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Analysis of exhaled air for early-stage diagnosis of lung cancer: opportunities and challenges

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Early detection of lung cancer usually markedly increases the efficiency of therapy. However, the currently employed diagnostic approaches are not sufficiently effective, resulting in late detection of the disease and high patient mortality. Therefore, development of a high-throughput and reliable diagnostic method is a priority task requiring fast solution. Analysis of exhaled air for a number of organic compounds recognized as lung cancer biomarkers seems to be a promising approach for early diagnosis of the disease. This issue attracts growing interest, as indicated by increasing number of publications on this topic. This review surveys contemporary analytical techniques for analysis of exhaled air, including various spectroscopic and mass spectral methods and also gas sensor-based methods. The key benefits and shortcomings of the techniques, sample injection and pre-concentration methods, and the potential applicability of the methods for lung cancer detection are discussed. The prospects of simultaneous application of several analytical techniques and approaches for the early diagnosis are demonstrated.
The bibliography includes 147 references.

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1. Introduction

Malignant diseases (MDs) constitute a highly acute medical and social problem all over the world, with the number of patients exceeding 140 million.[1] The cohort of patients with MDs in Russia is more than 3.5 million, that is, 2.5% of the population of the country. Cancer ranks second in the mortality rate, which reached over 15% in 2016.[2] The success of health care in the 20th century has provided the epidemiological transition to the 'era of non-communicable diseases', among which the lead position belongs to MDs. In general, statistical data indicate that in the 21st century, too, MDs will be a highly acute medical and social problem. One of the ways to address this problem is to improve early diagnosis. The incidence of lung cancer (LC) reaches 1.8 million cases in the world.[3,4] In Russia, LC ranks second in the general incidence of cancer (10.9%) and ranks first (17.6% in 2016) among the malignant tumours in men. The number of patients diagnosed with lung cancer every year exceeds 50 thousand, among which 90% – 96% individuals die, and more than 50% die within the same year they are diagnosed.[5]

Despite the vigorous development of diagnostic and treatment techniques, the five-year survival rate for lung cancer has not changed in the last 50 years in any of the countries, being not higher than 15% – 20%. Meanwhile, it is known that treatment efficiency is directly dependent on the tumour advancement at the time of diagnosis. Indeed, whereas the five-year survival rate can reach 70% – 80% for patients diagnosed with stage I lung cancer, that for stage IV does not exceed 5%.[5] Early-

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stage diagnosis of LC is still an unsolved problem, and for more than 2/3 of patients, specialized treatment starts when their lung cancer is locally advanced or disseminated.[2,6] Since the clinical presentations of LC are, most often, indicative of an advanced neoplastic process, it is reasonable to perform diagnostic procedures aimed at early diagnosis in the symptom-free period of tumour growth, *i.e.*, development of screening methods for risk groups is required.[5–13]

Despite the diversity of cancer types, only for some of them, the benefits of screening have been shown to exceed the possible undesired consequences. First of all, this is the case for breast cancer, cervical cancer and colorectal cancer. Lung cancer screening based on low-dose computed tomography has also proved to be efficient.

The traditional methods such as sputum cytology and chest X-ray are of low utility as screening methods, in particular, because of a large number of false-negative results. However, low-dose computed tomography, which has shown to be efficient for decreasing the mortality, gives a large number of false-positive results, which brings about numerous unnecessary biopsies associated with the risk of complications, the use of additional examination methods and increasing cost of diagnosis.[9]

Thus, the above facts indicate that development of new high-technology, economically feasible, safe, efficient, easily handled, compact and reliable tools for non-invasive lung cancer diagnosis and their subsequent testing as screening techniques is a relevant challenge of modern science. A fundamental difference of screening from early-stage diagnosis is examination and cancer detection in persons who would not consult an oncologist by themselves, because of the absence of cancer symptoms or signs or only minor symptoms present. Note that screening implies the use of simple tests among healthy population in order to reveal people who have already developed the disease, but still have no symptoms.[7]

Of obvious interest is utilization of the odour inherent in the patient (his/hers skin, exhaled breath, urine, feces and so on), which has been known as a diagnostic tool since ancient time. Hippocrates ($\sim$400 years BC) was among the first to describe the difference between the normal and pathological odour emitted by a patient.[7,14] Today, diagnosing patients by analysis of exhaled air (EA) components is a new developing area of medicine.

There are more than 3000 volatile organic compounds (VOCs) known to get into exhaled air from blood as a result of gas exchange in the lungs. Since these compounds are products of biochemical processes taking place in the body, a change in the VOC composition implies the possibility to diagnose various diseases by analyzing the exhaled air.[13] The advantages of analysis of exhaled air for health monitoring over the analysis of biological fluids include the simplicity and non-invasiveness of sample collection, relatively low matrix effects, the possibility of direct measurements at any frequency and the possibility of real-time online analysis.[8] A literature survey reveals quite a few papers devoted to the application of exhaled air analysis in the diagnosis of infectious diseases,[15–19] chronic non-specific respiratory diseases and cancer.[20–26]

This method can, in principle, be used not only for early diagnosis of lung cancer, but also for screening of large population groups for early detection of symptom-free cancer.

The analytical methods that are now used to analyze the exhaled air can be divided into two groups: (1) methods for identification and quantitative determination of VOCs in the exhaled air: gas chromatography–mass spectrometry (GC-MS), proton transfer reaction mass spectrometry (chemical ionization) (PTR-MS), selected ion flow tube mass spectrometry (SIFT-MS) and (2) methods in which analysis is based on an image recognition algorithm using the set of obtained analytical data as a whole without identification of particular compounds: electronic nose (E-nose) and multi-capillary column ion mobility spectrometry (MCC-IMS). In some cases though, MCC-IMS can be employed as a selective method.

While choosing a particular screening technique for scientific research in population programmes, it is necessary to take into account the following factors:

— sensitivity: the efficiency of a test in detecting cancer in those who have the disease;

— specificity: the probability of negative result for those free of the disease;

— positive predictive value: the probability that those who give a positive test result do have the disease;

— negative predictive value: the probability that those who give a negative test result are free from the disease;

— acceptability: the probability that individuals for whom the test is designed will agree to be tested.

— safety;

— relatively low cost.

The screening of cancer should be acceptable for large-scale use, relatively inexpensive, highly sensitive and specific and have high positive and negative predictive values,[14] as well as be painless and safe for a patient.[18–23]

High levels of specificity and positive predictive value are the most important requirements that determine the efficiency of any method for LC screening. These values should be at least 98%–99%; otherwise, the number of unnecessary biopsies associated with the risk of complications sharply increases, and the use of additional examination methods and the cost of examination also increase.[9] The requirements to the sensitivity and negative predictive value are less stringent, at least 90% and 85%, respectively.

In this review, we consider the possibility for development of a screening method for lung cancer on the basis of the most recent advances in exhaled air analysis. It is noteworthy that there are several reviews devoted to the diagnosis of LC based on exhaled air analysis.[25,27–29] The present review differs from the previous publications by the presence of a critical consideration of methods used for EA analysis evaluating their applicability for early diagnosis and screening of lung cancer. This approach is missing in the above-mentioned publications.

The following abbreviations are used:

Ac — asthma;

APCI-MS — atmospheric pressure chemical ionization mass spectrometry

AUC —area under the ROC curve

CNT — carbon nanotubes

COPD — chronic obstructive pulmonary disease

cps — counts (the number of pulses) per second
CRDS — cavity ring down spectroscopy
EA — exhaled air;
E-nose — electronic nose
ESI — electrospray ionization
GC — gastric cancer;
GC-MS —gas chromatography – mass spectrometry
IMS-MS — ion mobility spectrometry – mass spectrometry
LC — lung cancer;
LOD — limit of detection
MCC-IMS — multi-capillary column ion mobility spectrometry
MD — malignant disease;
PFA — perfluoroalkoxide polymers;
ppb — parts per billion
ppt – parts per trillion
PTFE — poytetrafluoroethylene;
PTR-MS — proton transfer reaction mass spectrometry;
PVDC — polyvinylidene chloride
PVF — polyvinyl fluoride;
SCFA — short-chain fatty acids;
SESI-MS — secondary electrospray ionization mass spectrometry;
SIFT-MS — selective ion flow tube mass spectrometry;
SiNW-FET — silicon nanowire field effect transistors;
SPME — solid-phase microextraction;
TOFMS — time-of-flight mass spectrometer;
VOC — volatile organic compound.

2. Volatile markers of lung cancer

Volatile compounds that are present in the exhaled air are formed in the exchange reactions that occur both in the human body and in the microbiota. In pathological states, metabolic shifts inevitably take place in the microbiota symbiosis and, as a consequence, the range of produced compounds, including low-molecular-mass ones, changes. These compounds can be detected in the exhaled air. In the case of pathology, changes in the range of low-molecular-mass microflora metabolites can, in principle, be detected to diagnose early cancer.

Human exhaled breath contains several hundred compounds, but only some of them can be useful for early-stage detection of LC. Below we consider only these compounds and methods used to detect them. It is noteworthy that the biomarkers found in healthy and diseased individuals differ in their concentration ranges rather than are merely absent or present. The mechanisms of generation of lung cancer biomarkers in the EA have been considered, for example, by Hakim *et al.*[27]

It is possible to distinguish a number of compounds, the informative value of which has been proved in a number of studies; these compounds are summarized in Table 1. The markers were selected from publications in which the difference between subjects with and without LC was statistically significant. Also, the presented markers are those reported in at least two publications.

As follows from Table 1, together with usual volatile components, the list of potential lung cancer markers includes short-chain fatty acids (SCFA) of the bacterial origin, for example, propionic acid[21] the concentration of which in the EA can exceed 10^{-2} relative to the concentration of acetone.[47] Microbiota, in particular, produces compounds that cannot be produced and fermented by eukaryotic cells. Certain groups of aromatic compounds, including indole derivatives and phenolic acids have been found in the human blood.[48,49] Acidic bacterial metabolites such as volatile carbocylic and phenylcarboxylic fatty acids and phenol- and benzene-derived acids have been reported.[50] Short-chain fatty acids (acetic, propionic, butyric, isovaleric and other) result from degradation of polysaccharides.[51] The intestinal anaerobic bacteria metabolize sugar molecules to form carbon dioxide and hydrogen[52,53] from short-chain fatty acids. Hydrogen is the only known inorganic gas of exclusively microbial origin.

The human microbiota, together with microorganisms, performs metabolic, detoxification and anti-infectious functions; therefore, its disorders result in, or result from, pathological conditions. Considerable interest is aroused in investigations of the microbiota metabolites, in particular, SCFA. The formation dynamics and composition of SCFA considerably change upon pathological

Table 1. Known lung cancer markers in exhaled air.

Compound (concentration)	Method	Cancer type	Ref.
Pentan-2-one (0.4 – 1.8 ppm)	GC-MS	Lung cancer	30
Pentanal	GC-MS	Non-small cell cancer	31
Pentanal (12.8 – 49 pM)	GC-MS, SIFT-MS	Adenocarcinoma and squamous cell cancer	26
Nonanal*	GC-MS, MCC-IMS	Squamous cell cancer	28, 31, 32
3-Hydroxybutan-2-one (2.0 – 50.3 ppb)	GC-MS	Adenocarcinoma and squamous cell cancer	22
Butan-1-ol (2 – 13 ppb)	GC-MS	Non-small cell cancer	22, 28
Butanal	SIFT-MS	Non-small cell cancer	27
Hexanal*	GC-MS, SIFT-MS	Squamous cell cancer	28, 31
Octanal*	GC-MS, SIFT-MS	Squamous cell cancer	28, 31
Propan-2-ol	PTR-MS	Small-cell and non-small cell cancer	33

Table 1 (continued).

Compound (concentration)	Method	Cancer type	Ref.
Ethylbenzene*	GC-MS	Adenocarcinoma and squamous cell cancer	34
Dodecane*	MCC-IMS	Small-cell and non-small cell cancer	28, 32, 35
Hexadecanal	GC-MS	Adenocarcinoma and squamous cell cancer	36, 37
Propionic acid*	GC-MS	Non-small cell cancer	38, 39
Acetic acid	GC-MS	Non-small cell cancer	38, 39
Propan-1-ol	GC-MS	Lung cancer	40
Acetaldehyde	SIFT-MS	Squamous cell cancer	41–44
Propane (3.19–9.7 ppb)	GC-MS	Adenocarcinoma and squamous cell cancer	34
o-Xylene	GC-MS	Adenocarcinoma and squamous cell cancer	34
Formaldehyde	PTR-MS	Small-cell and non-small cell cancer	33
Heptane	GC-MS	Non-small cell cancer	45, 46

Note. The most informative markers are asterisked.

processes;[54] however, there are still only few publications devoted to SCFA as LC markers; some data are available only for acetic, propionic and lactic acids.[38, 39] It is evident that studies along this line could identify new efficient biomarkers for some cancer types.

Below we will consider analytical methods that are used or can, in principle, be used for early diagnosis of some types of cancer, but, first of all, we will focus on sampling methods used in this area.

3. Analytical methods used to diagnose lung cancer from the analysis of exhaled air

3.1. Sampling methods used for the analysis of exhaled air

Sampling is a highly important stage of analysis of EA composition. There are several parameters that should be addressed in order to avoid erroneous assumptions about the origin of particular identified compounds. These parameters include the type of exhaled air (breath volume); breathing technique; sampling frequency; method of sampling; effect of the VOCs present in the room air; conditions of sample storage and transportation. All these parameters are considered in detail and discussed in the literature.[55–57] If the EA composition is analyzed in the online or real-time mode, the sampling and preconcentration stages can be omitted.

3.1.1. Sampling details

For analysis of EA composition, it is possible to sample mixed expiratory air or only alveolar air. In the former case, there is a high risk of sample contamination with exogenous compounds from the oral cavity and dead space (nasopharynx, trachea, bronchi and bronchioles until they are transformed to alveoli), which can compromise the results of analysis.[55] The alveolar air is richer in the volatile compounds of blood; therefore, the collection of alveolar breath is more accurate, the sample being representative and of constant quality.[58, 59]

The use of various breathing techniques such as breath-holding, hyperventilation, breathing against resistance and so on is, most often, directed towards either accumulation of the exhaled gases or separation of EA fractions in one exhalation.[58, 59]

The sampling can cover a single or several full exhalations. Analysis of the composition of multiple exhalations is more reproducible as regards the sample composition;[55] however, as a rule, a single exhalation takes less time and is more tolerable for patients.

A special mention should be paid to the problem of condensation of the water vapour present in EA and redistribution of EA components between the condensed and gas phases. Water vapour present in exhaled air (approximately 7 $cm^3\ kg^{-1}$ of body weight in 24 h) is involved in the transfer of many volatile and non-volatile compounds *via* dissolution (according to the partition ratios) and formation of new compounds in an aerosol particle.[60, 61] The water vapour accumulates all non-volatile compounds such as hydrogen peroxide, adenosine, leukotrienes, isoprostanes, peptides and cytokines.[62] Also, polar organic and inorganic compounds such as alcohols, ketones, carboxylic acids, ammonia and nitrogen oxides are concentrated completely or partially in the exhaled breath condensate.[63] The most comprehensive information about the exhaled breath composition is gained from analysis of volatile organic compounds and exhaled breath condensate. The uncontrolled condensation of water vapour in sampling devices and tubes is prevented by maintaining all parts of the system at 37–40 °C.[64]

3.1.2. Sample storage methods

Exhaled air samples can be stored in a number of ways.[11, 55] One approach is to use Tedlar® sampling bags. The bags are manufactured from chemically inert polymer materials such as polyvinyl fluoride (PVF), perfluoroalkoxide polymers (PFA), poytetrafluoroethylene (PTFE) and polyvinylidene chloride (PVDC).[65] These bags have a number of benefits: they are gas impermeable (when coated additionally by aluminium foil)[66] and convenient for use (can be used repeatedly when thoroughly purged with nitrogen or argon after the previous sample). Despite these benefits, the bags also

suffer from some drawbacks: the plasticizers and solvents used for polymer production (*e.g.*, phenol and *N*,*N*-dimethylacetamide) can be released in relatively high concentrations and thus contaminate the sample;[67] the bags are susceptible to punctures; some components, for example, hexanal and 2-methylbuta-1,3-diene, cannot be stored for more than several hours.[68,69]

One more method is the use of gas-tight syringes. A 50 ml syringe is connected to the cartridge in which the patient breathes. During the exhalation, approximately 20–30 ml of EA is withdrawn by the syringe and then transferred into an evacuated glass vial in which the sample is stored before analysis.[58]

One more way to store EA samples is the exhaled air condensate.[70]

Rather recently, a Bio-VOC breath sampler has been designed.[71] This device can be used to collect the alveolar air; after sample collection, VOCs are pre-concentrated using the solid phase microextraction (SPME) system[72,73] or a sorption tube. The major drawback is the small volume of the collected air (100–150 ml).[72–75]

3.1.3. Pre-concentration

As has already been noted, the content of VOCs in the EA can vary from several μmol litre^{-1} to several fmol litre^{-1} (Refs 58, 76). Therefore, depending on the method used to analyze EA, an intermediate stage between sample collection and analysis is needed to increase the contents of the target components.

The pre-concentration method used most often for VOC determination in exhaled breath is sorption on solid sorbents followed by thermal desorption.[77–79] The use of sorption pre-concentration makes it possible to attain limits of detection at ppt level for sample volumes up to 1 litre.[79,80] Despite the large assortment of solid sorbents with different retention ability, operating temperature and hydrophobicity, a single sorbent cannot adsorb all compounds present in a breath sample, which is due to the large range of volatility of the VOCs. Therefore, multicomponent tubes are used, which are packed with different sorbents arranged in the order of increasing retention ability.[81] The pre-concentration is conducted at room or lower temperature, while almost complete thermal desorption of the analyte from the sorbent surface is accomplished at 250–300°C.

The key sources of errors (loss of the analyte or artifacts) inherent in this pre-concentration method are the degradation of adsorbed analytes during storage,[82] thermal decomposition or isomerization of some compounds during thermal desorption[83,84] and degradation of the sorbent materials.[85,86]

3.2. Methods for exhaled air analysis clinically proven for lung cancer diagnosis

3.2.1. Gas chromatography–mass spectrometry

Gas chromatography–mass spectrometry (GC-MS) is perhaps the most versatile and sensitive method for analysis of VOCs in exhaled air suitable for analysis of numerous compounds in concentrations ranging from ppb to ppt. Therefore, GC-MS can be considered to be the gold standard for determining low contents of VOCs in human breath.[87,88]

Despite the versatility and low limits of detection, the GC-MS method has a number of drawbacks associated, first of all, with sampling and sample preparation. Meanwhile, analysis and processing of the results usually do not cause difficulties with the modern level of automation and availability of selective detectors and diverse chromatographic columns. However, clinical implementation of the GC-MS techniques has some limitations because of high cost, complexity of application and the need for highly skilled analytical chemists to run the equipment and interpret the results.

Furthermore, since GC-MS analysis takes considerable time (routine analysis takes up to 1 h), it is unsuitable for real-time analysis. The loss and degradation of analytes, in particular, reactive or thermally unstable metabolites, and possible contamination are important, still unsolved problems, which should be overcome to improve the quality of data gained in this analysis.[11,12,55,89,90]

It should be emphasized that GC-MS is used, first of all, for quantitative determination of VOCs such as alkanes, alkenes, alcohols, aldehydes and ketones. The study of Kischkel *et al.*[24] is a good illustration for the general approach to GC-MS analysis of exhaled air. The authors studied VOCs in the exhaled alveolar air of lung cancer patients (in total, 31 including 19 cases of non-small cell carcinoma, 12 cases of small cell carcinoma; 23 males and 8 females), healthy smokers (in total, 31 including 9 males and 22 females) and healthy non-smokers (in total 31 including 10 males and 21 females) by solid phase microextraction GC-MS analysis.[24] The background was corrected by parallel collection of room air (inhaled air) samples. The following physiological parameters were also measured for the participants: body height, body weight, blood pressure and heart rate. They were not on a special diet and did not consume food or smoke for at least 1 h before the trial. The authors quantitatively determined the set of 42 VOCs (including hydrocarbons, ketones, aldehydes, alcohols, nitriles, amides, furans, sulfides and aromatic compounds). Each compound was identified by the retention time and mass spectrometry. The age, gender, smoking history and inhaled compounds (contaminants) were shown to be the most important confounding variables in the group discrimination. Finally, the authors were unable to reliably and unambiguously discriminate the groups, except for identification of smokers. Considerable differences between the patient group and control group were obtained by using uni- and multivariate statistical data analysis without correction for the inhaled air. Thus, the compounds that determined the differences between the groups were mainly exogenous, coming from the clinical or laboratory air. For some compounds, the inspired concentrations were even higher than the expiratory concentrations. The authors classified dimethylformamide and carbon disulfide as contaminants. The results of this study also demonstrate that the concentrations of compounds in the exhaled air considerably depend on various parameters other than the disease being investigated, for example, patients' medical history. In other words, even the most advanced statistical methods fail if these confounding variables are not properly taken into account.[24]

Poli *et al.*[91] investigated the effect of surgery for lung cancer on the image of exhaled VOCs. The VOC levels were compared for non-small cell carcinoma patients before the surgery and 1 month and 3 years after the surgery. Statistically significant differences were found

for the following compounds: pentane, isoprene, benzene, toluene and ethylbenzene. The most important finding of this study is that two endogenous compounds — isoprene and pentane — show opposite concentration trends during 3 years after the surgical operation, which generally affected the VOC profile.[91] Furthermore, all aromatic compounds had higher concentrations before the surgical operation. One month later, a change in the level was found only for isoprene.

Subsequently, Wang *et al.*[21] proposed a special sampling system with surgical manipulations including placing a special gas collection tube on a tracheal bifurcation. The authors also related the following saturated hydrocarbons to oxidative stress and lipid peroxidation: 2,2-dimethyldecane, tetradecane, 2,2,4,6,6-pentamethylheptane, 2,3,4-trimethyldecane, nonane, 3,4,5,6-tetramethyloctane and hexadecane. The samples from the affected and healthy lungs were taken before and after the tumour resection. The analytes were pre-concentrated using solid phase microextraction. The results of some other studies on this issue are summarized in Table 2.

Table 2. Some cancer markers determined by gas chromatography – mass spectrometery.

Compound	Test group	Status/method/statistical processing	Ref.
VOC (profile of 22 compounds)	Lung cancer*	Differentiation from healthy (sensitivity, 100%; specificity, 81.3%)/GC-MS	45
Hexanal, heptanal, octanal and nonanal	Breast cancer	Increased level (AUC)/GC-MS	20
Decanal, butan-2-one, nonanal, styrene and hexadecane	Ovarian cancer (48 patients, 48 controls, 48 patients with benign tumours of gynaecologic organs)	Decanal and butan-2-one are increased for cancer patients with respect to the healthy control or patients with benign tumours; nonanal, styrene and hexadecane differentiate cancer from the control/GC-MS	92
Alkanes (C_5-C_{24}), benzene, acetone, isoprene, hexanal, heptanal, propylbenzene	17 (non-small cell cancer), 1 (small cell cancer), 10 (healthy control)	Discriminant analysis for 10 components resulted in 100% correct classification with 100% predictive value/GC-MS with SPME/analysis of major components	23
Possible biomarkers: butan-1-ol (sensitivity, 95.3%; specificity, 85.4%); 3-hydroxybutan-2-one (sensitivity, 93.0%; specificity, 92.7%)	43 patients with non-small cell lung carcinoma and 41 healthy controls (Chinese population)	Statistically significant differences between the patients and the controls; no difference between stages of the disease (I – II *versus* III – IV); difference between squamous cell cancer and adenocarcinoma/GS-MS with SPME/Wilcoxon rank sum test	22
Various VOCs	108 lung cancer patients,* 121 healthy volunteer, 24 persons with other lung diseases	Acetone and isoprene are increased for cancer and chronic obstructive lung disease/GC-MS with SPME/artificial neural network	46
Unbranched saturated C_3-C_9 aldehydes	38 non-smoker control group and 40 patients with non-small cell lung carcinoma (23 cases of adenocarcinoma, 13 cases of squamous cell carcinoma, and 1 case of large-cell carcinoma)	Butanal, pentanal, hexanal, heptanal and octanal are increased for cancer patients; correct differentiation from control for 90% of the patients/GC-MS with SPME, on-fibre derivatization/unilateral variance analysis	26
55 VOCs: methanol, acetaldehyde, ethanol, but-1-ene, acetonitrile, butane, prop-2-enal, furan, propane, acetone, carbon disulfide, propan-2-ol, dimethyl sulfide, 1-hydroxypropan-2-one, 2-methylbuta-1,3-diene, pentane, methacrolein, 2-methylpropanal, methyl vinyl ketone, butane-2,3-dione, 2-methylfuran, butanal, butan-2-one, ethyl acetate, 2-methylpentane, 3-methylpentane, benzene, methylcyclopentane, hexane, pentan-2-one, pentanal, toluene, 2,4,4-trimethylpent-1-ene, butyrolactone, ethylbenzene, *o*-xylene, nonane, decane and so on	23 lung cancer patients (2 cases of small-cell carcinoma, 21 cases of non-small cell carcinoma), 24 healthy non-smokers and 6 healthy smokers as the control; among patients, 2 non-smokers and 21 smokers	Considerable difference was observed for carbon dioxide, propane, *etc.*; the most pronounced differentiation was found for propan-2-ol, styrene, carbon disulfide, ethylbenzene, prop-2-enal and propane/Tedlar bag sampling, GC-MS with SPME/Mann – Whitney rank sum test	34

Note. The asterisk means that the type of tumour is not indicated.

3.2.2. Proton transfer reaction mass spectrometry

According to the proton transfer reaction mass spectrometry (PTR-MS), the reactant ion H_3O^+ is first generated in a low-pressure H_2O vapour discharge in a hollow cathode with a short drift tube. The ions enter the drift tube with a constant axial field, with the air to be analyzed being injected into the tube near the inlet. The main purpose of this tube is to reduce the intensity of the interfering cluster $H_3O^+(H_2O)$ (down to 1% relative to the H_3O^+ intensity) and some minor precursors ($O_2^+ < 1\%$, $NO^+ < 0.5\%$, $NH_4^+ < 2\%$ relative to the H_3O^+ intensity) by their collisional breakup.[93] At the end of this tube, there is a collisional cell in which the analyte (M) is protonated.

$$H_3O^+ + M \longrightarrow MH^+ + H_2O \quad (1)$$

Then the ions enter a mass spectrometer, most often, a quadrupole device. Quantitative determination is carried out by determining the signal intensity ratio of the analyte to the precursor H_3O^+.

The PTR-MS method has a high sensitivity: in some cases, the limits of detection are at the ppt level.[93] The key shortcomings of this method include the partial fragmentation of the analytes, multiple interferences and, as a consequence, complexity of interpretation of the mass spectra and quantitative determination of some compounds. In addition, the humidity of the air being analyzed considerably influences the sensitivity of the method and the relative intensities of the signals for fragments of the protonated analyte.[94] In recent years, the PTR-TOFMS procedure has been developed, the resolution of which is sufficient for eliminating a considerable part of interferences using the mass defect. Herbig *et al.*[47] and Sulzer *et al.*[95] employed a PTR-TOF 8000 (IONICON Analytik GmbH, Austria) mass spectrometer with a resolution of 5000 and a typical sensitivity of 150 cps ppb^{-1} for neutral analytes, which is substantially higher than that for the SIFT-MS method (10 cps ppb^{-1}), but somewhat lower than that for the conventional design of a PTR-QMS 500 instrument (400 cps ppb^{-1}). Furthermore, the sensitivity of PTR-QMS 500 decreases with increasing number of detectable components, whereas no such dependence is observed for PTR-TOF 8000. Therefore, when more than three compounds are to be determined simultaneously, the sensitivity is higher for PTR-TOF 8000 than for PTR-QMS 500.

The advantages of PTR-MS, like other real-time systems, are manifested especially clearly in determination of unstable compounds, in particular, aldehydes.[93] Note that for some compounds, for example, propanol, the protonated MH^+ molecule cannot be used, as it is unstable, although for many other compounds (Table 3), the protonated MH^+ components can be detected.

A major drawback of the PTR-MS technique is that the range of analytes is limited to those compounds for which the proton affinity in the MH^+ species is higher than that in the H_3O^+ species. However, soft ionization of a broad range of compounds can be attained by two other routes, instead of reaction (1).

1. Asymmetric charge transfer from a noble gas atom with the ionization energy (IE) exceeding the ionization energy of the analyte.[95]

$$Kr^+ + M \longrightarrow Kr + M^+ \quad (2)$$

2. Penning ionization involving metastable atoms of inert gases — Ar, Kr or Xe.[70]

$$Kr^* + M \longrightarrow Kr + M^+ + e^- \quad (3)$$

In these two cases, the molecular ion M^+ is detected instead of the protonated MH^+ molecule.

The asymmetric charge transfer procedure extends the range of possible analytes compared to that for the use of H_3O^+ as the reactant ion; however, the sensitivity of the method decreases several-fold.[95] In addition, because of the selectivity of process (2), the sensitivity for different compounds can differ by an order of magnitude, whereas in the case of the H_3O^+ precursor, the sensitivities are similar for a broad range of compounds.

When the Penning mechanism is used, the gas flow from the discharge cell is passed through a so-called 'conditioner' — a capillary in which ions and electrons are eliminated from the flow *via* ambipolar diffusion, which sharply suppresses the fragmentation of analyte M^+ ions.[96]

The Penning mechanism seems to hold a great promise for determination of a broad range of compounds in exhaled breath for lung cancer diagnosis; however, no studies on this subject are available as yet.

Table 3 presents the list of lung cancer markers that can be determined by PTR-MS based on both the protonated components MH^+ and their fragments. The Table also gives the relative signal intensities for the fragments of analyte compounds.[93] As can be seen in Tables 2 and 3, this list covers all of the main markers, which makes PTR-MS one of the most efficient methods for LC diagnosis. Meanwhile, relatively high signal intensities for fragments and relatively low signal intensities for MH^+ in the case of important markers such as propan-1-ol, propanal, pentanal, nonanal, propan-2-ol, octanal and some other substantially affect the quality of chemometric data processing, thus decreasing the specificity and sensitivity, which are important diagnostic parameters. In addition, according to Table 3, some markers form highly intense fragment peaks with the same m/z values (*e.g.*, 69 for, nonanal, pentanal, octanal and isoprene), which considerably reduces the selectivity of PTR-MS.

Wehinger *et al.*[33] employed the PTR-MS method to identify the potential LC biomarkers. This gave two candidate biomarkers: formaldehyde determined by the protonated molecule ($m/z = 31$) and propan-2-ol determined by the fragment with $m/z = 43$. The choice and the small number of compounds are probably caused by numerous interferences for many m/z values. In this study, a statistically significant difference was obtained between the mean concentrations ($\mu[x]$) of formaldehyde and propan-2-ol in healthy volunteers and lung cancer patients.

$$\mu[x_{31}](\text{patients}) : \mu[x_{31}](\text{controls}) \approx 2.3$$

$$\mu[x_{43}](\text{patients}) : \mu[x_{43}](\text{controls}) \approx 2.6$$

However, the scatter of the values is rather high and the two distributions (for individuals with and without the disease) partly overlap.

Table 3. Lung cancer markers that can be determined by PTR-MS and relative signal intensities for their fragments.[93]

Compound	Molecular mass (a.u.)	*m*/*z*	Relative signal intensities for ions (%)	Compound	Molecular mass (a.u.)	*m*/*z*	Relative signal intensities for ions (%)
Acetaldehyde	44	45	100	Propan-1-ol	60	43	100
		46	3.1			41	37.5
Propanal	58	59	100			44	3.4
		31	17.1			42	1.3
		41	7.1			27	0.4
		60	3.3	Propan-2-ol	60	43	100
Butanal	72	55	100			41	33.8
		73	7.6			44	3.6
		56	4.2			42	1.1
		46	2.2			27	0.1
Pentanal	86	69	100	*o*-Xylene	106	107	100
		41	34.2			108	8.8
		87	7.6	Isoprene	68	69	100
		70	5.6			41	83.5
		45	3.4			39	21.6
		39	2.9			70	5.8
Hexanal	100	83	100			67	3.8
		55	50.4			137	1.6
		84	6.7			68	1.1
		101	6.1	Acetonitrile	41	42	100
		56	2.3			46	2.1
Octanal	128	69	100	Benzene	78	79	100
		129	26.6			80	6.5
		70	6.3			78	0.4
		130	1.7	Toluene	92	93	100
		67	1.1			94	7.5
Nonanal	142	69	100	Formaldehyde	30	31	100
		143	37.2			35	5.7
		125	17.3			49	0.5
		72	9.6				
		70	5.9				
		67	3				

Wehinger *et al.*[33] evaluated the possibility of lung cancer diagnosis by means of the identified biomarkers by randomly forming groups of patients and healthy volunteers and determining the accuracy, sensitivity, specificity, positive predictive value and negative predictive value for these groups. The resulting values — 0.96 ± 0.02 for the accuracy, 0.54 ± 0.20 for the sensitivity, 0.99 ± 0.01 for the specificity, 0.90 ± 0.16 for the positive predictive value and 0.96 ± 0.02 for the negative predictive value — demonstrate that formaldehyde and isopropyl alcohol can, in principle, serve as lung cancer markers, but these values are too low for practical application of this approach.

Isoprene as a possible biomarker was used by Steeghs *et al.*,[97] who attempted to classify a small group of LC patients (11 individuals) and healthy individuals using the PTR-MS method. However, the researchers did not determine the sensitivity and specificity; therefore, it is difficult to evaluate the results. Apparently, the use of

only one and, moreover, unvalidated, biomarker is insufficient for gaining adequate results.

Bajtarevic *et al.*,[98] who focused attention on isoprene, methanol and acetone (although 50 more components were also measured), did not obtain statistically significant difference between healthy individuals and lung cancer patients (two methods, GC-MS and PRT-MS, were used). Meanwhile, this is not surprising, because the mentioned compounds are not recognized as lung cancer markers.

It is noteworthy that, despite its potential benefits such as accessibility of online analysis and high sensitivity, PTR-MS has not been used in recent years to diagnose LC. The cited publications appeared 8–10 years ago. The possible reason may be related to numerous interferences that hamper quantification of some markers.

3.2.3. Selective ion flow tube mass spectrometry

One more method suitable for determining the contents of VOCs in exhaled air is selective ion flow tube mass spectrometry (SIFT-MS). According to this method, one of the reactant ions, H_3O^+, O_2^+ or NO^+, is selected (using a quadrupole mass filter) from a mixture of components excited in a radio-frequency discharge in moist air; this is followed by chemical ionization of a broad range of compounds in a drift tube with ion detection by a mass spectrometer.[27,41–44,99,100] At the outlet of the drift tube, the analyte M can form a variety of molecular ions, particularly, M^+, $MH^+(H_2O)_n$, $(M-H)^+(H_2O)_n$ ($n = 0-2$) and so on. The above-listed reactant ions are efficient for analysis of exhaled air, because they slowly react with the major air component, but rapidly react with volatile organic compounds.[101] In the operation principle, SIFT-MS resembles PTR-MS. The difference is that in the SIFT-MS technique, one reactant ion is separated by a mass filter, whereas in PTR-MS, only one reactant ion, H_3O^+, is formed in the ion source, and the conditions are adjusted in such a way that the signal intensities of other molecular ions are much lower. This approach not only simplifies the system, but accounts for higher sensitivity and lower limits of detection of PTR-MS compared with SIFT-MS.[94]

Meanwhile, SIFT-MS is one of the few methods being used for quantitative determination of a number of known and potential lung cancer biomarkers, particularly, acetaldehyde, propan-1-ol, propan-2-ol, acetic acid, methyl formate, ethylbenzene, isoprene and some other.[27,42] Particular attention is paid to acetaldehyde determination in EA and in the gas phase containing growing cancer cells.[41–43,99]

It is worth noting that in the early stage of development of this method, the SIFT-based instruments were stationary (weighing 2000 kg), while later much lighter-weight devices appeared, such as Profile 3 (120 kg) and Voice 200, suitable for operation in clinics.[101] The detection limits of some few-atom VOCs are at the level (several ppb) sufficient for quantification of LC biomarkers in exhaled air, and the dynamic range reaches 10^7. However, no results that would allow evaluation of the specificity and sensitivity of SIFT-MS have yet been obtained.

3.2.4. Multi-capillary column ion mobility spectrometry

Ion mobility spectrometry (IMS) is used, most often, for EA analysis in the multi-capillary column version (MCC-IMS).[32,102–106] The method gives two-dimensional images of exhaled air. This approach was utilized by Baumbach *et al.*[32] to analyze the exhaled breath of 19 patients with confirmed histologically different non-small cell lung carcinoma using flexible video-chip bronchoscopy. Altogether 72 peaks were recorded, of which five were considerably different for LC-affected and healthy lungs. A peak corresponding apparently to the n-decane dimer was distinguished in the case of adenocarcinoma, while for squamous cell cancer, two peaks attributable to butan-2-ol or 2-methylfuran and nonanal were chosen. The sensitivity, specificity, and positive and negative predictive values were 100%, 75%, 80% and 100% for adenocarcinoma, and 78% and 78%, 67% and 78%, 70% and 80%, and 75% and 88% (butan-2-ol and nonanal, respectively) for squamous cell cancer.

Note that the procedure proposed by Baumbach *et al.*[32] can hardly be considered non-invasive, because it requires insertion of probes into damaged and healthy lungs in order to measure the peak intensity difference for various components present in the breath.

The application of MCC-IMS for exhaled air-based diagnosis of malignant pleural mesothelioma[102] produced results similar to those cited above:[32] the sensitivity, specificity, and positive and negative predictive values were 96%, 67%, 76% and 93%, respectively. This positive predictive value is insufficient for MCC-IMS to be used as a single screening method, because this would require additional examination for quite a few individuals without cancer.

The use of non-MCC IMS to diagnose cancer or other diseases based on EA is inefficient,[107,108] because of low selectivity of IMS.

An important feature of the MCC IMS system is the possibility of direct analysis without the use of Tedlar® or SPME technique,[35,102] which is very important for screening.

3.2.5. Electronic nose with a gas sensor array

Apart from the above-listed methods, a promising approach for early diagnosis of lung cancer based on exhaled air is so-called electronic nose (E-nose). This term is used to designate a compact and relatively inexpensive gas analyzer, which consists of an array of non-selective chemical sensors and an image recognition system.[109] The E-nose operation is based on the same principle of gas mixture recognition as the olfaction system of mammals. The essence is the generation of a multidimensional response from a set of sensors possessing different cross-sensitivity and the subsequent chemimetric processing of this response to obtain so-called image of the given gas mixture, in our case, the exhaled air. These images can be called breathprints by analogy with fingerprints. A training data set obtained from a group of patients with a particular disease and a control group is used to construct a classifier mathematical model, which employs the breathprint to assign an individual to the healthy or diseased group.[110] The classifiers are constructed using discriminant analysis,[111–113] decision trees,[114] logistic regression,[115] random forest[116] or artificial neural networks.[117,118] Data analy-

Table 4. Advantages and drawbacks of various types of sensors (based on our analysis of published data).

Manufacturing technique	Advantages	Drawbacks
Conducting polymer composites	Sensitivity to various VOCs; fast operation	Sensitivity to humidity and temperature; short lifetime
Colorimetric sensors	High sensitivity and selectivity; minor effect of moisture	Single-shot measurements; difficulties in determination of hydrocarbons
Quartz crystal microbalance	High accuracy and sensitivity; a broad range of sensing coatings is available	Low signal-to-noise ratio; moisture and temperature sensitive; complexity of manufacture
Au/Pt nanoparticles	High sensitivity; small time constant	Moisture sensitivity
CNT	Fast operation; reversibility	Moisture sensitivity; weak signal
SiNW-FET	Fast operation; low energy consumption; the possibility of using several NW-FET sensors with different cross-sensitivity	Moisture sensitivity

sis for multisensor systems is considered in more detail in reviews.[119, 120]

The selection of sensors plays a key role in the development of diagnostic tools based on E-nose. The sensors used to detect lung cancer are based on Au/Pt nanoparticles,[121] silicon nanowire field effect transistors (SiNW-FET),[112] carbon nanotubes (CNT),[122, 123] conducting polymer composites,[114] quartz crystal microbalance[115] and colorimetric sensors.[124] The advantages and shortcomings of these types of sensors are closely related to different mechanisms of formation of the analytical signal. Table 4 presents the key advantages and drawbacks for the above types of sensors.

For example, sensors based on conducting polymer composites are sensitive to a broad spectrum of VOCs and have a short response time, with the limits of detection for compounds such as toluene, ethanol, acetone and isoprene being in the 80–240 ppb range.[125] However, these sensors are moisture- and temperature-sensitive and also have short shelf life. Quartz crystal microbalance-based sensors have reasonably good sensitivity and rather low limits of detection (LODs) (33, 44 and 100 ppb for ethanol, hexane and ethyl acetate, respectively);[126] however, like conducting polymer composites, these sensors are susceptible to moisture and temperature change. Apart from high sensitivity (LODs of about 10–20 ppb for propan-2-ol, hexanal and ethylbenzene), an advantage of colorimetric sensors is lower moisture sensitivity as compared with other sensors; however, the detection of alkanes with these sensors is problematic.[127] Sensors based on nanomaterials such as Au/Pt nanoparticles, CNT or SiNW are fairly sensitive — the limits of detection for them are in the range of 1–5 ppb for many VOCs; apart from high sensitivity, the use of nano-sized materials ensures short response and recovery times.[128, 129]

By attachment of functional groups to the sensor surface, it is possible, in principle, to attain a higher sensitivity and selectivity to particular LC biomarkers or groups of biomarkers. More detailed information about various types of sensors is available in reviews.[129, 130]

A variety of multisensor systems have been used to diagnose lung cancer. For example, McWilliams *et al.*[114] applied the Cyranose 320 E-nose, which comprises a 32-sensor array based on conducting polymers, to elucidate the effect of the gender and smoking history on the possibility of early lung cancer diagnosis. They analyzed samples from 25 LC patients (stages I and II) and from a high-risk group consisting of 166 active and former smokers without LC. The results demonstrated a considerable influence of the smoking status and the gender of test subjects: the efficiency was higher for former smokers than for active ones, at least in the case of adenocarcinoma [the area under the ROC curve (AUC) was 0.846 for male former smokers; 0.816 for female former smokers; 0.745 for male active smokers; and 0.725 for female active smokers]. In the authors' opinion, the changes in the VOC composition caused by active smoking somewhat mask the VOCs associated with the appearance of the tumour; in addition, the smoking-induced changes in VOCs are more pronounced for males than for females. The sensitivity and specificity of the developed method were 88% and 81.3%, respectively.

Mazzone *et al.*[124] used an array of 24 one-shot colorimetric sensors to identify LC patients and classify the histological type. This trial included 229 individuals, 92 being LC patients (41 with stage I or II non-small cell carcinoma and 42 with stage III or IV non-small cell carcinoma) and 137 forming the control group of high-risk individuals. All LC patients were split into groups in terms of the histological type of cancer (adenocarcinoma, squamous cell carcinoma, small-cell carcinoma), and EA for each group was compared with the control group EA. The possibility to discriminate the early (I and II) and advanced (III and IV) stages of the disease and the possibility of predicting the survival were evaluated. It was shown that models designed for each type of cancer separately are more accurate than the common model combining all types of tumours. The attained sensitivity and specificity varied in the ranges of 70%–91% and 73%–95%, respectively, depending on the histological type. The differences between the early and late stages were identified with the sensitivity of 81% and specificity of 93%, while the survival (<12 months or >12 months) was estimated with the sensitivity of 70% and specificity of 86%.

Gasparri *et al.*[111] used a sensor array based on quartz crystal microbalance coated by various metal porphyrins to differentiate 70 LC patients and 76 healthy volunteers. The attained sensitivity and specificity were 81% and 91%, respectively. The sensitivity to stage I lung cancer was higher than that to stage II–IV lung cancer (92% and 58%, respectively).

Sensors based on molecularly modified SiNW-FET were employed by Shehada *et al.*[117] in order to detect and classify lung cancer, gastric cancer, bronchial asthma (Ac) and chronic obstructive pulmonary disease (COPD) (the total number of test subjects was 374). The sample size for LC was 149 subjects; that for gastric cancer (GC) was 40 subjects, the number of asthma or COPD patients was 56, and the control group included 129 individuals. The test subjects with lung or gastric cancer were additionally split into two groups in terms of the stage of the disease, early (I and II) or late (III and IV) stage. Finally, the sensitivity and specificity for the obtained binary classifiers were: 87% and 82% (LC–control group), 92% and 80% (LC–Ac), and 97% and 90% (LC–GC), respectively. It was noted that the ability of the sensors to distinguish between the Ac and control groups was rather low (48% sensitivity and 91% specificity); the authors attributed this result to the fact that, unlike cancer, Ac is characterized by only one marker, pentane, rather than a set of markers. The sensitivity and specificity attained in the staging of lung cancer were 34% and 95%, respectively.

Mention should be made of the study of Nakhleh *et al.*,[131] who employed E-nose (NaNose) with an array of sensors based on Au nanoparticles with various surface modifiers and sensors based on carbon nanotube networks. In total, they examined 813 patients with 17 diseases and 591 healthy volunteers in five countries: Israel, France, the USA, Latvia and China. The diseases were classified using a series of binary classifiers generated by discriminant factor analysis. The blind validation of the developed tool demonstrated different sensitivity (57% to 100%) and specificity (43% to 100%) for each classifier. It is noteworthy that NaNose is able to distinguish between LC subtypes (small-cell lung cancer, adenocarcinoma and squamous cell cancer)[132] and mutation status[133] and can be used as a prognostic and predictive method.[134]

Thus, it can be seen that studies in which the use of sensors of various type provided high specificity and selectivity characteristics in the detection of LC, including early stage detection, are currently available. In view of their compactness, relatively low cost and easy application, sensor arrays have high potential for the application in non-invasive diagnosis of early stage LC.

However, since it is impossible to identify and quantify any particular VOCs, no relationship can be established between the exhaled air composition and the target disease. Therefore, in the preliminary determination of EA composition by the E-nose, it is necessary to use, in parallel, other methods such as GC-MS, PTR-MS or SIFT-MS in order to generate a database of VOC contents (profiles) to rely upon for making hypotheses about the relationship between VOCs in exhaled air and the disease and for adjusting the sensor array.[135]

4. Methods for exhaled air analysis potentially suitable for lung cancer diagnosis

4.1. Atmospheric pressure chemical ionization mass spectrometry

According to the atmospheric pressure chemical ionization mass spectrometry (APCI-MS) techniques, ionization of analytes is accomplished by air corona discharge (in a special cell) at atmospheric pressure. The air to be analyzed is pumped through the corona discharge area in which H_2O, N_2 and O_2 molecules are ionized. The resulting H_2O^+ ions react with water molecules to give the $H^+(H_2O)_n$ ions with different n values. These clusters can be involved in the proton transfer reaction with VOCs.

$$H^+(H_2O)_n + M \longrightarrow MH^+ + (H_2O)_n \quad (4)$$

The probability of reaction (4) depends on a number of factors, including the proton affinity of the analyte and the moisture content. APCI-MS is a highly sensitive method for the determination of VOCs in air and in EA with limits of detection of < 1 ppb; for some compounds, for example, nicotine, the LOD can be as low as 10 ppt.[136] The APCI-MS spectrum mainly consists of molecular ion peaks of analytes (M^+) and their fragments, although highly intense protonated MH^+ peaks are present for some compounds. A key benefit of APCI-MS is the possibility to detect non-volatile compounds in the EA. Berchtold *et al.*[137] used APCI-MS to determine one potential (but unvalidated) lung cancer marker, isoprene, on the basis of the MH^+ ion and pyridine. The obtained limit of detection for isoprene (0.2 ppb) was much below its content in the EA (100–1000 ppb); therefore, APCI-MS is, in principle, applicable for solving the diagnostic task. However, note that the mass spectrum obtained in the APCI-MS procedure is much more complex than those obtained in PTR-MS or SIFT-MS; in particular, in the case of atmospheric ionization, polyatomic clusters are easily formed, and ions can also be produced in reactions with ozone, which is generated by corona discharge.[137] This method can be utilized to determine particular LC markers, but, in our opinion, it is inapplicable for determination of the minimum number of compounds required to attain reasonable specificity and sensitivity values.

4.2. Secondary electrospray ionization mass spectrometry (chemical ionization)

One more method suitable for real-time analysis of exhaled air is secondary electrospray ionization mass spectrometry (SESI-MS). According to this method, the exhaled air flow is mixed with a flow of sprayed solvent from a nano-spray emitter, which results in soft ionization of the components to be detected. Sinues *et al.*[138] used this method to monitor the condition of the human body with time. SESI-MS in the negative ion mode was successfully used[139,140] for online determination of both short-chain and heavier fatty acids in EA with limits of detection of about several ppt. The acids were detected in the deprotonated form $[M-H]^-$. The determined concentrations of fatty acids were in the range of 50–200 ppt. One more advantage of the SESI technique is high speed: the time of single analysis, including

recording of the background spectrum, is 1 min, which is almost two orders of magnitude shorter than the time of single GC-MS analysis. However, in the positive ion mode, the obtained spectra are very complex, which substantially hampers simultaneous determination of several compounds.

Like the APCI-MS technique, SESI-MS can be applied to determine particular LC markers (for example, SCFA), but, in our opinion, it can hardly be used to determine the minimum number of compounds required to attain reasonable specificity and sensitivity values.

4.3. Ion mobility spectrometry with time-of-flight mass spectrometer detection

Exhaled air analysis using a combination of ion mobility spectrometry and time-of-flight mass spectrometer detection (IMS-TOFMS) has been reported.[141, 142] Sabo and Matejčík[141] used a quadrupole mass spectrometer and a β-active ionization source (^{63}Ni), while Reynolds *et al.*[142] applied a time-of-flight mass spectrometer with a relatively low resolution (400) and corona discharge. Unlike other mass spectral methods for determination of EA components that use chemical ionization, IMS-TOFMS is able to produce two-dimensional spectra, which increases the information content of the technique.[141] However, when the IMS drift tube was filled with usual air, the sensitivity of this method was rather low and the limits of detection were rather high (tens of ppb).[142] This is due to the fact that $H_3O^+(H_2O)_n$ species act in this case as the reactant ions according to reaction (5):

$$H_3O^+(H_2O)_n + M \longrightarrow M^+ + H + H_2O + (H_2O)_n \qquad (5)$$

instead of H_3O^+ ions, which are involved in reaction (1) with protonation of monomer M in the PTR-MS and SIFT-MS techniques.

The probability of reaction (5) is much lower than that of reaction (1), which accounts for the difference in the limits of detection. However, when artificial air is employed for the IMS drift tube, NO^+, which is involved in much more efficient reaction (6), becomes the main reactant ion.

$$NO^+ + M + N_2 \longrightarrow MNO^+ + N_2 \qquad (6)$$

In this case, the limits of detection can be reduced to values typical of the APCI-MS technique (about 1 ppb).[137, 142] Reynolds *et al.*[142] achieved quite low LODs: 1.5 ppb for benzene, 1.4 ppb for toluene and 2.6 ppb for xylene, which is a lung cancer marker. Note that IMS-TOFMS as an analytical 2D system can, in principle, be used to determine the minimum required number of LC markers; however, there are no publications on this topic as yet.

4.4. Cavity ring down spectroscopy

The rapidly developing cavity ring down spectroscopy (CRDS) has been used in recent years for online determination of a number of low-molecular-mass components such as ammonia, acetone, acetylene and some other in human breath.[143, 144]

The CRDS method is based on measurements of changes in the attenuation curve of pulsed laser radiation in an external cavity upon injection of the analyte. Owing to large effective thickness of the absorbing layer (hundreds of metres), very low limits of detection (<1 ppb) of compounds in the exhaled breath can be attained in the real-time mode with a very large dynamic range (6–7 orders of magnitude).[143] However, determination of each component requires its own laser, which moreover should be wavelength tunable; otherwise, it is impossible to take into account absorption caused by other compounds present in the EA. This restricts the potential of CRDS to simultaneous determination of only a few EA components. One more restriction of CRDS is the narrow range of compounds that can be detected by this method. Since efficient determination of a compound requires the presence of allowed vibrational-rotational structure, this method is actually inapplicable to determination of multiatomic compounds, because their vibrational-rotational spectra are highly diffuse. Therefore, only molecules consisting of a few atoms can be determined in this way. Nevertheless, both validated and potential cancer markers are present among these molecules, that is, propan-2-ol, butan-1-ol, isoprene[145] and short-chain fatty acids, in particular, propionic and valeric acids. The CRDS method is mainly used in the near-IR region in which the spectra of some few-atom compounds are well resolved. However, it can also be applied in the UV region, which contains the electronic absorption bands of analyte molecules. In particular, Wang and Surampudi[146] employed the fourth harmonic of a compact pulsed Nd-YAG laser at 266 nm to determine acetone in EA. According to the authors, wavelength modulation was not required in this case, because no interfering absorption bands were present in this spectral range. The sensitivity of this procedure was sufficient for acetone determination in EA at the level of a few ppm, but insufficient for determining the background concentrations of acetone in air. Sahay *et al.*[145] were able to achieve the limit of detection for acetone in air of about 0.1 ppm at 266 nm. The isoprene LOD was of the same order of magnitude, although two orders of magnitude lower LODs can be attained with the use of shorter-wavelength laser radiation (215 nm, fifth harmonic of the Nd-YAG laser) due to a larger absorption cross-section of isoprene in this spectral region.[145]

From our standpoint, the potential of CRDS could be considerably extended by using a tunable pulsed laser in the UV spectral range (since the absorption cross-section is much greater in the UV than in the IR range) and reduced pressure in the analytical cell (to increase the selectivity).

The CRDS method is apparently suitable for being included into a diagnostic screening system (as one of the system elements), because along with high sensitivity and selectivity, this method is suitable for online analysis of exhaled air, which ensures high productivity of the method. However, the small number of detectable components requires that other analyzers be also included in the analytical system, for example, E-nose and MCC-IMS.

5. Comparative possibilities of diagnostic methods for lung cancer based on exhaled air analysis

Among the above exhaled air-based methods for lung cancer diagnosis, positive results have been obtained for

Table 5. Sensitivity and specificity ranges for the considered methods of exhaled air analysis.

Method	Sensitivity (%)	Specificity (%)	Ref.
GC-MS	66.7 – 100	73 – 100	22, 45, 46
PTR-MS	54	99	33
E-nose	73.3 – 97.5	63.3 – 94	111, 113, 114, 116
MCC-IMS	78 – 100	67 – 78	32

GC-MS, PTR-MS, E-nose and MCC-IMS, although the MCC-IMS procedure described by Baumbach *et al.*[32] can hardly be classified as non-invasive, because the analysis is based on the differential signal, which is obtained by probes inserted into the healthy and damaged lungs. However, MCC-IMS has also been successfully used [35, 147] for the online lung cancer diagnosis. Table 5 summarizes the sensitivity and specificity values and positive and negative predictive values attained in these methods.

The PTR-MS method is most sensitive and does not require pre-concentration. The sensitivity of GC-MS is lower; therefore, pre-concentration is needed, which is accomplished using sampling bags, SPME and liquid nitrogen traps; this considerably increases the labour intensity and time of analysis. However, in the PTR-MS method, it is often difficult to identify the spectrum components. The combined use of these methods reported by Bajtarevic *et al.*[98] markedly increases the information content, but simultaneously complicates the analysis.

A rather high potential for LC diagnosis is also inherent in SIFT-MS (this method resembles PTR-MS in its parameters), SESI-MS and CRDS, although CRDS should apparently be combined with other methods of exhaled air analysis because of the narrow range of quantifiable components. However, the use of highly sensitive methods, such as GC-MS and PTR-MS, for screening examination is very cumbersome and expensive. Furthermore, the productivity of one GC-MS instrument, without sampling, is only 2 or 3 samples per hour. Therefore, although GC-MS is now the most efficient method for exhaled air-based diagnosis of lung cancer, it is hardly possible to design a screening procedure based on this method.

A rather significant drawback of PTR-MS, which has to be mentioned, is the limited (in comparison with GC-MS) range of compounds that can be determined in this way. In particular, the method is inapplicable to alkanes, which are fairly important markers and are determined rather easily by GC-MS.

It can be seen from the above that some of the methods, GC-MS, PTR-MS, MCC-IMS and E-nose, are, in principle, suitable for diagnosing lung cancer. However, currently this problem can be solved apparently for late stages of cancer, III and IV, where the probability of patient survival is relatively low. Handa *et al.*[35] state that the specificity and sensitivity of LC diagnosis do not depend on the stage of the disease. We have found only one more publication [22] that supports this opinion. Song *et al.*[22] presents the results of comparison of a control group (41 subjects) and LC patient group (43 subjects) in only two components, butan-1-ol and 3-hydroxybutan-2-one, which were determined by GC-MS. According to the results of this study, the researchers observed both considerable differences between the average contents of these components for the healthy and diseased test subjects and similarity of the concentrations found for I – II stage and III – IV stage patients, with the attained sensitivity being 93% – 95% and specificity being 85% – 93%. It should be noted, however, that butan-1-ol is considered as a lung cancer marker only in this particular publication [22] in which the group of I – II stage patients is rather small. In our opinion, to obtain reliable data about the effect of LC stage on the contents of the key biomarkers in exhaled air, additional investigations are required with the use of biomarkers summarized in Table 1 and a sufficiently large group of patients should be tested.

The detection of lung cancer at least at stage II would considerably decrease the mortality caused by this disease. It should be emphasized that currently there are no efficient screening methods for early lung cancer diagnosis. In order to develop such a method suitable for early-stage diagnosis of LC based on exhaled air analysis, it is necessary, first, to arrange extensive studies with the participation of sufficiently large risk groups and, second, to enhance the informative value of these methods, since the expected changes in the contents of biomarkers in the exhaled air may prove to be smaller for stages I and II than for stages III and IV. In addition, as noted above, the methods should allow operation in the online mode and the time required for a single analysis should not exceed several minutes. One more requirement is mobility of the analytical system, the possibility of system installation in clinics and fast system displacement in the case of necessity. An important, although hardly practicable, requirement is a sufficient number of LC biomarkers that can be determined, directly or indirectly, by the given analytical system. Meanwhile, the components that provide little information or no information at all should not be detected (or should not be used in further processing). Otherwise, the method specificity and sensitivity can markedly decrease. Among the methods considered above, the formulated requirements are satisfied for E-nose and MCC-IMS methods; in some cases, despite the relatively large instrument weight ($\sim$200 kg), PTR-MS and related SIFT-MS methods can also be used. An additional method (that cannot be applied separately) increasing the information content for lung cancer detection is CRDS. However, the specificity and positive predicted values (see Table 5) achieved by each of these methods are insufficient for the design of an efficient screening procedure. The problem may be solved by the simultaneous use of several methods (that meet the above conditions), which will increase the information content and, as a result, will make it possible to attain the required specificity and predictive values. Furthermore, methods, the analytical characteristics of which are unsuitable for their separate use for LC detection (for example, SESI-MS, CRDS), can also be utilized according to this approach.

As can be seen in Table 5, the sensitivity and specificity values vary over broad ranges, with few literature sources being available. It should be emphasized that a specificity close to 100% is, first of all, important for an efficient screening procedure. However, the only study [45] in which the method specificity was higher than 95% demonstrated a moderate sensitivity of 66.7%.

6. Conclusion

In conclusion, we can state that analysis of exhaled air is a relatively new promising research area. This analysis may serve as the basis for a screening procedure for early diagnosis of lung cancer. However, the development of such a method should meet a number of criteria, which are often contradictory. The method should be characterized by short sampling and analysis times or better operate in the real-time mode, and be relatively cheap and non-invasive. A very important requirement for not only the considered methods, but also any other LC screening method is high specificity and high positive predictive value. These values should be at least 98% – 99%; otherwise, the number of unnecessary biopsies sharply increases, which is associated with the risk of complications, and the use of additional examination methods and the cost of examination also increase. The requirements to the sensitivity and negative predictive value are less stringent, at least 90% for sensitivity and at least 85% for the negative predictive value.

At the given stage of development of this approach, any of the analysis methods used to detect LC (GC-MS, PTR-MS, MCC-IMS, E-nose) does not fully satisfy the requirements, to one or another extent: GC-MS has a low productivity and is too labour-consuming and can be used only in the offline mode. For the methods applicable in the online mode (PTR-MS, MCC-IMS, E-nose), the specificity levels and the positive predictive values are insufficient (this, however, is also true for GC-MS) for the development of an efficient screening procedure. A possible solution is the simultaneous use of several methods, including those that are inapplicable as single techniques for LC detection because of inappropriate analytical characteristics (*e.g.*, SESI-MS, CRDS), with the common chemometric processing of the results and a common database. This will increase the information content of the method and, hence, reach the required specificity and predictive levels.

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