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SLEEP BREATHING PHYSIOLOGY AND DISORDERS • SHORT COMMUNICATION

A detection dog for obstructive sleep apnea

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Abstract

Purpose We sought to assess whether a dog can be trained to distinguish obstructive sleep apnea patients from healthy controls based on the olfactory detection of urine.

Methods Urine samples were collected from 23 adult male obstructive sleep apnea patients and from 20 voluntary adult male volunteers. Three dogs were trained through reinforced operant conditioning.

Results Two of the three dogs correctly detected two thirds of obstructive sleep apnea patients ($p < 0.000194$ and $p < 0.000003$, respectively).

Conclusions We found that dogs can be trained to distinguish obstructive sleep apnea patients from healthy controls based on the smell of urine. Potentially, dogs could be utilized to identify novel biomarkers or possibly screen for obstructive sleep apnea.

Keywords Canine · Diagnostics · Olfactory detection · Screening

Introduction

The olfactory sensitivity of a dog is 10,000 to 100,000 times that of humans. This stems from the olfactory organ size, neuronal density, the number of functional genes, and the anatomic structures influencing the odorant transport [1]. Olfaction-based detection dogs are utilized by the military, police, and customs authorities to detect explosives, missing persons, or narcotics. In addition, detection dogs have been successfully trained to sniff and identify a number of more peculiar things such as screwworms [2], bed bugs [3], fire ants [4], sarcoptes-infected animals (*Sarcoptes scabiei*) [5], and even cows in estrus [6].

Any disease, such as cancer, diabetes, or infections, causes metabolic changes leading to volatile organic compounds that may be detected from the blood, breath, or urine [7]. Medical detection dogs stem from the olfactory detection of these metabolic changes. Reports on the use of medical detection dogs cover diabetes, cancers, and infectious diseases. For instance, diabetes alert dogs have been trained to react to hypoglycemia. Both successes and failures have been reported [8,9]. The ability of dogs to detect a malignancy, such as melanomas [10,11], prostate cancer [12,13], or lung cancer [14,15], has been studied yielding sensitivities ranging from 56 to 99% and specificities ranging from 8 to 99%. Dogs have also been used to detect infections. A trained beagle had sensitivity and specificity scores of 100% in stool samples from *Clostridium difficile*-infected patients [16]. Furthermore, two independent studies reported the success of dogs to detect urinary tract infections with an overall sensitivity of around 90% [17,18].

Obstructive sleep apnea (OSA) results in hypopnea/apnea, oxygen desaturation, and sleep arousals. Patients with severe untreated OSA have a twofold increased risk of all-cause mortality. Other adverse outcomes of OSA include an increased risk of motor vehicle accidents and diminished cognitive performance and quality of life. The estimated prevalence of sleep apnea in the USA is approximately 16% for mild OSA and 10% for moderate to severe OSA. Risk factors for OSA include being male, older, and postmenopausal, as well as having a higher body mass index (BMI) and anatomical abnormalities [19,20].

The diagnostic standard for OSA is overnight polysomnography (PSG). However, PSG can interfere with sleep quality and is considered labor-intensive, uncomfortable, and expensive. Thus, screening questionnaires have been developed to identify OSA, two of which

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have been properly been evaluated in primary care or among general populations. The sensitivity ranges from 37 to 91% and specificity from 80 to 84% depending on the apnea-hypopnea index (AHI) cut-off point and patient population [19,20].

Proteomics and metabolomics have been used to identify biomarkers for OSA. Xu and co-authors reviewed 30 studies evaluating putative OSA biomarkers, including 13 urine studies. The abnormal expression of more than 100 proteins or metabolites was reported among OSA patients and controls [21]. For example, leukotriene E4 and neurotransmitter metabolites homovanillic acid and 3,4-dihydroxyphenylacetic acid are increased in selected OSA patients [22–24]. Despite changes in urine proteins and metabolites, reports remain inconsistent and cannot be utilized in clinical practice. Thus, we sought to study if the metabolic changes of OSA patients could be identified through olfactory-based detection dogs.

Methods

The dogs Three dogs were trained: a German Spitz Mittel (dog 1), a Labrador retriever (dog 2), and a German shepherd (dog 3). All dogs and trainers had previous experience in olfactory-based detection. The German Spitz Mittel was previously trained and tested on the detection of pediatric urinary tract infections caused by *E. coli* [18].

The training The training period was 2 to 3 months, approximately three to five times a week, with each session lasting about 10 min. The training was based on rewards in which correct detection was rewarded with treats (reinforced operant conditioning). The dog was taught to pick up the desired cup from among four cups based on smell detection. The cups were placed in a line of four, each separated by a distance of approximately 30 cm.

Patients and urine samples Urine samples were collected from 23 adult male OSA patients who visited the Sleep Unit of Helsinki University Hospital between November 2016 and May 2017. We included patients who were male, > 18 years and AHI > 15. Control samples were collected from 20 voluntary adult male volunteers, age > 18 years, BMI < 30, and with no symptoms or any suspicion of OSA. Urine samples were fractioned into small microcentrifuge tubes and frozen (− 18 °C, aliquots of approximately 0.5 ml each). Eight patient samples and ten control samples were used for training only. The remaining 15 patient samples and 10 control samples were used for testing only. Demographic data, BMI, medical comorbidities, and smoking data were asked from the patients and confirmed from the hospital records (Table 1).

Table 1 Patient and control demographics

	Age	AHI	BMI	Smoking status	Comorbidity
Patients used in training					
1	80	24.7	24.8	Ex-smoker	Hypertension
2	36	17.8	31.9	–	–
3	32	49	23.9	Ex-smoker	–
4	47	80	40.6	Ex-smoker	Hypertension
5	50	99	41.7	–	Asthma
6	48	137.3	48.3	Yes	Hypertension
7	34	15	31	Ex-smoker	Hypertension
8	36	68	29.1	–	Hypertension
Patients used in testing					
9	35	23	28.4	Yes	–
10	41	35	25.7	Yes	–
11	56	29	26.9	Ex-smoker	–
12	73	62	35.2	Ex-smoker	Hypertension, CAD
13	55	18.8	33.3	Ex-smoker	Hypertension
14	39	71	32	Ex-smoker	Bipolar
15	88	62	37	Ex-smoker	Hypertension, diabetes
16	64	43	29.3	–	Hypertension
18	55	49.8	31.3	–	Hypertension, atopy
19	54	105	53.7	Ex-smoker	Hypertension
20	64	63	34.7	–	Hypertension, diabetes
21	72	35	21.2	–	Parkinson
22	65	75	37.7	–	Hypertension
23	68	73	28	–	Hypertension, CAD, diabetes
24	65	87	29	Ex-smoker	Renal insufficiency
Controls used in testing					
17	29	n/a	23.1	–	–
30	38	n/a	23.0	–	–
31	46	n/a	26.8	Ex-smoker	–
32	51	n/a	23.0	–	–
33	40	n/a	26.3	–	–
34	18	n/a	21.4	–	–
35	20	n/a	23.2	–	–
36	23	n/a	21.5	–	–
37	56	n/a	26.1	–	–
38	36	n/a	25.2	–	–

n/a not applicable

The test Testing was performed at the home of the detection dog. The owner was given 30 rows, with four urine samples per row. The rows were numbered 1 to 30 and each tube in a row was labeled from A to D. One of the tubes in each row contained urine from a patient with OSA (positive sample), with the remaining three samples taken from negative

controls. All 15 positive samples were included in two rows. The owner of the dog was blinded to avoid any possible signaling between the owner and the dog. The owner sent the letter of each dog's choices (A–D) by mobile phone. Only one selection per row was allowed with no possibility of refusal (Table 2).

Statistics An independent consultant from Elisa Appelsiini Oy (Helsinki, Finland) completed all statistical analyses. Confidence limits and hypothesis tests based on the binomial distribution of a single proportion were calculated. The measured proportions for the correct selection were compared to the hypothesized proportion of 0.25.

The ethics The study protocol was approved by the ethics committee of the Helsinki University Hospital (Dnro 168/13/03/00/16). Written informed consent was obtained from all participating patients.

Results

OSA urine vs. controls All 15 positive samples appeared twice in the test (30 rows). Dog 1 correctly detected 18/30 rows (60%, $p < 0.000194$, 95% confidence limits 0.41–0.77). She correctly detected 10/15 patient samples at least once and 8/15 correctly both times. Dog 2 correctly detected 20/30 rows (67%, $p < 0.000003$, 95% confidence limits 0.47–0.67). She correctly detected 13/15 patient samples at least once and 7/15 correctly both times. Dogs 1 and 2 detected 6/15 positive samples twice (samples 11, 15, 18, 20, 21, and 23). Dogs 1 and 2 failed to detect 2/15 samples both times (samples 12 and 19). Dog 3 performed no better than by chance, correctly detecting only 5/30 rows (17%, $p < 0.33$, 95% confidence limits 0.06–0.35; Table 2). The dogs succeeded or failed to identify OSA patients regardless of their age, AHI, BMI, medical comorbidities, or smoking status.

Two dogs were consistent and unanimous in detecting 6 of the 15 positive samples. These patients did not differ from other patients in terms of age, BMI, other diseases, medication, or smoking habits.

Table 2 Dog 1 correctly detected 10/15 patient samples at least once and 8/15 correctly both times. Dog 2 correctly detected 13/15 patient samples at least once and 7/15 correctly both times. Dog 3 performed no better than by chance

Patient	Dog 1	Dog 2	Dog 3
9	0	1	0
10	2	1	1
11	2	2	1
12	0	0	0
13	2	1	0
14	0	1	0
15	2	2	0
16	1	1	0
17	0	2	0
18	2	2	0
19	0	0	0
20	2	2	2
21	2	2	1
22	1	1	0
23	2	2	0
p	0.000	0.000	0.33

0, no detection; 1, correct detection (once); 2, correct detection (twice)

Discussion

In this study, we demonstrated that dogs can be trained to distinguish OSA urine from control urine samples based on olfaction. Two of our three dogs correctly detected two thirds of the selected OSA patient samples with an impressive *p* value ($p < 0.000194$ and $p < 0.000003$). This detection rate is comparable to questionnaires, clinical prediction tools, and portable sleep monitors, in which the sensitivity varies from 43 to 64% and the specificity varies from 80 to 92% [25]. Furthermore, the use of detection dogs is more economical and faster, although we did not specifically evaluate this in our study.

The dogs in our study consisted of the adult pet dogs of the members of the research team. In previous medical detection dog studies, the age and breed of the dogs have varied greatly. Polgar and co-authors demonstrated that breeds selected for scent seem to have a better olfactory capacity than short-nosed or non-scent breeds [26]. However, odor-detection work is far more complicated than olfactory capacity. That is, the dogs also need to have a motivation to work. This complexity may lead to surprises. For instance, Hall et al. compared the olfactory discrimination ability of German shepherds, pugs, and greyhounds. Contrary to expectations, pugs outperformed the German shepherds, and unsurprisingly, nine out of ten greyhounds failed the training because of a lack of motivation [27].

Our proof-of-concept study does have limitations. First, the set-up of "one out of four" is not clinically meaningful. We did not perform a PSG on our control subjects, although it is unlikely that they have moderate or severe OSA given that they were asymptomatic and not overweight [28]. If our controls did have OSA, this would have caused a negative bias, rendering these results even more impressive. In addition, we only studied male patients since we wanted our proof-of-concept population as homogenous as possible. In addition, freezing might have affected the metabolomics of the OSA urine samples. However, control and patient urine samples were handled identically, thus minimizing

any such distortions. Since dogs are living creatures, the training is always exposed to a subjective bias. Finally, a dog's detection accuracy may vary due to many factors, such as a decreased motivation or energy levels or a confounding test environment.

To conclude, future screening tools for OSA might consist of objective laboratory parameters. The altered expression of numerous different proteins and metabolites has been identified in the urine of OSA patients, reflecting the activation of the sympathetic nervous system followed by oxidative stress and systemic inflammation. Despite multiple studies investigating possible biomarkers for OSA, these results are inconsistent and far from being clinically applicable [21]. Two of our dogs consistently and unanimously identified 6 of the 15 positive samples. We believe that these OSA patients share an unknown protein or metabolite which was detected by the dogs. Metabolomic analyses of these patients' urine might reveal novel metabolites suitable for more traditional laboratory diagnostics for OSA.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval All procedures performed in studies involving human participants were carried out in accordance with the ethical standards of the institutional research committee (Helsinki University Hospital, 168/13/03/00/16) and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards.

Informed consent Informed consent was obtained from all individual participants included in this study.

Animal experiments, ethical approval All applicable international, national, and/or institutional guidelines for the care and use of animals were followed. All procedures performed in studies involving animals were carried out in accordance with the ethical standards of the institution or practice at which the studies were conducted.

References

1. Craven BA, Paterson EG, Settles GS (2010) The fluid dynamics of canine olfaction: unique nasal airflow patterns as an explanation of macrosmia. J R Soc Interface 7:933–943
2. Welch JB (1990) A detector dog for screwworms (diptera: Calliphoridae). J Econ Entomol 83:1932–1934
3. Pfiester M, Koehler PG, Pereira RM (2008) Ability of bed bug-detecting canines to locate live bed bugs and viable bed bug eggs. J Econ Entomol 101:1389–1396
4. Lin HM, Chi WL, Lin CC, Tseng YC, Chen WT, Kung YL, Lien YY, Chen YY (2011) Fire ant-detecting canines: a complementary method in detecting red imported fire ants. J Econ Entomol 104: 225–231
5. Alasaad S, Permunian R, Gakuya F, Mutinda M, Soriguer RC, Rossi L (2012) Sarcoptic-mange detector dogs used to identify infected animals during outbreaks in wildlife. BMC Vet Res 8:110
6. Fischer-Tenhagen C, Tenhagen BA, Heuwieser W (2013) Short communication: ability of dogs to detect cows in estrus from sniffing saliva samples. J Dairy Sci 96:1081–1084
7. Turner C (2016) Techniques and issues in breath and clinical sample headspace analysis for disease diagnosis. Bioanalysis 8:677–690
8. Hardin DS, Anderson W, Cattet J (2015) Dogs can be successfully trained to alert to hypoglycemia samples from patients with type 1 diabetes. Diabetes Ther 6:509–517
9. Dehlinger K, Tarnowski K, House JL et al (2013) Can trained dogs detect a hypoglycemic scent in patients with type 1 diabetes? Diabetes Care 36:98–99
10. Pickel D, Manucy G, Walker D, Hall S, Walker J (2004) Evidence for canine olfactory detection of melanoma. Appl Anim Behav Sci 89:107–116
11. Willis CM, Britton LE, Swindells MA, Jones EM, Kemp AE, Muirhead NL, Gul A, Matin RN, Knutsson L, Ali M (2016) Invasive melanoma in vivo can be distinguished from basal cell carcinoma, benign naevi and healthy skin by canine olfaction: a proof-of-principle study of differential volatile organic compound emission. Br J Dermatol 175:1020–1029
12. Cornu JN, Cancel-Tassin G, Ondet V, Girardet C, Cussenot O (2011) Olfactory detection of prostate cancer by dogs sniffing urine: a step forward in early diagnosis. Eur Urol 59:197–201
13. Taverna G, Tidu L, Grizzi F, Torri V, Mandressi A, Sardella P, la Torre G, Cocciolone G, Seveso M, Giusti G, Hurle R, Santoro A, Graziotti P (2015) Olfactory system of highly trained dogs detects prostate cancer in urine samples. J Urol 193:1382–1387
14. Ehmann R, Boedeker E, Friedrich U, Sagert J, Dippon J, Friedel G, Walles T (2012) Canine scent detection in the diagnosis of lung cancer: revisiting a puzzling phenomenon. Eur Respir J 39(3): 669–676
15. Amundsen T, Sundstrom S, Buvik T, Gederaas OA, Haaverstad R (2014) Can dogs smell lung cancer? First study using exhaled breath and urine screening in unselected patients with suspected lung cancer. Acta Oncol 53:307–315
16. Bomers MK, van Agtmael MA, Luik H, van Veen MC, Vandenbroucke-Grauls CM, Smulders YM (2012) Using a dog's superior olfactory sensitivity to identify clostridium difficile in stools and patients: proof of principle study. BMJ 345:e7396
17. Maurer M, McCulloch M, Willey AM, Hirsch W, Dewey D (2016) Detection of bacteriuria by canine olfaction. Open Forum Infect Dis 9:ofw051
18. Koskinen A, Koskinen H, Back L, Saxen H, Klockars T (2017) A detection dog for paediatric urinary tract infection caused by Escherichia Coli. Infect Dis (Lond) 49:874–877
19. Teodorescu M, Barnet JH, Hagen EW, Palta M, Young TB, Peppard PE (2015) Association between asthma and risk of developing obstructive sleep apnea. JAMA 313:156–164
20. Jonas DE, Amick HR, Feltner C, Weber RP, Arvanitis M, Stine A, Lux L, Harris RP (2017) Screening for obstructive sleep apnea in adults: evidence report and systematic review for the US preventive services task force. JAMA 317:415–433
21. Xu CY, Li DJ, Wu CL, Lou HJ, Jiang HW, Ding GQ (2015) Serum sLOX-1 levels are correlated with the presence and severity of obstructive sleep apnea. Genet Test Mol Biomarkers 19:272–276
22. Stanke-Labesque F, Back M, Lefebvre B et al (2009) Increased urinary leukotriene E4 excretion in obstructive sleep apnea: effects of obesity and hypoxia. J Allergy Clin Immunol 124:364–370

23. Jurado-Gamez B, Gomez-Chaparro JL, Munoz-Calero M et al (2012) Serum proteomic changes in adults with obstructive sleep apnoea. J Sleep Res 21:139–146
24. Paik MJ, Kim DK, Nguyen DT, Lee G, Rhee CS, Yoon IY, Kim JW (2014) Correlation of daytime sleepiness with urine metabolites in patients with obstructive sleep apnea. Sleep Breath 18:517–523
25. Preventive Services Task Force US, Bibbins-Domingo K, Grossman DC et al (2017) Screening for obstructive sleep apnea in adults: US preventive services task force recommendation statement. JAMA 317:407–414
26. Polgár Z, Kinnunen M, Újváry D, Miklósi Á, Gácsi M (2016) A test of canine olfactory capacity: comparing various dog breeds and wolves in a natural detection task. PLoS One 11(5):e0154087
27. Hall NJ, Glenn K, Smith DW, Wynne CD (2015) Performance of pugs, German shepherds, and greyhounds (Canis lupus familiaris) on an odor-discrimination task. J Comp Psychol 129(3):237–246
28. Franklin KA, Lindberg E (2015) Obstructive sleep apnea is a common disorder in the population—a review on the epidemiology of sleep apnea. J Thorac Dis 7:1311–1322