Detection of human ovarian carcinoma from blood samples using scent dogs

Detecção de carcinoma do ovário humano a partir de amostra de sangue com o uso de cães de detecção de odores

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Abstract

Ovarian cancer is the most lethal of all common gynecologic malignancies, with more than 204,000 new cases and 125,000 deaths/year worldwide. Currently, there are no acceptable screening techniques available. Body odors are the result of volatile organic compounds that are originally secreted from various cells. Tumors likely have distinctive odors that can be recognized by trained dogs.

In a double-blinded test, three detection dogs were trained to identify blood samples obtained from patients with ovarian carcinoma. Animals where presented to three equal copies of five different test sets, each comprising five samples. Each set had a positive target sample, non-ovarian malignant tumor sample(s), healthy donors sample(s) or benign tumor sample(s).

Individual success rate was 40%, while if an identification was considered when two or more dogs mark the same sample, success reached 60%. A malignant sample was identified 64.45% of the times. If the identification was made by two dogs at the same time, malignant samples were identified 80% of the times.

The present study consists, in the authors' knowledge, the first description of the use of scent dogs to detect ovarian tumors from blood samples, when up against blood samples containing any other possible type of tumor.

Keywords: Dog; Tumor detection; Blood; Ovarian carcinoma.

INTRODUCTION

Ovarian cancer is the most lethal of all common gynecologic malignancies, with more than 204,000 new cases and 125,000 deaths each year, accounting for 4% of all cancer cases and 4.2% of all cancer deaths in women around the world[1]. In Portugal, according to the National Oncologic Registry in 2001, the incidence of ovarian cancer was 8.3 per 100,000 people[2].

Early ovarian cancer is not associated with symptoms therefore detection is often by fortuity. Mortality rate is high, as approximately 70% of ovarian cancers are diagnosed at an advanced stage [International Federation of Gynecology and Obstetrics (FIGO) stage III or IV], and only 30% of women with such type of cancers can expect to survive 5 years[3].

Currently, there are no acceptable screening techniques available. Though, early detection strategies to identify ovarian cancer precursor lesions or early-stage carcinomas should theoretically have a major impact on mortality and survival in patients[1,3]. New insights and approaches should be considered, leading to the development of ground-breaking detection techniques

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and therapeutic interventions.

Body odors are the result of volatile organic compounds (VOCs) that are originally secreted from various cells inside the body via metabolic pathways[4]. Consequently tumors, which secret VOCs, are likely to have distinctive odors that can be recognized by dogs, with their outstanding olfactory acuity[5–7]. Scent information is helpful in elucidating the cause of disorders. The therapeutic targets for some oncologic or metabolic diseases could be identified if we elucidate the mechanisms underlying the production of specific odors. Microanalyses of VOCs from biological samples and investigation of the biosynthetic pathways that produce the relevant VOCs from patients may lead to a better understanding of pathophysiological mechanisms that cause a particular disease[4].

Fluids secreted or excreted from a living organism provide a unique window into its biochemical status since the composition of a given biofluid is a consequence of the function of the cells that are intimately concerned with the fluid's manufacture and secretion[8]. The composition of a particular fluid can carry biochemical information on details of organ dysfunction and disease and ascertain potential tumor markers.

Prior work suggested that dogs trained to smell human samples could recognize bladder, breast, colorectal, lung, prostate and ovarian cancer with various success rates[6,9].

The majority of studies available rely on detection from body samples that were in direct contact with the target tumor. Willis et al reported that dogs can distinguish urine from patients with bladder cancer with a mean success rate of 41%[6], whilst Cornu et al reported that trained dogs could be conditioned to recognize prostate cancer among controls by sniffing urine (both sensitivity and specificity of 0.91)[10]. Sonada et al reported sensitivity of 0.97 and specificity of 0.99 in the canine scent detection of colorectal cancer in watery stool samples, furthermore, the accuracy was even higher for early-stage cancers[9]. McCulloch et al reported that ordinary household dogs can be trained to distinguish breath samples of patients with lung and breast cancer from those of control volunteers with high accuracy (sensitivity and specificity of 0.99 and 0.99 in lung cancer and 0.88 and 0.98 in breast cancer, respectively)[11].

In studies that approach the detection of ovarian cancer[12,13], dogs were able to correctly identify ovarian cancer tissues and blood samples collected from those patients, when compared to other gynecological tumors.

The objective of this study was to evaluate whether trained dogs could detect ovarian cancer from human serum samples.

MATERIALS AND METHODS

The protocol of this study was approved by the Ethical Committee of the IPOLFG (Proj. UIC/772 extended in May 3rd 2012) and complies with the NIH guidelines for Humane Care and Use of Animals.

Three drug detection dogs (Canis lupus familiaris) from the Grupo de Intervenção Cinotécnico da Guarda Nacional Republicana (GIC-GNR) where chosen. These were Labrador Retriever female dogs, with a mean age of 6 years old (range 5-7 years), housed in Grupo de Intervenção Cinotécnico facilities, receiving regular technical and physical training, as regular veterinary assistance and care.

Biological samples

Blood samples were obtained from patients with clinical suspicions of an ovarian tumor. Written, informed consent, was obtained from all patients. The samples were stored in polymer tubes and after coagulation, centrifuged for 3000 rpm for 10min. Plasma was then collected to be used in the training phase after histological confirmation of an ovarian carcinoma. CA-125 values of all blood samples diagnosed as ovarian carcinomas used were >500 IU. Training and testing samples had a volume of 40µl, were contained in regular Eppendorf containers and were prepared in a similar fashion. Blood samples for training and testing were collected and stored within the same time period (2015 and 2016).

All samples submitted for training and testing had a histological diagnosis that was reviewed and confirmed by one author (AF). In all testing samples used from patients with malignant tumor not ovarian carcinoma, the CA125 value ranged from 6,8 to 472 IU with a median of 25.15 IU (mean value 82.02 IU). The CA125 values of malignant ovarian carcinomas used for testing ranged between 200 IU and ≥ 19 779 IU with a median value of 702.4 IU and a mean of 5067.6 IU.

All adnexal carcinomas used for testing were high grade serous type, diagnosed at stage 2 to 4 (FIGO) from ovarian and/or Fallopian Tube.

Animal training

Due to the specific mission of these dogs, they were already conditioned to give a desired response (pointing, during which the dog should stay with his head fixed a few centimeters towards the opening of the target odor recipient) when in the presence of a target odor. They were also trained to conduct search in metallic stands (that allowed the animal to scent but not to touch the sample). Using an indirect target and positive reinforcement, the odor of blood serum obtained from patients with ovarian cancer was introduced as a target odor. A discrimination process was then conducted, presenting the animal with different other serum samples, prepared in a similar fashion as target samples, obtained from patients with different types of tumours or healthy donors. All training and testing sessions were conducted in an odor discrimination lab available at the GIC-GNR facilities and followed the training guidelines of the FRONTEX Agency (European Border and Coast Guard Agency). All samples where manipulated by the same operator.

Double blinding

In a double blinded test, the animals where then presented to three equal copies (one to three) of five different test sets (codded one to three), each comprising five samples (identified with the set number followed by the sample position). Each set had a positive target sample (containing a blood sample from a patient diagnosed with ovarian carcinoma) and a non-ovarian malignant tumour sample. The remaining samples were randomly divided between samples from healthy donors or patients with a benign tumour. The order by which the samples were arranged within each set was randomly assigned.

All animals went through all sets, one at a time and the order in which they entered the test was randomly assigned. The sample they marked as positive was then registered and when all animals have passed through a set, the samples were discarded and a new set was placed for test. The animals were presented with the same sets three times, in different days, and all sessions were video recorded. With regard to try to guaranty an optimal performance, testing and training sessions were intercalated randomly.

Statistical Analyses

Results were then analyzed in order to identify success rates and concordance correlation coefficient. Stata software (StataCorp. 2017. Stata Statistical Software: Release 15. College Station, TX: StataCorp LLC) and MedCalc Statistical Software version 15.8 (MedCalc Software bvba, Ostend, Belgium; https://www.medcalc.org; 2015) were used to analyze data.

RESULTS

Each one of the animals included in this study conducted 15 test situations, which were conducted over a period of four nonconsecutive weekdays.

When considering individual success rate (as defined as a correct identification of the target sample), it was of 26.67% in the first copy and of 46.67% on the second the third copies (mean value 40%). The positive sample of set 1 was the one identified correctly the largest number of times (success rate of 78%). If a successful identification is considered when two or more dogs mark the same sample, the success rate reaches the highest value in the second copy of sets (60%).

It is interesting to analyze, in the cases were the target sample as not being correctly identified, which samples were. In most of times, it was the sample obtained from a patient with a malignant tumor. If one accounts of the number of time in which a malignant sample was identified (target sample + malignant tumor sample), a mean value of 64,45% was observed, 80% in the first copy of samples, 66.67% in the second and 46.67% in the third. Individually, Dog 1 identified a malignant sample 80% of the times, while the remaining 2 dogs 60% of the times.

In the identification of malignant samples, if we consider a successful identification when two or more dogs mark the same sample, a malignant sample was identified 80% of the times.

DISCUSSION

To the authors' knowledge, this is the first description of a test that accesses the ability of dogs to identify a specific tumor from blood samples from a set that contains samples from a wide range of origins, varying from healthy donors, to patients with benign or any given malignant tumor. Previous studies, describing the efficacy of detection of ovarian carcinoma have been published, but amongst sets containing other gynecological neoplasias[12,13].

The success rate in this study was higher than would be expected by chance, suggesting that tumor detection

based on blood samples is feasible. Still, success rate is lower than the ones presented in previous studies. This may be due to a variety of reasons, as further depicted.

Previous reports have accounted for the ability of detecting "directly" (i.e. a histological portion of the removed tumor[13]) or from body fluids or exhaled air that would be pretty much in direct contact with the tumor[6,9–11,14] (detecting bladder tumors from urine samples, lung tumors from exhaled air of colorectal tumors from stool samples). This may allow for a higher accumulation of volatile components in the sample, making the detection "easier". In this study, we used blood samples based on the premise volatile organic compounds (VOCs) that are originally secreted to the blood stream from various cells inside the body[4]. Even though the detection is possible it may also be "harder", since VOCs from all metabolic process will accumulate in the blood, alongside with the ones produced by a specific tumor.

This confounding effect, added to the fact that different tumor may produce similar VOCs, may account for a majority of incorrect identifications that we observed, since in most of them the dogs were signaling malignant tumors. The malignant tumor that was more frequent signaled was a retroperitoneal sarcoma with a 472IU of CA125, the others that were also sign at least one time were an ovarian metastasis of a breast carcinoma (CA125 = 7.8IU) and a uterine pleomorphic sarcoma (172 IU – CA125). No differences were found regarding CA125 and disease stage and detection.

An additional difficulty is related with the nature of the samples using during training. If training is conducted using samples obtained from a small number of patients, with is possible that the animals will learn to identify the odor that specifies those sample and not a specific target odor. This discrimination process is essential and has to be kept and reinforced during the entire dog active life of detection. Larger sample volume, in contrast to the volume we used (40µl), may aid in this process.

The fact that the animals included in this study were active working animals, and that this specific detection training was and addition to the normal workload may account for lower target odor detection rates. It is reasonable to assume that an animal fully dedicated and trained to this specific purpose will have better results.

An additional observation was that some samples seem to be "easier" to identify. The positive sample that was included in set 1 has individually identified correctly 78% of the times and, if considering a correct identification when 2 or more dogs marked that sample, 100% of the times. This may lead to an alternative use of dogs in this field, that is has a preliminary step in the definition of a screening laboratorial test. After a positive identification by a dog or group of dogs, sample composition may be analyzed thus arriving to a specific marker. In this study, this variation of ease may also be related with the origin of the sample itself. The samples used in the animal training were not purposely collected, but were the remaining volume after diagnostic and biochemical testing were made for that patient. It is also possible that the least manipulated samples would contain higher VOC concentration.

Dogs, as humans, show variations in performance through time. A decrease in performance, particular in this field of application, may not be acceptable. This can be counteracted by the use of more than one dog, leading to increased consistency and detection rate, as showed by our results.

CONCLUSIONS

The present study consists, in the authors' knowledge, the first description of the use of scent dogs to detect ovarian tumors from blood samples, when up against blood samples containing any other possible type of benign or malignant tumor.

Overall efficacy was lower than that of previous reports, making additional studies, enrolling a larger sample and variety of samples, necessary in order to ascertain the feasibility of the use of scent dogs in the screening of different tumors from blood samples.

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