, taboo, fear of stigmatization, etc. [20, 21].

There are several technical limitations of the Pap smear screening as the specimen collection that imply collection and processing time, the procedure as the smear is taken, the quality of the samples, processing standards, lack of training of cytotechnologists for the accurate interpretation of results, the loss of concentration and fatigue that suffer by a repetitive task (up to 50 times a day) of visualization of slides. This provokes the rates of false-negative that can conduce to an increase in the cost CC screening and bad prognosis for the patients, as well as, the false-positive results that could cause psychological stress, overdiagnosis and overtreatment [9, 13].

5. OMIC era in the diagnosis of the diseases: volatilome and volatolome

To find alternative tools in cancer diagnosis in an earlier and more precise manner, researchers have explored the use of Metabolomics, specifically the volatile organic compounds (VOC) to detect these complex diseases.

VOC are carbon-based chemicals, volatile at room temperature and pressure, and source of most odors. Being produced during metabolic processes in millions of cells simultaneously, thus they are potentially releasing in an extracellular way on a detectable scale and may be emitted from different areas of the body prone to odor production e.g., scalp, axillae, feet, groin, oral cavity. These also can be
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excreted through different biofluids as saliva, breath, blood, sputum, feces, sweat, urine and may serve as ideal clinical biomarkers for several pathophysiological processes. The entire set of VOC produced by an organism is called Volatilome, and their accumulation inside and outside of the body reflects a unique metabolic state in an organism [6, 22, 23]. This knowledge is too old; the ancient Greek and Chinese human noses were the first to identify and describe the diagnostic potential of VOC in the diseases through the smell of different biological samples such as urine and sputum. Based on this ancestral knowledge, we know the VOC potential in the medical field, and ever since, our sense of smell has been used in medical practice as a more precise and less invasive diagnostic tool for the detection of several diseases [24]. Among the several diseases characterized by a specific odor are diabetes (rotten apple odor in the breath), scurvy (putrid body odor), cholera (rice water), trimethylaminuria (rotten fish-like odor in the breath, vaginal fluid, sweat and urine), phenylketonuria (musty odor), cystic fibrosis (chloride), or typhoid fever (baked bread body odor), etc. [25, 26].

Interestingly, in the last few decades, the diagnosis potential of VOC has focused on the search of the volatile profiles of many cancers in all the biofluids as the urine, feces, exhaled breath, and saliva of patients. The rationale for this is that cancer cells have different metabolic or biochemical requirements in comparison from normal cells, due to the genetic alterations that acquire and that allow them to proliferate outside the context of normal tissue development [27]. Therefore, the metabolic and bioenergetic alterations presented by tumor cells lead to a VOC profile different from that of healthy cells. These VOC profiles are useful for the diagnostic of cancer, predict patient response towards chemotherapies or treatment and monitor disease recurrences [28].

For example, the lack of sensitive and specific biomarkers for the early detection of prostate cancer led a Portuguese research group to investigate the performance of VOC present in the urine of patients as potential markers for this cancer in a metabolomic approach based on the analytical tool, the Gas Chromatography–Mass Spectrometry (GC–MS), finding a urinary profile of VOC different from that of cancer-free subjects with 78% sensitivity, 94% specificity and 86% accuracy [29].

A research group in the UK assessed the utility of VOC as feces biomarkers for colorectal neoplasia by headspace extraction followed by GC–MS. This group found that Propan-2-ol was the volatile organic compound most strongly associated with cancer, and 3-methylbutanoic acid or DL-menthol was the only volatile organic compound negatively associated with cancer. These VOC showed a diagnostic ability of sensitivity 87.9% and specificity 84.6% in the identification of colorectal adenocarcinoma [30].

Another example was research in which the gastric cancer was correlated with specific VOC biomarkers in the exhaled breath of a South American population. The exhaled VOC were analyzed by GC–MS and by a chemical gas sensor based on gold nanoparticles functionalized with octadecylamine ligands. Six VOC showed statistically significant differences between the cancer patients and the controls group (e.g., hexadecane and octadecane in the gastric cancer group, while eicosane and 1-cyclohexyl-2-(cyclohexylmethyl) pentane were identified as biomarkers in the control group). The sensor data responses to the breath samples yielded 97% accuracy, 100% sensitivity and 93% specificity [31].

Recently in Japan, the salivary metabolomic profile of oral squamous cell carcinoma was established through VOC analysis as potential biomarkers for the diagnosis of oral cancer through a method combining thin-film microextraction based on a ZSM 5/polydimethylsiloxane hybrid film coupled with GC–MS in saliva samples of oral cancer patients and healthy controls in which eighty kinds of volatile metabolites that were detected and identified and were classified as alcohols,
ketones, hydrocarbons, aldehydes, organic acids, esters, phenols, etc. Among them, twelve COV were selected as potential oral cancer biomarkers for their use as non-invasive tools for the possible diagnosis of this cancer [32].

The above were just some examples of the vast literature that currently exists on the analysis of VOC for the diagnosis of different cancers through non-invasive samples and analytical methods.

6. Analytical chemistry (electronic noses) in VOC detection

As it was mentioned already, the analysis of VOC as cancer biomarkers in diverse biofluids is desirable because it allows a repeated sampling and a non-invasive and quick analysis. In this sense, there is great potential for the development and clinical application of VOC analysis in the diagnosis and monitoring of cancer [33].

The number of studies demonstrating the potential of VOC in cancer diagnosis has increased in the last decades due to analytical chemistry advancements that have made possible the quantitative analysis and comparison of VOC of cellular origin [6, 23].

From the several analytical techniques that exist, the GC–MS, Selected Ion Flow Tube Mass Spectrometry (SIFT-MS), Proton Transfer Reaction Mass Spectrometry (PTR-MS), Proton Transfer Reaction Time of Flight (PTR-ToF) and Ion Mobility Spectrometry (IMS) have been the most used to separate and identify VOC. These are sophisticated and valuable stationary analytical chemical instruments for the discovery of biological scents [34].

However, despite the low detection limits and high sensitivity offered by these methodologies, they present certain limitations that have prevented their routine application as screening methods. These require high levels of technical expertise and lengthy instrument run times (tens-of-minutes to hours) for detailed chemical analysis. Most of them, exceptionally high-resolution mass spectrometers, are extremely expensive and require expert maintenance. Furthermore, data interpretation, especially for non-targeted analyzes, may initially take many hours per sample until sufficient statistical results are accumulated to develop a targeted approach. Finally, they require infrastructure and trained personnel for their operation [35].

7. Dogs (biological noses) as clinical tools in cancer detection

Dogs have excellent odor detection capabilities in a vast range of fields. Their olfaction is a fundamental sense that let them perceive and comprehend the world around them. Humans have harnessed the canine sense of smell for the detection of different targets as an orthodox manner, such as explosives, land mines, narcotics, missing persons (forensic area), and invasive or endangered species [34, 36]. Right now, in this pandemic situation worldwide, dogs have been trained for COVID-19 early detection [37]. The question arises, why not use the canine olfactory for cancer detection?, nevertheless in the last decades, the use of canine olfaction as a diagnostic tool for identifying preclinical disease, especially cancer in biological samples has increased [34, 38].

Nowadays, there are a considerable number of publications using trained dogs to sort biological samples for follow-up and future diagnostics [31]. Several authors have published research suggesting that dogs can sort dozens of samples, including blind replicates and known control samples in a few minutes and may be able to detect lung, breast, prostate, ovarian, and melanoma cancers by smelling skin lesions, urine, exhaled breath, and surgically extracted tumors [35, 39].
The first report of dogs’ potential to detect cancer was published in 1989 in the UK when a pet dog spontaneously detected its owner’s melanoma, saving her life. After it, several additional cases of spontaneous cancer detection by dogs were reported, this caught the attention of the scientific community, and canine olfaction began to be used in the search for increasingly sensitive and specific diagnostic techniques for diseases as cancer where mass screening and early diagnosis could be improved [40].

In pilot work, a research group demonstrated the validity of using dogs as a biological system to examine exhaled breath in the diagnostic identification of lung and breast cancers. Its results showed an overall 99% sensitivity and specificity for canine scent detection among lung cancer patients and controls compared to biopsy-confirmed conventional diagnosis and 88% of sensitivity and 98% of specificity among breast cancer patients and controls [41].

Another research group in France, trained a Belgian Malinois shepherd by the clicker training method for prostate cancer detection on human urine samples. Its results showed that dogs could be trained to detect prostate cancer by smelling urine with a significant success rate (91% of sensitivity and specificity) suggesting that prostate cancer gives an odor signature to urine and it could be used as a potential screening tool [42].

In another research study, the ability of dogs to detect ovarian cancer from plasma samples was evaluated and how the odor associated to this cancer is affected by the treatment to reduce tumor burden, including surgery and five courses of chemotherapy. The dogs showed high sensitivity (97%) and specificity (99%) for the detection of ovarian cancer patients’ plasma and indicated positive samples from patients who had recurrences. For the above, the dogs offer an outstanding assessment of ovarian cancer prognosis based on the specific odor in the blood which could enhance primary diagnosis and enable earlier relapse diagnosis and consequently an increase in patient’s survival [43].

At present, Medical Detection Dogs in the UK an organization that is at the forefront of innovative research in the dogs’ ability to detect the smell of human diseases and save lives. This organization focuses on detecting the VOCs associated with prostate cancer and colorectal cancer using trained dogs as a non-invasive method that can detect cancer at an early stage could both increase uptake of the screening and improve health outcomes [44].

Another current medical innovation research program is KDOG sustained by French Institute Curie (Paris) who is elaborating a simple, non-invasive, and cost-effective breast cancer screening method, based on canine detection. This method is contactless between the animal and the patient and the dogs’ success rate in cancer detection that has been reported was about 100% [45].

8. Canine detection of cervical cancer

Issues concerning CC detection make it necessary searching for alternatives that help to increase the early screening coverage with greater percentages of sensitivity and specificity in screening and diagnostic tests. The introduction of methods capable of detecting virtually invisible -a single molecule among a billion or trillions of compounds- changes in the cell through the analysis of cells “odor” have much in their favor in practical applications. In this way, the key could rely upon the poorly explored field of the metabolomics of the cervicovaginal epithelium. Biotechnological and analytical systems such as dogs and analytics as GC–MS may be alternatives to current tests which leaves us with two good panoramas: 1) a laboratory analytical test; 2) a biotechnological field test; both of which use a “volatile
biopsy”, basically a scent sample, obtained without penetrating the human body at all. Our work team has proposed a device specifically made for this purpose [46, 47].

This device that our research group has developed is a gadget worn by the patient for a defined period, after which is simply stored in a container -provided by us- and mailed to the recipient, avoiding the stress of queuing in a hospital. Our device quickly collects in an unorthodox manner the VOC of the genitourinary tract allowing us, to use analytical devices GC–MS system for sample examination, with the surplus of being simple to dispose of after analysis is done. Each sample being scanned in one hour. Afterwards, the metabolic profile is reviewed by an expert to determine if there are any volatile biomarkers associated with the sample.

In this unorthodox scenario (for some people), this device is scanned by a trainer-dog binomial test carried out in seconds. In such an analysis, our gadget eliminates the shock some people could have by watching a dog “deciding their fate”. Our results in both scenarios show that a sample's VOC profile result by the analytic test and detected by a trained dog, discriminates between cancerous and non-cancerous samples with more than a 90% sensitivity and specificity. These data are correlated afterwards to histopathological observation as the gold standard, suggesting that the device has a great value proposition [46, 47].

These proposals represent the ideal diagnostic tests for screening CC because they are non-invasive, low cost, accurate and partially portable, therefore meeting the requirements for a good screening test according to WHO. This established that a screening test must be sufficiently accurate to detect the condition earlier than in the absence of screening [48].

Recently another research group in Japan trained a dog to distinguish urine samples from cervical cancer patients from those of the controls, showing that cancer detection by dog sniffing can be a non-invasive, cost-effective screening technique for CC [49]. This report supports our proposal that the canine nose can be used and developed for CC detection.

The use of screening dogs is a real issue, for instance, they play vital roles helping in natural disasters or detecting drug or weapons trafficking as we have mentioned before; in the case of GC–MS itself is used again in the detection of drug trafficking, anti-doping or as a standard test in food products; then, why then should not they be used in the health-care industry? An example is the exhalomic test “Hearts Breath Test for Grade 3 Heart Transplant Rejection detection”. Dr. Phillips et al. at Menssana Research Inc. in New Jersey USA developed this FDA approved test. This test detected a specific metabolomic profile and had opened a vast opportunity in the marketing of metabolomic or volatolomic tests [50].

9. A big challenge and an alternative

Pap test was not specifically developed to detect neither human cervical lesions nor HPV infection [44]. Moreover, it has not been subjected to a rigorous analysis regarding its sensitivity and specificity; however, it is the accepted test for detecting cytomorphological changes in the cervicovaginal epithelium but not for HPV. Epidemiological studies show that HPV detection does not necessarily indicate cancer, so it is considered necessary but not enough for CC development [51].

On the other hand, the CC research has served to define that prior to this type of invasive lesions, there are precursor or pre-invasive lesions as SIL, thanks to epidemiological studies, it has been determined that less than 10% of women infected with HPV will develop CC [52]. So far, it is unknown what factors are indispensable for the progression of these lesions causing “headaches” among oncologist and gynecologists alike, for nobody knows which SIL will progress to a more aggressive
lesion. Many questions result in no concrete answer. Given these facts, the new OMICs area opens the opportunity for the early detection of pre-invasive lesions. Combining analytical and biotechnological procedures as aforementioned will permit the basis for new portable nanosystems.

In summary, our proposals are affordable and accessible for any women and could be an important weapon in this war against CC as a preventive measure deployed by health services, all this granting the public and government departments accept them.

10. Conclusions

Early detection saves lives. Therefore, it is necessary to implement new and alternative technologies that allow the development of accessible diagnostic methods that cover at least some of the limitations presented by conventional screening tests for the detection of diseases such as cancer, especially Cervical Cancer.

The canine detection of odors or volatile profiles emitted by cancer cells is a portable, highly sensitive and specific tool which could be used as a screening alternative (as fast track) in marginalized or areas of difficult access (even in the urban regions) to increase coverage in high-risk populations. Additionally, the use of a trained dog for screening could facilitate prevention campaigns, saving money, time, labor, and lives due to an early diagnosis.

The CC and its precursor lesions detection is a priority health concern in different countries, therefore having an analytical alternative (GC–MS) and a biological alternative (canine smell) for screening could be a great support technique for conventional methods offering a non-invasive, fast, and accurate detection that can be carried out repeatedly and that would also be useful for monitoring the disease.

Applications for these tools extend to providing much needed medical attention for women from cultural backgrounds imposing several prohibitions, deep-rooted cultural taboos, religious beliefs, shame, or lack of health coverages. We thought that a “with a little help” to current methods by using improved, non-expensive and innovative procedures will conduct to accurate and timely diagnostics for this cancer type. Unfortunately, for both analytical and bio-detection methods, there is no consensus in the methodologies used or in the results obtained, thus this is a call to join forces with the scientific and social communities (research groups) for the replication of the studies that lead to the future implementation of these methodologies for clinical diagnosis.

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