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Preliminary Communication

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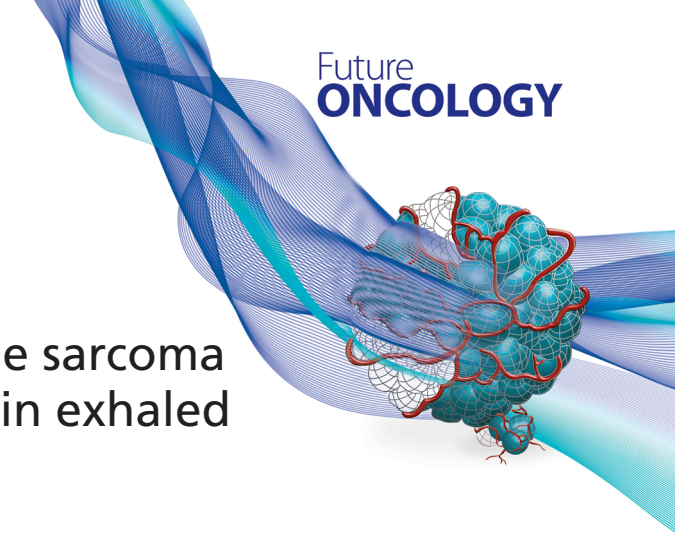

Noninvasive detection of soft tissue sarcoma using volatile organic compounds in exhaled breath: a pilot study

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Aim: The aim of this pilot study was to assess whether an electronic nose can detect patients with soft tissue sarcoma (STS) based on volatile organic compound profiles in exhaled breath. **Patients & methods:** In this cross-sectional pilot study, patients with primary STS and healthy controls, matched on sex and age, were included for breath analysis. Machine learning techniques were used to develop the best-fitting model. **Results:** Fifty-nine breath samples were collected (29 STS and 30 control) from March 2018 to March 2022. The final model yielded a c-statistic of 0.85 with a sensitivity of 83% and specificity of 60%. **Conclusion:** This study suggests that exhaled volatile organic compound analysis could serve as a noninvasive diagnostic biomarker for the detection of STS with a good performance.

Plain language summary: Diagnosing soft tissue sarcoma (STS) among the large number of benign soft tissue tumors is challenging. There is a serious need for a novel and easy tool that could accurately detect patients with STS. This study aimed to assess how well an easy-to-use electronic nose could differentiate between patients with STS and those without STS based on their exhaled breath. This is the first pilot study to reveal that an electronic nose could serve as a diagnostic tool for the detection of STS with a good performance. Future studies are needed to validate the findings in larger cohorts.

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Keywords: breath analysis • diagnostic test • electronic nose • soft tissue sarcoma • volatile organic compounds

Differentiating soft tissue sarcomas (STSs) from benign soft tissue tumors is challenging in daily practice. The incidence of STS is less than 4.7 per 100,000 persons per year in Northern Europe [1], and it has been estimated that benign soft tissue tumors occur 300-times more often than their malignant counterparts [2–4]. Besides their rarity, STSs often present as asymptomatic or unspecific lumps. These difficulties explain why STSs are often thought to be benign. This results in the frequent performance of unplanned excisions in which the STS is inadvertently and inadequately removed without an appropriate diagnosis, preoperative imaging or planning [5]. Referrals after unplanned excisions account for 8–53% of the new patients treated in sarcoma centers [3, 6–11]. These patients often require re-excision due to incomplete surgical margins [12, 13].

Although core needle biopsy is an invasive procedure that is prone to various complications, it is the gold standard for differentiating STS from benign soft tissue tumors [12, 31]. Because benign tumors are very common, there is a serious need for novel noninvasive diagnostic tools that accurately detect patients with STS. Achievement of a higher pretest probability for STS could reduce the number of unplanned excisions and re-excisions, but could also reduce the number of imaging assessments and biopsies during routine follow-up.

In the past years, volatile organic compounds (VOCs) have been widely investigated as a new diagnostic biomarker in medicine. VOC profiles can be detected in breath, blood, saliva, semen, milk, feces and urine, and

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on the skin [14]. Several studies have been performed with a noninvasive electronic nose (eNose) in which VOC profiles were detected from exhaled breath, and VOC analyses seem promising for the detection of several cancer types such as lung, breast, prostate, colorectal and head and neck carcinomas [15–17]. However, no studies have been performed investigating the discriminative ability of the eNose for STS. Therefore, the aim of this pilot study was to assess whether the eNose can discriminate between patients with and without STS based on VOC profiles in exhaled breath.

Patients & methods

Study design

This prospective proof-of-principle study was conducted in a specialized sarcoma center outpatient clinic in a tertiary hospital in the Netherlands (Leiden University Medical Center) between March 2018 and March 2022. Ethical approval was obtained from the institutional review board prior to the study (no. P18.046). All study participants provided written informed consent before breath testing. The measurements were performed in parallel with the regular diagnostic workup. No formal sample size calculation was performed for this pilot study. Based on previous studies with an eNose, a sample size of 25 participants per study arm was considered sufficiently powered for a pilot study [18, 19]. The primary outcome of this pilot study was the discriminative ability (area under the receiver operating characteristic [ROC] curve) of the VOC profiles recorded by the eNose.

Participants

Patients who were referred to our outpatient clinic for suspected primary STS were approached to participate in this study. Patients were included in this study if they had a histologically proven primary high-grade STS. Patients were excluded if they were younger than 18 years, had a history of cancer or chronic respiratory conditions (e.g., chronic obstructive pulmonary disease or asthma), were previously treated with radiotherapy and/or chemotherapy, received any prior treatment for STS or were diagnosed with distant metastasis within 3 months after inclusion. Also, patients who did not complete the breath test were excluded. Individuals with no suspicion for STS who visited our outpatient clinic for other conditions or who accompanied a patient to our outpatient clinic, and employees at our department, were asked to participate in this study as healthy controls. Individuals with a suspected STS that turned out to be a benign tumor (e.g., schwannoma, lipoma, hemangioma) were excluded from the analysis. The control group was matched to our STS population based on age and sex in a 1:1 ratio. The same exclusion criteria were applied to the control group. In addition, we performed a secondary analysis with less stringent inclusion criteria in order to expand the sample size. In this analysis we also included patients with a low-grade STS in the sick group and patients with a rejected STS diagnosis in the control group.

Materials & study procedure

The eNose used in this study (Aeonose, The eNose Company, Zutphen, The Netherlands) is a handheld, battery-powered eNose which enables the analysis of VOCs. Participants were instructed to breathe through a disposable connecting mouthpiece for 5 min. The mouthpiece contained a carbon filter, and a nose clip was placed on the nose of the participants to avoid entry of nonfiltered air during the measurement to eliminate exogenous influences on VOCs. In addition, the mouthpiece contained a high-efficiency particulate air filter and one-way valves to prevent viral and bacterial contamination of the device. In the first 2 min of each measurement, the lungs were rinsed with clean filtered air to further eliminate exogenous VOCs. During the remaining 3 min, the exhaled breath was guided over three micro-hotplate metal oxide sensors with different material properties. The hotplate was periodically heated between 260 and 340°C, simulating multiple identical sensors that are operating at different temperatures. The VOCs in the exhaled breath induce a redox reaction on the metal oxide sensor surfaces, causing a conductivity change. These changes in conductivity over time result in a unique VOC profile for each participant. The total measurement took 15 min, consisting of 5 min breathing followed by 10 min of regeneration of the eNose. Supplementary Figure 1 depicts the eNose and test setup.

For the measurement, all participants were asked to abstain from food, drink (except water) and smoking for at least 3 h prior to the study visit to minimize exogenous VOCs [20]. Tumor characteristics (histological subtype and tumor grade) and medical history (previous malignancies and chronic respiratory conditions) were collected from clinical records.

Table 1. Baseline characteristics.

	Model 1			Model 2		
	STS (n = 25)	Control (n = 25)	p-value	STS (n = 29)	Control (n = 30)	p-value
Age (years)			0.669			0.679
Median (IQR)	57 (39–65)	54 (44–61)		56 (39–63)	59 (45–62)	
Sex			1			0.693
Female	8 (32.0%)	8 (32.0%)		11 (37.9%)	10 (33.3%)	
Male	17 (68.0%)	17 (68.0%)		18 (62.1%)	20 (66.7%)	
Histological subtype						
LPS	6 (24.0%)	–		6 (20.7%)	–	
LMS	2 (8.0%)	–		3 (10.3%)	–	
MFS	5 (20.0%)	–		7 (24.1%)	–	
MPNST	5 (20.0%)	–		5 (17.2%)	–	
SS	2 (8.0%)	–		2 (6.9%)	–	
UPS	2 (8.0%)	–		2 (6.9%)	–	
Other	3 (12.0%)	–		4 (13.8%)	–	
Tumor size (mm)						
Median (IQR)	60 (46–84)	–		60 (46–84)	–	
Tumor grade						
1	–	–		4 (13.8%)	–	
2	14 (56.0%)	–		14 (48.3%)	–	
3	8 (32.0%)	–		8 (27.6%)	–	
High-grade not otherwise specified	3 (12.0%)	–		3 (10.3%)	–	
Location						
Extremity	21 (84.0%)	–		24 (82.8%)	–	
Trunk wall	3 (12.0%)	–		4 (13.8%)	–	
Uterus	1 (4.0%)	–		1 (3.45%)	–	

IQR: Interquartile range; LMS: Leiomyosarcoma; LPS: Liposarcoma; MFS: Myofibrosarcoma; MPNST: Malignant peripheral nerve sheath tumor; SS: Synovial sarcoma; STS: Soft tissue sarcoma; UPS: Undifferentiated pleomorphic sarcoma.

Statistical analysis

Baseline characteristics were described with proportions for categorical variables and means with standard deviations or medians with interquartile ranges for continuous variables. Differences in continuous variables were assessed with the Mann–Whitney U test. Differences in categorical variables were assessed with the Pearson's $\chi 2$ test.

An eNose measurement resulted in a time series of conductivity values for each sensor. Multiple machine learning models were built using different sensor combinations and classifiers. Data compression was performed using a Tucker 3-like tensor decomposition technique. While applying tenfold cross-validation, models were ranked on area under the curve (AUC). The validation results were averaged over the ten rounds, resulting in a combined AUC. The random forest classifier turned out to be most favorable. Data compression and data analyses were integrated in a proprietary software program (Aethena, The eNose Company, Zutphen, The Netherlands). Descriptive statistics were performed in R (v. 4.1.2) [21].

A p-value ≤ 0.05 was considered statistically significant. Results from the final models were described by means of the most optimal AUC with corresponding sensitivity and specificity with 95% CIs. For each analysis, two cutoff values for the predicted response value of the final model were presented with corresponding sensitivity and specificity. The cutoff value was set manually at a predictive value of 0.0 and at a value at which the sensitivity was maximized with an acceptable specificity ($\geq 50\%$). The predicted response value for the STS and control population was presented in a scatter plot.

Results

Twenty-five patients with high-grade STS and 25 controls, matched on age and sex, were included for the first analysis. For the second analysis with less stringent inclusion criteria, 29 patients with STS and 30 controls were included. Baseline demographics and clinical characteristics are presented in Table 1.

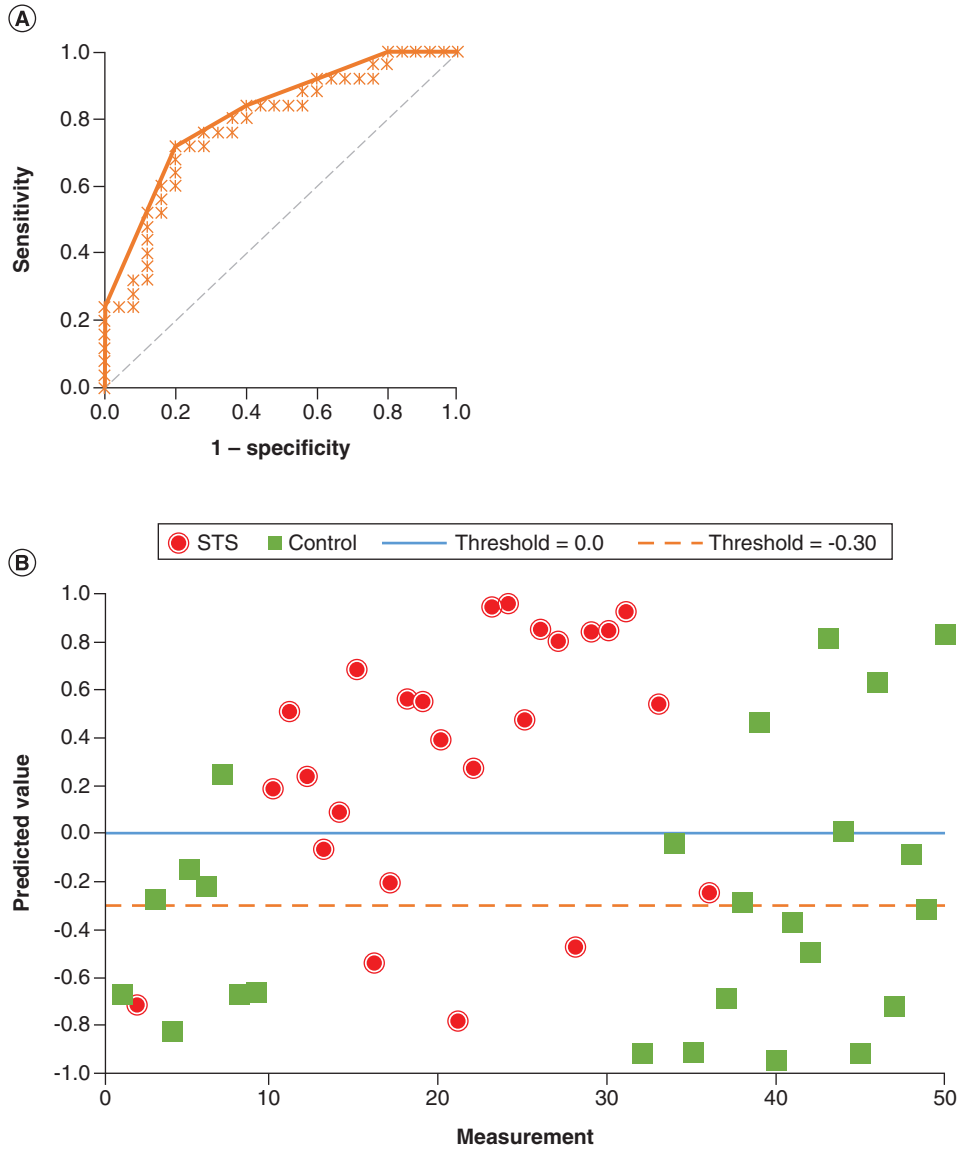

Figure 1. Performance of model 1. (A) Receiver operating characteristic curve for the best fit of model 1 (AUC: 0.78) **(B)** Scatter plot of individual predicted values based on the cross-validated model 1. The red circles represent patients with STS. The green squares represent the controls.
AUC: Area under the curve; STS: Soft tissue sarcoma.

Figure 1A depicts the ROC curve of model 1, which had a fair discriminative ability, with an AUC of 0.78. Figure 1B depicts a scatter plot of the predicted value of each measurement of model 1. Setting the predictive value at 0.0 resulted in sensitivity and specificity of 72% (95% CI: 50–87) and 76% (95% CI: 54–90), respectively. A threshold of -0.3 resulted in sensitivity and specificity of 84% (95% CI: 63–95) and 52% (95% CI: 32–73), respectively. Figure 2 depicts the ROC curve (Figure 2A) and scatter plot (Figure 2B) of model 2 with less stringent inclusion criteria. This model showed that an increased sample size resulted in a better discriminative ability, with an AUC of 0.85. At a threshold of 0.0, the sensitivity and specificity were 72% (95% CI: 53–87) and 90% (95% CI: 72–97), respectively. A threshold of -0.2 resulted in sensitivity and specificity of 83% (95% CI: 64–93) and 60% (95% CI: 41–77), respectively. A scatter plot of individual predicted values stratified by histological subtype is presented in Supplementary Figure 2.

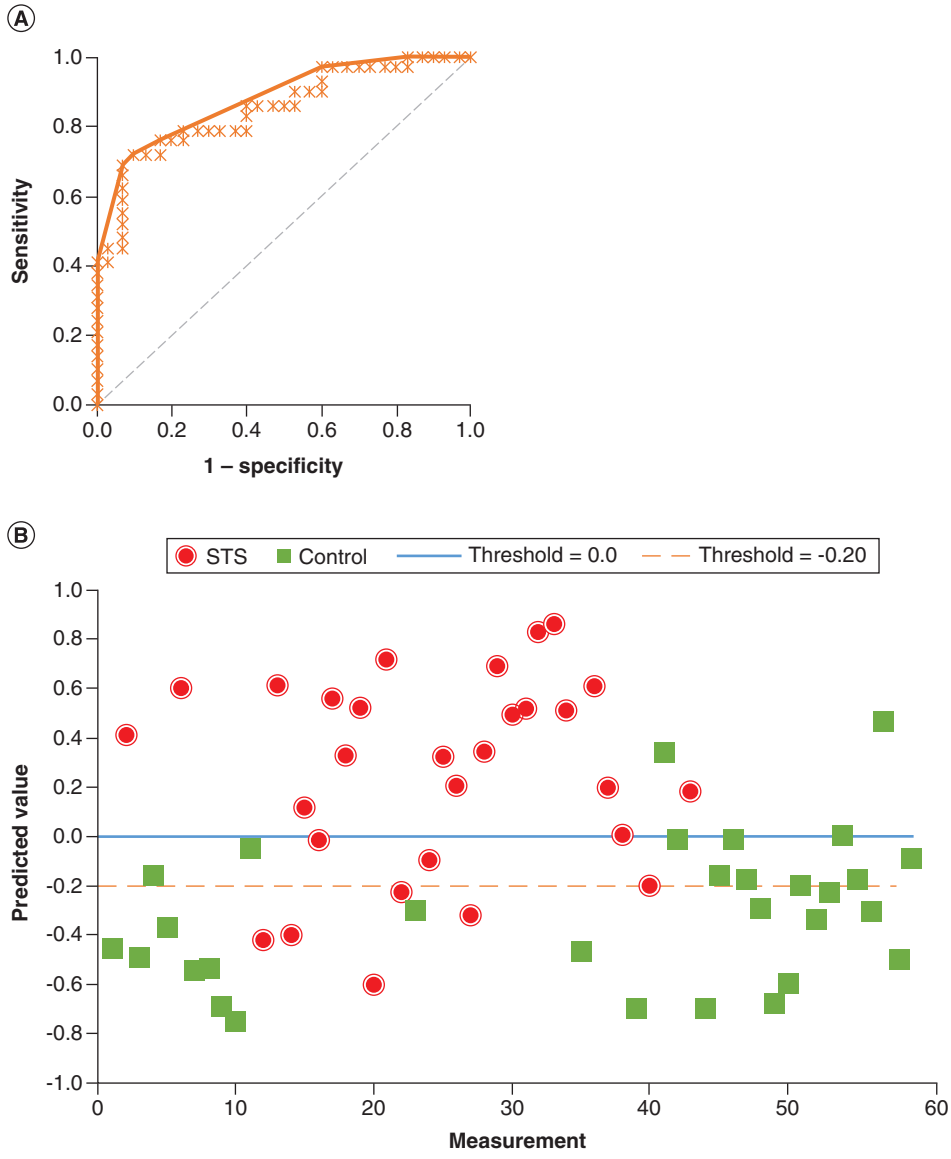

Figure 2. Performance of model 2. (A) Receiver operating characteristic curve for the best fit of model 2 (AUC: 0.85) **(B)** Scatter plot of individual predicted values based on the cross-validated model 2. The red circles represent patients with STS. The green squares represent the controls.
AUC: Area under the curve; STS: Soft tissue sarcoma.

Discussion

In this proof-of-principle study, we determined that the eNose could well distinguish patients with and without STSs, suggesting that exhaled VOC analysis with eNose could become a promising noninvasive diagnostic tool to achieve a higher pretest probability for STS and potentially reduce the number of unplanned excisions, re-excisions and biopsies. With an AUC of 0.85 of the second model and a corresponding sensitivity and specificity of 83 and 60%, respectively, the discriminative ability could be considered good. Larger multicenter studies are needed to confirm current findings, improve accuracy and extend the validity of the current models.

In the last years, several phase I studies have demonstrated the diagnostic ability of VOC patterns in exhaled breath for several cancer types [15–17] but no studies so far have assessed the diagnostic performance of VOC profiles as diagnostic biomarker for STS. VOCs are a group of organic carbon- and hydrogen-containing compounds that are found in various cellular functions such as oxidative stress and energy metabolism. Oxidative stress and altered cellular energy metabolism have been implicated in the pathophysiology of cancer in order to support

continuous cell growth and proliferation [22, 23]. Changes in VOC concentrations reflect these altered metabolic and pathophysiological processes in the human body [24]. In breath there are almost 1500 VOCs reported [14]. For most of the VOCs, the biochemical process for their production remains unknown. Several studies have shown that different cancer types and diseases reveal different VOC profiles, suggesting that VOC profiles could be diagnostic biomarkers for a broad range of diseases [25, 26]. Analysis of VOCs in exhaled breath is not yet implemented in clinical practice for any of the studied diseases [15, 16].

Some limitations of this pilot study must be overcome in future studies. This study showed an overall good discriminative ability of the eNose. However, due to the limited sample size, the machine learning models built on our data could partially be based on artifacts in the data (e.g., due to contamination with exogenous VOCs) instead of true differences in VOC profile that were caused by the pathophysiology of the malignancy. Therefore, future larger studies are needed to update and externally validate the models. As shown in our second model, the discriminative ability of the model might even further improve with larger sample sizes. In most diagnostic studies, such as this study, the primary target is endogenous VOCs. However, human breath contains a mixture of endogenous and exogenous VOCs. Exogenous VOCs could arise from room air, but dietary habits and medication could also influence the exhaled VOC profiles [24, 27]. In a large cohort of healthy volunteers, smoking behavior – and, to a lesser extent, age, BMI and gender – were shown to influence VOC profiles in the general population [28]. To minimize the effect of these influencing factors, we matched the STS population with the control group by age and gender, performed all eNose measurements in the same testing area and asked participants to abstain from food, drink and smoking for 3 h before testing. Furthermore, as radiotherapy and chemotherapy cause oxidative stress, inflammation and tissue damage, participants who had a history of receiving these treatments were excluded [29, 30]. We did not match patients based on BMI or other influencing factors, such as smoking status and comorbidities, because of the small sample size. The likelihood that these and other (unknown) influencing factors were not well distributed between the STS and control groups is higher than in larger cohorts. For future studies, standardization of the study procedure and breath collection, especially for training models, is crucial. Furthermore, studies should include controls from the same target population as the STS population (e.g., benign soft tissue tumors) to inform clinical application and should be externally validated in other target populations to assess generalizability of the models. In this pilot study, only internal cross-validation was performed.

This proof-of-concept study aimed to assess the feasibility of using an eNose for detection of STS at the beginning of a patient's workup, when referred to a sarcoma center with a primary nonspecific tumor of the soft tissue. The reported results were based on maximizing the sensitivity with acceptable specificity. Depending on the use of the eNose in clinical practice, other cutoff values might be preferable. In a primary or secondary healthcare setting, the prevalence of benign soft tissue tumors is much higher than the prevalence of STS. The physician needs to decide whether to treat the tumor as a benign tumor or to refer the patient to a tertiary sarcoma center for biopsy, which is the gold standard for diagnosis of STSs [12, 31]. This decision is nowadays based on physical examination and imaging. However, the large number of unplanned excisions in patients with STSs reflects the inaccuracy of the current diagnostic workup [3, 6–11]. Patients with asymptomatic benign tumors often do not need any treatment, while patients with primary STSs need appropriate treatment in a sarcoma center, including an oncological resection with wide surgical margins and often (neo)adjuvant therapy [12, 31]. As a core needle biopsy is an invasive procedure and benign tumors are very common in this setting, not all patients with a nonspecific tumor of the soft tissue get a biopsy. A noninvasive diagnostic tool, such as a breath test, could help to decide which patients should get a biopsy in a tertiary sarcoma center. In this case, maximizing the sensitivity in order to minimize the risk of untreated or unplanned excisions for STSs (false negatives), at the expense of more false positives referred to a sarcoma center, would be clinically most desirable, especially given that in superficial and small STSs the proportion of unplanned excisions is high [6, 8–11]. Therefore the use of a noninvasive breath test in this target population and setting seems most promising and desirable.

Besides the use of an eNose as a pretest at the beginning of each patient's workup to decide whether further diagnostic tests, such as a biopsy, are needed, the eNose could also play a role in monitoring the response to cancer therapy, surveilling patients after successful treatment or differentiating between high- and low-risk STSs. It is likely that, for each application, different VOC models with different cutoff values will need to be built and validated. Furthermore, further studies are needed to assess the minimum detectable tumor volume for the eNose.

Conclusion

This study suggests that VOCs in exhaled breath could become a new diagnostic biomarker for the detection of STSs. Future studies are needed to validate these promising preliminary findings before VOC analyses could be incorporated in clinical practice.

Summary points

- Differentiating soft tissue sarcomas (STSs) from benign soft tissue tumors is challenging in daily practice.
- Volatile organic compounds (VOCs) are widely investigated as a new diagnostic biomarker in medicine.
- This study aimed to assess the diagnostic ability of VOC profiles in exhaled breath in patients with STSs.
- Patients with primary STSs and healthy controls, matched on sex and age, were included for breath analysis.
- Machine learning techniques were used to develop the best-fitting model.
- This proof-of-principle study showed that the VOC profiles in exhaled breath could well distinguish patients with and without STS.
- This study suggests that exhaled VOC analysis could serve as a noninvasive diagnostic biomarker for the detection of STSs with a fair performance in future.
- Larger multicenter studies are needed to confirm the current findings, improve accuracy and extend the validity of the current models.

Supplementary data

To view the supplementary data that accompany this paper please visit the journal website at: www.futuremedicine.com/doi/suppl/10.2217/fon-2022-1122

Author contributions

Conceptualization: V van Praag, C Mostert, R Neijenhuis and M van de Sande; methodology: I Acem, V van Praag, C Mostert, R Neijenhuis and M van de Sande; formal analysis: I Acem; investigation: I Acem, V van Praag, C Mostert, R Neijenhuis, C Verhoef, D Grünhagen and M van de Sande; data curation: I Acem; writing (original draft preparation): I Acem; writing (review and editing): I Acem, V van Praag, C Mostert, R van der Wal, R Neijenhuis, C Verhoef, D Grünhagen and M van de Sande; visualization: I Acem; supervision: C Verhoef, D Grünhagen and M van de Sande All authors have read and agreed to the published version of the manuscript.

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The authors have no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.

No writing assistance was utilized in the production of this manuscript.

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