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Trained dogs can identify malignant solitary pulmonary nodules in exhaled gas

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ABSTRACT

Objectives: To investigate the capacity of a trained dog to identify LC in patients with malignant SPN.
Methods: We collected 90 exhaled gas samples from 30 patients with SPN (3 samples/patient). As controls we used 61 healthy volunteers and 18 COPD patients without SNP or LC, in each of whom we collected 5 exhaled gas samples (n = 395). The dog (Blat, a 4-year-old crossbreed between a Labrador Retriever and a Pitbull) and the methodology used were the same as previously reported by our group (see: https://drive.google.com/open?id=1R4mOtOtuZkTeb5iOEEv0K9r2kHKlPhWd).
Results: Of 30 patients with SPN, Blat recognized 27 of them as positive for LC and 3 as negative for LC. These results fully matched post-surgical pathological results. Sensibility was 0.97, Specificity 0.99, Positive Predictive value 0.97 and negative predictive value 0.99. The AUC of the ROC curve was 0.985.
Conclusions: Trained dogs can identify accurately the malignant origin of SPN. It is now time to develop technology that can match canine olfaction and facilitate the implementation of this diagnostic approach in the clinic.

1. Introduction

Lung cancer (LC) can be cured if diagnosed in early stages and removed surgically [1]. Unfortunately, this is not the case in most patients, who are diagnosed when the disease is already advanced [2]. Several potential strategies can improve early LC diagnosis, including population screening with computed tomography (CT) [3–6], exhaled breath analysis [7], and circulating tumor markers assessment [8,9]. A large, multi-center study in the USA showed that CT screening improves early LC diagnosis and reduces all-cause mortality by 20% [3]. These results have been recently confirmed in another large European study [5]. Both studies, however, often identified small solitary pulmonary nodules (SPN) whose precise diagnosis may require invasive procedures which, in turn, are not exempted of potential complications.

We recently showed that a trained dog can recognize the presence of LC by smelling the exhaled gas of patients with a high sensitivity (0.95 [95%CI 0.93,0.96]), specificity (0.98 [95%CI 0.98,0.99]), positive (0.95 [95% CI 0.93,0.96]) and negative predictive value (0.98 [95% CI 0.98,0.99] [7]. However, about 70% of patients included in that study had advanced LC stages [7]. Hence, the capacity of the trained dog to diagnose SPN remains untested. The current study sought to evaluate this possibility.

2. Methods

2.1. Study design and ethics

This is a prospective and controlled study that explores the diagnostic performance of a trained dog to identify the presence of LC in patients with and without SPN (defined as lung lesions of unknown pathology identified by computed tomography (CT) of the chest, with a diameter < 3 cm, without significant size mediastinal nodes and no

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other distant lesions) recruited from Hospital Clinic and Hospital Universitari Sagrat Cor of Barcelona (Spain). According to the recommendations of our multidisciplinary LC committee, all these SPN required diagnostic and/or therapeutic surgery for their appropriate clinical management, so the results of the pathologic report after surgery could be contrasted with those obtained by the dog. This project was approved by the Ethics Committee of our institution (2012/7899) and all participants signed their informed consent.

2.2. Participants

During one year (from April 2017 until May 2018) we collected exhaled gas samples (3/ participant) from all patients seen in our institutions with SPN defined as above (n = 30) that were removed by surgery. As controls we used 61 healthy volunteers and 18 COPD patients, all of them without SPN on CT (obtained for a variety of clinical reasons other than LC). In each of these controls, we collected 5 exhaled gas samples (n = 395) because in each test the dog is exposed to one patient but four controls, so a higher number of available control samples is necessary for testing Exclusion criteria were a pulmonary lesion bigger than 3 cm, any malignant disease other than LC, previous treatment with chemotherapy or thoracic surgery, tracheostomy and any endoscopic procedure during the seven days that preceded the collection of exhaled gas.

2.3. Exhaled gas sampling

Exhaled gas samples were obtained following the same methodology we used in our previous publication [7]. In brief, participants refrained from ingesting any food or drinks, as well as smoking, 30 min before they exhaled inside a 15 cm crystal cylinder tube opened on both ends and filled with hydrophilic and hydrophobic wool, sealed with silicon taps and stored until exposed to the dog, no longer than three months, as we did in our previous report [7].

2.4. Exhaled gas testing

The trained dog used in this study ("Blat", a four-year-old crossbreed between a Labrador Retriever and a Pitbull) was the same as the one used in our previous publication [7]. The training methodology followed is detailed in this earlier publication, but it involved being exposed to individuals with LC (n = 20), most often with advanced stages of the disease (n = 15) and without it (n = 5) [7].

The canine testing method used here was also the same as reported earlier [7]. In brief, tubes containing the exhaled gas samples were inserted in wooden boxes that had an open end to allow the sniffing of the sample. Each time, the dog was confronted with 1 sample obtained from a participant with SPN (n = 1) and 4 samples obtained from individuals without SPN. The location of the wooden box with the SPN sample was randomly distributed among the other four ones. The dog was asked to identify the latter (SPN) by sitting next to that wooden box. This process was repeated (altering the distribution of the wooden boxe) 10 times for each sample from a patient with a SPN (30 patients x 3 samples/patient = 90 samples), adding up to a total of 900 tests.

2.5. Statistical analysis

Results are presented as n, proportions and/or mean ± standard deviation, as appropriate. The sensitivity (Sn), specificity (Sp), positive (PPV) and negative predictive values (NPV), as well as the Area Under the Curve (AUC) of the Receiving Operator Characteristics (ROC) curve were calculated using standard formulas, as reported in our previous publication [7].

Table 1
Demographic and clinical characteristics of participants.

	Patients with SPN (n = 30)	Healthy subjects (n = 61)	COPD Patients (n = 18)
Male, n (%)	22 (73.3%)	27 (44.3 %)	6 (33.3%)
Age, years	66.4 ± 9.4	44.2 ± 11.1	60.4 ± 7.3
Smoking status			
Never	2 (6.7%)	27 (41.0%)	0
Current	12 (40.0%)	13 (16.4%)	10 (55.6%)
Past Smoker	16 (53.3%)	21 (31.1%)	8 (44.4%)
FEV1/FVC (%)	70.0% ± 15.2	ND	62.9% ± 11.3
FEV1(% reference)	78.0% ± 14.9	ND	65.1% ± 18.1%
Pathological diagnosis			
Lepidic adenocarcinoma	10 (33.3%)		
Adenocarcinoma	11 (36.7%)		
Squamous	3 (10.0%)		
Large Cell	2 (6.3%)		
SCLC	1 (3.3%)		
Benign	3 (10.0%)		
Lung cancer stage			
IA1	4 (13.3%)		
IA2	12 (36.7%)		
IA3	9 (30.0%)		
IB	1 (3.3%)		
IIA	0		
IIB	1 (3.3%)		

ND. Not done.

3. Results

3.1. Patient characteristics

Table 1 presents the main demographic and clinical characteristics of the patients and controls included in the study. Patients with SPN were mostly males with a mean age of 66.4 ± 9.4 years. By contrast, controls included more younger females. There were more smokers within patients with SPN than in controls, but differences failed to reach statistical significance (p = 0.45). After surgery, pathology confirmed that the SPN was malignant in 27 patients (90%) and benign in 3 (10%). Table 1 details the proportion of different LC pathological types. Twenty five of the 27 malignant SPN were stage IA (Table 1).

3.2. Dog diagnostic performance

Blat was confronted with 90 exhaled gas samples from patients with SPN and 382 from controls; 13 of these samples were lost because of broken tubes 6, unable to be opened 3 tubes and 4 samples because the taps were not sealed properly. As reported in our previous study [7], here Blat also sat by the tube corresponding to what he believed was an exhaled breath sample corresponding a malignant SPN (https://drive.google.com/open?id=1R4mOtOtuZkTeb5iOEEv0K9r2kHKlPhWd). When he could not identify it immediately among the five tubes, he kept searching for a while (without sitting by any tube) and the test was interpreted as "no LC" detected.

Of 30 patients with SPN, Blat recognized 27 of them as positive for LC and 3 as negative for LC, matching pathology results most of the time (879/900 tests). Fig. 1 shows that he did not made any mistake in the case of benign SPN and failed in 21 out of the 789 tests (2.6%) when the SPN was finally proved to be malignant. These 21 mistakes occurred in 15 patients (55%) and were distributed as follows: 5 (24%) occurred in the same patient, 4 (19%) occurred in 2 different patients (2 per patient) and the rest (n = 12, 57%) occurred once in the remaining 12 patients. Overall, therefore, the diagnostic performance of Blat showed a Sn of (0.97 [95% CI 0.96,0.98]), a Sp of (0.99 [95% CI 0.99,0.996]), a PPV of (0.97 [95% CI 0.96,0.98]) and a NPV of (0.99 [95% CI 0.99,0.996]), and the AUC of the ROC curve was 0.985 (Fig. 2).

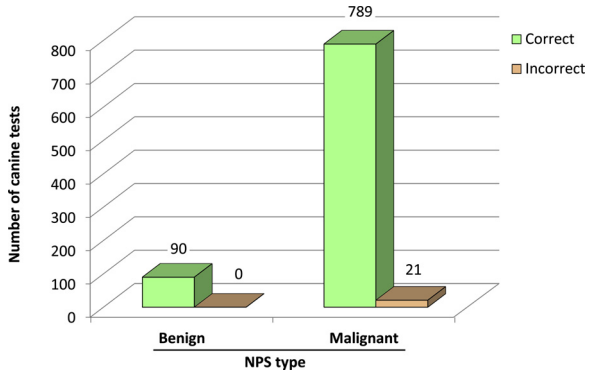

Fig. 1. Number of correct (green columns) and incorrect (red columns) diagnosis by the trained dog in patients with SPN that were eventually proved by pathology to be benign or malignant. For further explanations, see text (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

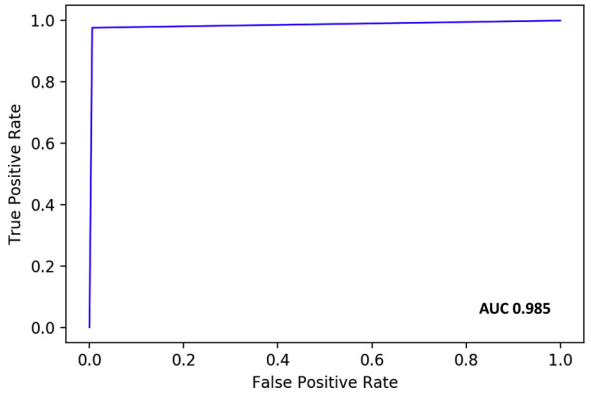

Fig. 2. Receiver Operating Curve and Area Under the Curve (AUC). For further explanations, see text.

4. Discussion

This study shows that a trained dog can identify malignant SPN with extraordinarily high accuracy. Combined with our previous report in patients with larger lung tumors [7], these results prove the principle that the exhaled gas of patients with LC (even when small SPN) contains a distinct odor that can be recognized by a trained dog (today) and ideally, by appropriate technology (tomorrow).

4.1. Previous studies

Numerous previous papers have shown that the olfactory capacity of dogs can detect the presence of several types of human cancer, including LC [7,10–14]. We previously showed that a trained dog had an excellent diagnostic performance in patients with advanced LC [7]. Here we test the hypothesis that the same dog (Blat) can also detect SPN reliably.

4.2. Interpretation of new findings and future challenges

Our results fully confirm our working hypothesis by showing that Blat matched lung pathology of 27 malignant and 3 benign SPN with high accuracy. In fact, the AUC of the ROC curve was almost ideal (Fig. 2). Having established the principle (exhaled breath of patients with LC, independently of size, can be detected precisely), the challenge is now to identify what molecular pattern is the dog smelling. In this context, it is of note that Boedeker et al compared the diagnostic capacity of trained dogs vs. an electronic nose but had to conclude that dogs were more specific and sensitive than the e-nose device tested [15,16]. Further research is needed to characterize the molecular pattern in exhaled breath of patients with LC, so in the future we can rely more on technology than in canine capacity and this diagnostic technique can be implemented worldwide both as an addition to CT LC screening programs or in clinical practice.

On the other hand, it is of note that the dog could identify the malignant origin of the SPN regardless of its pathological classification by histology (Table 1). Whether this argues in favor of the identification by the dog of the malignant tumor itself or, alternatively, in favor of identification of a potential interaction of the tumor with the immune responses to it cannot be addressed from our results and requires further research.

4.3. Potential limitations

Three potential limitations of our study deserve comment. First, we did not calculate formally the sample size of the study. This was because this was a pilot, exploratory, non-interventional study, for which is very difficult to calculate it. Yet, based on our previous observations [7], where the diagnostic accuracy of the trained dog was so high, we speculated that studying 30 patients with SPN and 79 controls should be enough. Further, given that we obtained several exhaled gas samples per participant, the dog was actually confronted to 900 samples, which should be sufficient to support our conclusions. On the other hand, the incidence of SPN is, unfortunately, much lower than that of advanced LC, so there was also a logistic limitation for a higher recruitment into the study; in fact, we included in this analysis all patients with SPN (as defined above) seen in our institutions over a year. Second, it can be argued that any study on diagnostic accuracy of any test requires external validation. To some extent, however, the current study is a "validation" of our previous publication [7] because it included different patients with, actually, much smaller tumors. Third and finally, there were more males among participants with SPN than in controls. This is likely due to the higher prevalence of LC in males than in females, at least in our country today [17]. Thus, we cannot exclude the possibility that the dog may be identifying gender differences rather than the presence of LC, since there were more males among patients with malignant (21 males, 6 females) than benign SNP (1 male, 2 females). Yet, in our previous study, which included a larger population of patients and controls, we did not observe any significant difference in LC diagnostic accuracy by gender ($p = 0.46$) [7].

4.4. Conclusions

Trained dogs can detect LC in exhaled breath gas analysis with high accuracy, even in tumors smaller than 3 cm presenting as solitary pulmonary nodules. This proofs the principle that LC (even at early stages) can be detected by breath analysis. Now, the scientific community needs to develop appropriate technology (breathomics) [18] to transfer this knowledge to clinical practice.

Note

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Transparency document

The Transparency document associated with this article can be found in the online version.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.lungcan.2019.06.008.

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