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Determination of breath isoprene in 109 suspected lung cancer patients using cavity ringdown spectroscopy

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Background: Lung cancer is one of the most common malignant tumors worldwide. Currently, effective screening methods for early lung cancer are still scarce. Breath analysis provides a promising method for the pre-screening or early screening of lung cancer. Isoprene is a potential and important breath biomarker of lung cancer. **Material and Methods:** To investigate the clinical value of isoprene for diagnosing lung cancer patients, a cavity ringdown spectroscopy (CRDS) based near-real time, sensitive analysis method of breath isoprene is developed in our lab. In this paper, 92 breath samples from lung cancer patients, 17 breath samples from patients with benign lesions, and 107 breath samples from healthy people were collected. **Results:** Research indicates that breath isoprene concentration is significantly higher in healthy individuals (221.3 ± 122.2 ppbv) than in patients with lung cancer (112.0 ± 36.6 ppbv) and benign lung lesions (127.9 ± 41.2 ppbv). The result of Receiver Operating Characteristic (ROC) curve suggests that the concentration of isoprene is meaningful for the diagnosis of lung cancer (AUC = 0.822, sensitivity = 63.6%, specificity = 90.2%, $P < 0.01$). **Conclusion:** This study demonstrates that the CRDS breath isoprene analysis system can effectively analyze a large sample of human breath isoprene, and preliminarily confirms the use of breath isoprene as a biomarker for lung diseases.

Keywords: Cavity ringdown spectroscopy; breath isoprene; early diagnosis of lung cancer.

1. Introduction

Lung cancer is one of the most common malignant tumors in the world.[1,2] The incidence and mortality rate rank first among all cancer, and the incidence rate is still on the rise. Every year, about 1.8 million people die of lung cancer.[3] According to the statistics

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on cancer released by the American Cancer Society, Lung cancer kills more people than breast, prostate, colon, and leukemia.[4] Since the insidious onset of lung cancer, early lesions are difficult to find.[5] About 70% of lung cancer patients are in the advanced stage at the first diagnosis, making the survival rate of lung cancer less than 30%.[6] However, local treatment of early lung cancer can significantly improve the survival rate of patients, which is as high as 60–80% for stage I patient.[7] Therefore, early screening of lung cancer is of great value for disease diagnosis, monitoring, and efficacy observation.[8]

The present standard technique for early detection of lung cancer is a low-dose computer tomography (LDCT). Imaging tests still rely on the doctor's experience, and high sensitivity makes the screening results prone to false positives.[9] In addition, there is a very low probability of getting cancer from the radiation exposure by cancer screening.

Breath analysis provides a promising method and gains increasing attention recently for early screening of lung cancer with its noninvasive, simple, and rapid nature. In 1985, Gordon[10] first proposed and confirmed the application of breath gas analysis in the diagnosis of lung cancer. They used Gas Chromatography-Mass Spectrometry (GC-MS) to analyze the mass spectrum peak distribution between 12 lung cancer samples and 17 control samples, and screened out 28 types of breath gas characteristic VOCs with diagnostic accuracy of 80%. Hanna[11] systematic review and meta-analysis identified 63 relevant publications. They concluded that breath testing may have the potential for noninvasive cancer diagnosis. The overall diagnostic accuracy remains unknown.

Isoprene (2-methylbuta-1,3-diene, C_5H_8) is widely present in the breath gas of the human body,[12] Jansson and Larsson[13] first used GC-MS in 1969 to confirm the presence of isoprene in human exhaled breath, with a concentration of about 50–200 part per billion volume (ppbv).[14] Several groups have identified this compound in exhaled breath as one of the biomarkers of lung cancer. The review by Yannick Saalberg[15] lists and ranks VOC biomarkers for lung cancer identified in the papers published from 1985 to 2015. Among them, isoprene was repeatedly identified four times and ranked 2. Agnieszka Ulanowska *et al.*[16] found that in the breath of 137 patients with lung cancer, increased concentration of ethanol, acetone, isoprene, propanal, and so on was observed in comparison to 143 healthy nonsmokers. Fuchs[17] analyzed isoprene concentrations in breath samples in Tedlar bags collected from 79 lung cancer patients. The result showed that decline of exhaled isoprene in lung cancer patients correlated with immune activation. Japanese researchers analyzed the breath exhaled by 107 lung cancer patients and 29 healthy subjects (controls) using GC-MS and observed that a combination of five VOCs, including CHN, methanol, CH_3CN, isoprene, and 1-propanol, is sufficient for 89.0% screening accuracy.[18] Although GC-MS used in published articles for isoprene analysis is the gold standard for the detection of human breath components, the chemical separation process in GC technology takes a long time, and the analysis time for a single sample is as long as dozens of minutes, which is not suitable for real-time online breath testing.

Cavity ringdown spectroscopy (CRDS) has the characteristics of high specificity, high sensitivity, high stability analysis of quantitative substances, combining online sampling and real-time detection.[19] The detection limit can reach part per million volume (ppmv), part per billion volume (ppbv), or even part trillion volume (pptv) level without pre-concentrating the sample. In the previous work, our laboratory has built an experimental platform for near real time measuring breath isoprene concentration based on CRDS technology, and verified the stability, linear response, accuracy and detection limit of the experimental platform to meet the needs of clinical breath sample detection.[20] David Perez-Guaita[21] used substrate-integrated hollow waveguide mid-infrared sensors to determinate isoprene in human breath, which has a limit of detection (LoD) of 106 ppbv. Based on CRDS in the ultraviolet region, Sahay[22] measured isoprene at 266 nm with the LoD of 4 ppmv. In the previous work, the LoD in our experimental system reached 0.47 ppbv.

In this paper, breath samples from 109 suspected lung cancer patients (92 confirmed lung cancer and 17 confirmed benign lung lesions) before surgery and from 107 healthy people were collected and analyzed by using a high sensitivity, near-real-time CRDS isoprene analyzer. The association of the index such as gender, age, body mass index (BMI), fasting, and smoking or not and exhaled isoprene was analyzed. The purpose is to explore the factors of breath isoprene concentration in patients with lung cancer and to provide reference for the clinical

application of early diagnosis of lung cancer in the future.

2. Instrument and Method

2.1. *CRDS analysis system*

The CRDS system consists of four main components, including laser light source, vacuum ringdown cavity, photoelectric detection, and data acquisition. Figure 1 is a schematic diagram of the overall structure of the CRDS expiratory isoprene analysis system. The experiment was carried out at room temperature and a standard atmospheric pressure. A Nd: YAG pump laser (Spectra-Physics, Quanta Ray DLS 8020, U.S.) pumped dye laser (Spectra-Physics, Cobra-Stretch-LG, U.S.) is used as ultraviolet laser light sources. The final output wavelength is 226.56 nm and its nearby wavelength range. The absorption cell consists of a vacuum ringdown cavity (CRD Optics, 903-1001, US), a pair of mirror frames (CRD Optics, 902-0010, US) for fixing and adjusting the collimation of the mirror, and a pair of high-reflectivity lenses with a reflectivity of 99.8% (MLD Technologies LLC, US). Three stainless steel tubes are connected to the vacuum ringdown chamber, which is connected to the gas sample, vacuum pump (Oerlikon Leybold Vacuum Gmbh, SCROLLVAC SC5D, Germany), and pressure sensor (MKS 870B, US, range 0–1000 Torr, Resolution 1 Torr). A high-sensitivity photomultiplier tube (PMT) (HAMAMATSU, Japan) was used to collect the ringdown signal and an oscilloscope (Tektronix, US, MDO3012) to digitally process the ringdown signal was used in order to transmit it to the computer. The CRDS measurement system software developed in the early stage of the laboratory analyzes and processes the ringdown curve and extracts the required data.

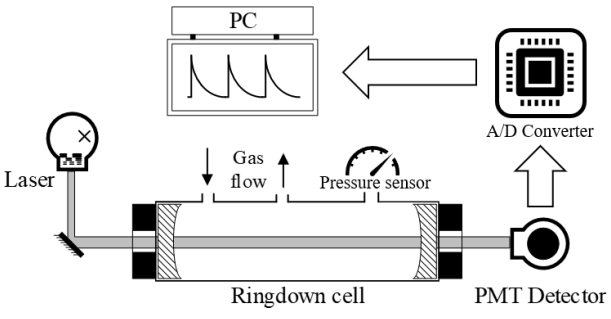

Fig. 1. Schematic of the experimental CRDS system.

2.2. *Breath sample collection*

Lung cancer inclusion criteria: (1) Patients with abnormal chest CT and diagnosed as lung cancer by pathological analysis, (2) Unlimited gender and age, (3) Sign informed consent. Exclusion criteria: (1) History of other malignancies, (2) Have other lung diseases, and (3) Dyspnea, unable to collect breathing gas normally.

Healthy people included in the criteria: (1) No pulmonary abnormalities detected by CT, (2) Unlimited gender and age, (3) Sign informed consent. Exclusion criteria: (1) Have other lung diseases, and (2) Unable to cooperate normally to collect breathing gas.

The experiment adopts an offline sampling method. The sampling belt is filled with fluorinated ethylene propylene (FEP) gas sampling bag with a volume of 1L, which is repeatedly flushed with high-purity nitrogen three times before and after use. Through explanations and demonstrations, the subjects were instructed to take a proper breath and then breathe the end-tidal volume of a single breath into the sampling bag through the disposable mouthpiece. The sampling bag can keep the gas in the bag stable for 6 h. Subjects were collected by one exhalation sample by one subject. Since the composition of the background air is stable, approximately one background air sample is used for every three subject samples. After sampling, connect the sampling bag to the injection port of the CRDS breath analysis system and introduce the sample into the sample chamber of the measurement instrument.

The process of collecting human breath gas is as follows: (1) Prepare FEP gas sampling bag for the subject; (2) After the subject inhales through the nose, holds his nose, exhale the gas from his mouth at one time, and blow the whole gas sampling bag as full as possible; (3) After blowing, the subject turns off the switch of FEP gas sampling bag; (4) The experimenter wrote the subject number and the sampling time on the label of FEP gas sampling bag, and then put it into the incubator to keep it at a constant temperature and away from light. After the collection, the incubator is brought back to the laboratory for CRDS measurement; (5) Note that during exhalation, the subject shall not have the action of repeated mouth breathing, nor shall he exhale with his nose when blowing.

In this paper, a total of 216 breath samples were collected, including 92 breath samples from lung

cancer patients, 17 breath samples from patients with benign diseases, and 107 breath samples from healthy people. The gender, age, BMI, whether to smoke, fasting, and other indicators were recorded.

2.3. *Measurement and analysis methods*

The CRDS breath analysis system uses the background subtraction method to calculate the isoprene concentration, that is, the air is regarded as the background of human breath, and the cavity ringdown time between the breath sample and the air is alternately measured so that the effective absorption of air and breath can be obtained.[23]

$$A_{\text{atm}} = \sigma(\nu)\text{nd} = \frac{d}{c}\left(\frac{1}{\tau_{\text{atm}}} - \frac{1}{\tau_0}\right), \quad (1)$$

$$A_{\text{breath}} = \sigma(\nu)\text{nd} = \frac{d}{c}\left(\frac{1}{\tau_{\text{breath}}} - \frac{1}{\tau_0}\right), \quad (2)$$

$$A_{\text{breath}} - A_{\text{atm}} = \sigma(\nu)\text{nd}. \quad (3)$$

A_{atm} is the absorption intensity of the atmosphere, A_{breath} is the absorption intensity of the breathing gas, τ_{atm} and τ_{breath} are the ringdown time of background air and breathing gas under 1 atm in the ringdown cavity, respectively, τ_0 is ringdown time constant under vacuum. $\sigma(\nu)$ is the absorption cross-section of matter. d is the length of the ringdown cavity, c is the speed of light. Through the absorption difference between breathing gas and background air, the isoprene concentration n (molecule/cm^3), Particle number density, can be calculated.

In this study, Statistical Product Service Solutions (SPSS) software was used for statistical analysis. Statistical data uses mean ± standard deviation ($\bar{x} \pm s$) to represent its distribution. In the correlation analysis, r is used to represent the correlation coefficient, Pearson correlation analysis is used for normal distribution and rank correlation. Spearman correlation analysis is used for non-normal distribution. Through the analysis method of fitting multiple linear regression model, we explore the influencing factors of isoprene concentration.

3. Results and Discussion

3.1. *Basic information*

The trial was registered with the Institutional Review Board (IRB) of the Chinese Clinical Trial Registry (registration number: chiCTR1900023659). All methods were carried out in accordance with relevant guidelines and regulations, and informed consent was obtained from all participants. The breath samples of 92 patients with lung cancer and the breath samples of 17 patients with benign lung lesions are all from Tianjin Cancer Hospital. The 107 breath samples of healthy volunteers from the Institute of Chinese Academy of Medical Sciences. The ratio of male to female is close to 1:1. Table 1 shows the basic information of volunteers.

Table 1. Subject information.

	Cancer	Benign lung lesion	Healthy volunteer
Counts	92	17	107
Age/year	58.2 ± 8.4	55.5 ± 10.3	25.4 ± 4.2
BMI/kg ∗ m^{-2}	24.1 ± 3.0	24.5 ± 2.9	21.9 ± 3.5
Smokers	43 (46.7%)	5 (29.4%)	5 (4.7%)
Male	38 (41.3%)	10 (58.8%)	49 (45.8%)
Expiratory isoprene concentration/ppb	112.0 ± 36.6	127.9 ± 41.2	221.3 ± 122.2

The statistical analysis finds that the breath isoprene concentration of healthy people was higher than that of lung cancer patients, and there is a statistical difference between the two ($P < 0.05$). The breath isoprene concentration of healthy people is higher than that of benign lung lesions, and there is a statistical difference between the two ($P < 0.05$). The breath isoprene concentration of patients with benign lung lesions and lung cancer patients is not statistically different ($P = 0.109$).

The breath isoprene of 92 lung cancer patients showed a normal distribution, as shown in Fig. 2(b), with an average concentration of 112.0 ± 36.6 ppbv, a range of 23.5 − 202.7 ppbv. The breath isoprene of 17 patients with benign lung disease showed a normal distribution (127.9 ± 41.2 ppbv, range 64.6 − 215.3 ppbv), as shown in Fig. 2(c). The breath isoprene of 107 healthy people showed a non-normal distribution, as shown in Fig. 2(d), ranging from 51.1–642.0 ppbv. Synthesis of the mevalonate pathway intermediate dimethylallyl-diphosphate is associated with the production of the volatile molecule isoprene which has been linked to a plethora of pathophysiological conditions. Numerous studies have reported the presence of increased levels of isoprene in the breath of lung cancer patients, compared to healthy controls or patients with other lung disorders. An analysis by Bajtarevic[24]

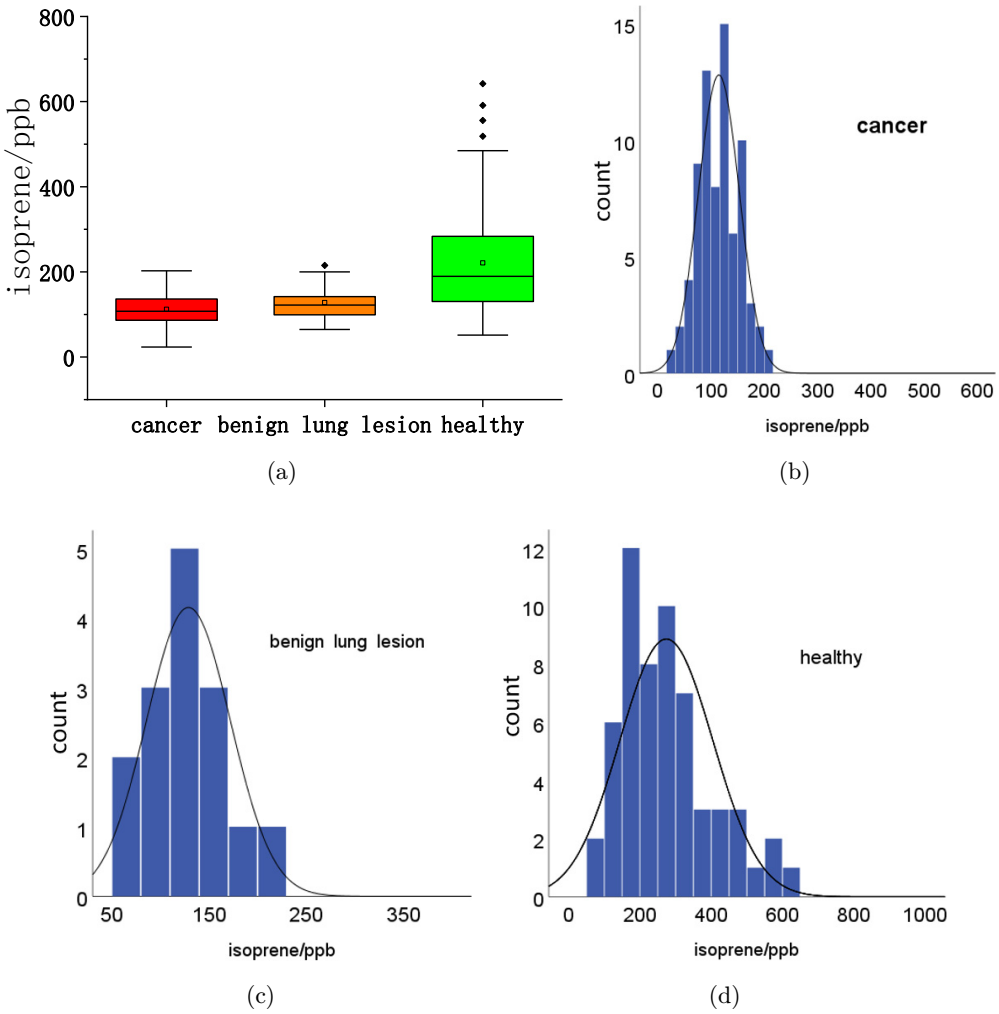

Fig. 2. (a) Three groups of volunteers' breath isoprene concentration; (b) Distribution of isoprene concentration in breath of lung cancer patients; (c) Distribution of isoprene concentration in breath in patients with benign lung disease; (d) Distribution of isoprene concentration in breath of healthy people.

has shown that compared with healthy controls, patients with lung cancer have lower levels of breath isoprene. In addition, there are also other diseases related to inflammation or immune activation that have decreased breath isoprene.[25,26]

3.2. *The relationship between breath isoprene and gender, age, BMI*

We analyzed the concentration distribution of breath isoprene in all subjects of different genders. Figure 3 shows that there is no significant difference between the breath isoprene concentration in male (163.9 ± 95.1 ppbv) and the breath isoprene concentration in female (170.2 ± 112.0) ($P = 0.665$).

Figure 4 shows the relationship between isoprene concentration and age in different groups. The breath isoprene concentration of lung cancer patients decreases with age. However, there is no correlation found between the two ($y = -0.2626x + 127.3125$, $R^2 = 0.0074$). The breath isoprene concentration of patients with benign lung lesions increases with age. However, the correlation is not obvious ($y = 1.3765x + 51.5050$, $R^2 = 0.0594$). The breath isoprene concentration of healthy people shows a downward trend with age, but there is no correlation found between the two ($y = -5.1475x + 352.20$, $R^2 = 0.0215$). All in all, there is no correlation found between breath isoprene concentration and age.

Figure 5(a) shows the relationship between isoprene concentration and BMI in different groups. It is usually considered that BMI > 25 is overweight. All volunteers are divided into two groups

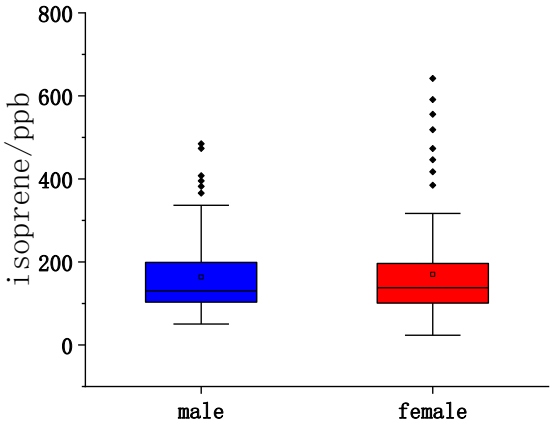

Fig. 3. Breath isoprene concentration of different genders in all subjects.

according to BMI. The breath isoprene concentration of volunteers with BMI < 25 is slightly higher than that of volunteers with BMI > 25, but there is no significant difference ($P = 0.096$). At the same time, in Fig. 5(b), among all the volunteers, as the BMI increases, the concentration of breath isoprene shows a downward trend ($y = -4.2641x + 265.555$, $R^2 = 0.0153$), but there is no correlation.

The results of the three groups of volunteers did not show differences in exhaled isoprene between gender and age. Although there is no consensus in the literature on the relationship between isoprene and age or gender, many studies have shown that the amount of isoprene exhaled by the human body is not affected by age and gender.[27] There is no correlation between the breath isoprene concentration and BMI of healthy volunteers. This result is consistent with the reports in the literature.[28] So far, it has not been possible to fully explain this difference through literature research. Therefore, in-depth research on a large sample of human breath isoprene is the key to a more comprehensive understanding of the mechanism of isoprene production.

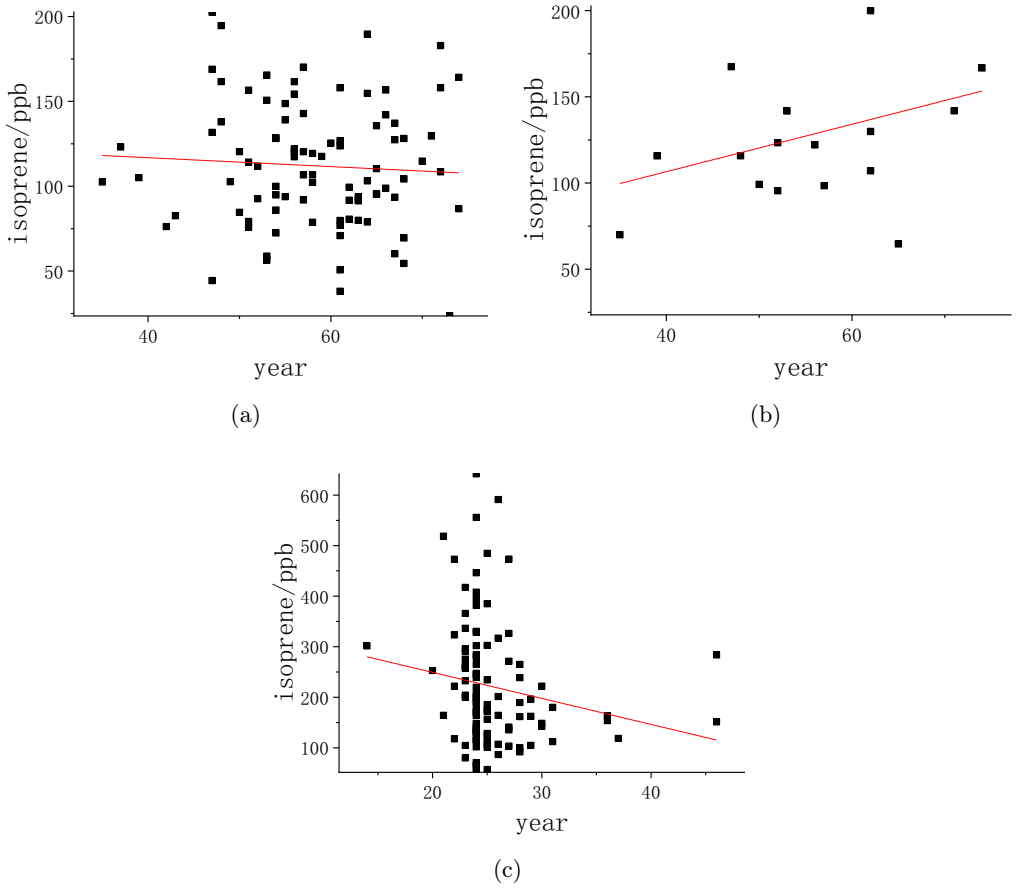

Fig. 4. (a) Relationship between breath isoprene concentration and age in patients with lung cancer; (b) Relationship between breath isoprene concentration and age in patients with benign lung lesions, and (c) Relationship between breath isoprene concentration and age in healthy people.

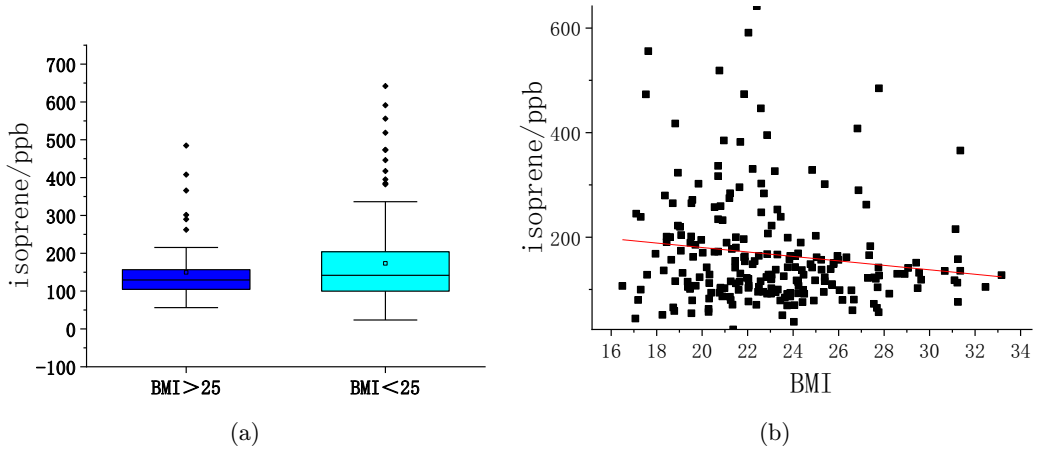

Fig. 5. The relationship between isoprene concentration and BMI.

3.3. *Effect of fasting or not on breath isoprene*

Because the sample size of patients with benign lung tumors is small, and there is no significant difference in breath isoprene between patients with lung cancer and patients with benign lung lesions, we call lung cancer patients and benign lung lesions patients as lung disease patients. Among the patients with lung diseases, 59 subjects were fasting and 50 subjects were not fasting. Among the healthy subjects, 53 subjects were fasting and 54 subjects were not fasting. Figure 6 shows the relationship between fasting and breath isoprene concentration. The results showed that there was no significant difference in the concentration of breath isoprene in patients with lung diseases ($P = 0.38$, > 0.05), and there was no significant difference in the concentration of breath isoprene in healthy subjects ($P = 0.96$, > 0.05). It can be seen whether fasting has no effect on breath isoprene concentration.

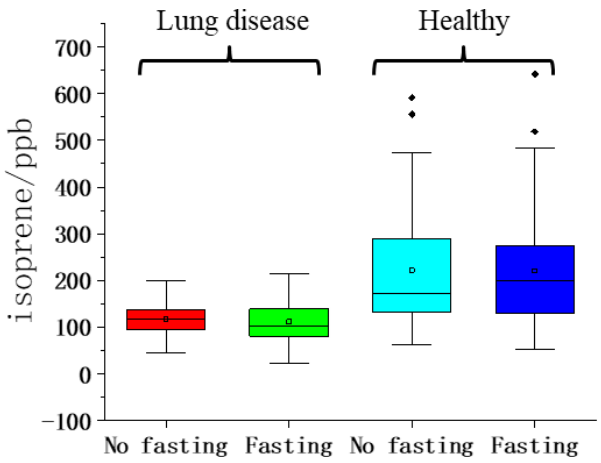

Fig. 6. The relationship between fasting and breath isoprene concentration.

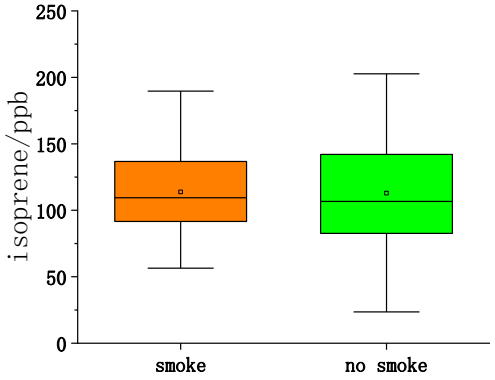

Fig. 7. Comparison of isoprene concentrations between smoking and nonsmokers in patients with lung cancer.

3.4. *The relationship between breath isoprene and smoking*

Healthy volunteers hardly smoke, and the sample size of patients with benign lung lesions is small. Therefore, only the relationship between respiratory isoprene and smoking in lung cancer patients is analyzed. Figure 7 shows the relationship between smoking and breath isoprene concentration. Among lung cancer patients, the average breath isoprene concentration (113.84 ± 34.8 ppbv) of smokers is lower than the average breath isoprene concentration (112.9 ± 40.1 ppbv) of nonsmokers. However, there is no statistical difference between the two.

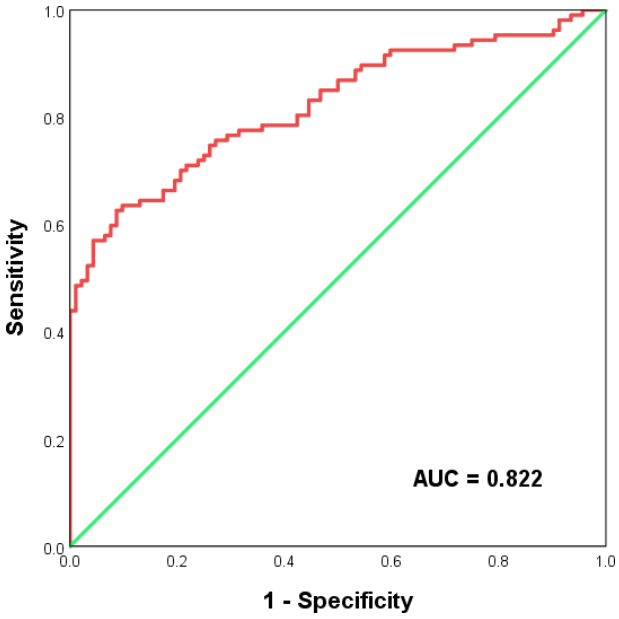

Fig. 8. The ROC curve of exhaled isoprene in the diagnosis of lung cancer.

3.5. *The diagnostic significance of breath isoprene for lung cancer*

Since the expiratory isoprene levels of the three groups of subjects are not affected by individual factors, the Receiver Operating Characteristic (ROC) is drawn to evaluate the significance of breath isoprene for the diagnosis of lung cancer. We used 107 healthy and 92 lung cancer breath samples to plot ROC curves. ROC curve results show (Fig. 8) that breath isoprene concentration is meaningful for the diagnosis of lung cancer (The area under the ROC curve AUC = 0.822, the sensitivity is 63.6%, the specificity is 90.2%, $P < 0.01$).

4. Conclusion

The purpose of this paper is to explore the effect of isoprene on lung cancer screening. The breath samples of 107 healthy people, 92 patients with lung cancer, and 17 patients with benign lung lesions are measured, which confirms the feasibility of the CRDS system in the measurement of large sample size clinical data. The results show that the breath isoprene level of healthy people (221.3 ± 122.2 ppbv) is significantly higher than that of lung cancer patients (112.0 ± 36.6 ppbv) and patients with benign lung disease (127.9 ± 41.2 ppbv), which may be related to oxidative stress and immune activation during the disease process. There was no correlation between the breath isoprene concentration and BMI, age, gender, and smoking. Since the breath isoprene level of the three groups of subjects is not affected by individual factors, the ROC curve results show that the breath isoprene concentration is meaningful for the diagnosis of lung cancer (AUC = 0.822, the sensitivity is 63.6%, the specificity is 90.2%, and $P < 0.01$).

The actual endogenous origin of isoprene in human breath is yet uncertain. The metabolic source of respiratory isoprene in the human body has not been determined. So, it cannot be used as a diagnostic biomarker in clinical practice so far.[29] This study demonstrates that the CRDS breath isoprene analysis system can effectively analyze a large sample of human breath isoprene, and preliminarily confirms the use of breath isoprene as a biomarker for lung diseases. So far, no VOC only exists in the exhaled breath of patients with lung cancer. The VOCs used for the diagnosis of lung cancer are not a single VOC, but a group of related VOCs. How to achieve more effective and reliable lung cancer breath biomarkers requires more in-depth research.

Authorship Contribution

Xin Wei contributed in Methodology, writing the original draft, forming the analysis and visualization. Qingyuan Li validated the writing, reviewed it and edited it. Yinghua Wu, Jing Li, and Guangkuo Zhang made the formal analysis and visualization. Jing Li and Meixiu Sun supervised the resources, project administration, funding acquisition, and also wrote, reviewed, and edited this paper.

Conflict of Interest

The authors declare that they have no financial and personal relationships with other people or organizations that can inappropriately influence their work, there is no professional or other personal interest of any nature or kind in any product, service and/or company that could be construed as influencing the position presented in, or the review of, the manuscript entitled.

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