Detection heart failures (HF) biomarkers by proton transfer reaction - mass spectrometry and ion mobility spectrometry

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Abstract. Exhaled breath contains 1% of volatile organic compounds. The concentration of individual biomarkers in hundreds of volatile organic compounds lies within the range ppm-ppb. In compare with control group the concentrations of acetone, acetic acid, ethanol, propylene biomarkers is significantly higher in HF-PEF group.

1. Introduction
Heart failure (HF) is a preeminent cause of cardiovascular morbidity and mortality. The 5-year survival rate for patients with heart failure, regardless of ejection fraction (EF), is less than 50%. Data from epidemiologic studies of heart failure with preserved EF (HF-PEF) find that the annual mortality is approximately 10%. The diagnosis of HF-PEF is more complicated than the diagnosis of HF with reduced EF (HF-REF). In our previous studies [1-5], we found out that concentration of certain voluntary organic compounds (VOCs) in exhaled breath in patients (pts) with and without HF differs.

2. The experimental scheme
From October 2013 to September 2015 it was enrolled 21 pts with HF-PEF in heart failure group and 16 pts without HF in the control group. HF-PEF is diagnosed according to ESC active guidelines. It is collected fasting exhaled breath samples of all patients in 1L Tedlar bags. Exhaled breath is analyzed using PTR-MS (Compact PTR-MS, Ionicon, Austria). The PTR-MS is gaining popularity modern technique [6-8]. Correlations were evaluated by the method of Pearson and Spearman. All statistical operations were carried out with the help of IBM SPSS Statistic Software, version 22.

The experimental setup is designed and assembled for determination of several volatile organic compounds (VOCs) concentration in the exhaled air samples. The unit consists of the following components: proton transfer reaction - mass spectrometer compact PTR-MS, zero air generator Sonimix 3057, the thermostat, solenoid valve, manometer, flow meter, valves, connecting pipes, computer. The thermostat is taken from chromatograph Shimadzu GC-8A.

The air sample in Tedlar bag, DuPont, USA (bag made of polyvinyl fluoride film) is placed in the thermostat at constant temperature 42 °C. The solenoid valve is mounted inside the thermostat which is also connected with air channels from zero air generator and PTR-MS input. The design allows excluding probe condensation on channels and solenoid surface. The solenoid valve switching makes

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it possible to supply to PTR-MS an air from zero air generator or exhale probe. The generator air consumption is supported at level 0.5 lpm for optimum operation of generator and avoidance of room air entry. The air consumption is regulated by a valve and controlled by variable area flow meter. The pressure-gauge controls the air pressure on generator output.

The PTR-MS probe consumption contents 0.05 lpm thus the whole time of 1 l probe analysis is 20 min. The experimental study shows that the device consumes 90-95 % of the sample volume during 20 min of analysis. A small amount of air left in the bag. This mode of analysis should be considered as optimal. The analysis of each probe of exhale starts in zero air flow takes 10 min. The sample temperature control mode is maintained during this time. Then the solenoid valve is switched to exhale probe support.

The PTR-MS device has specific feature. The output data of some channels concentration characterized by individual m/z couldn't be unambiguously identified as certain chemical compounds. The output signal is proportional to the number of concentrations of VOCs sum and products of their chemical breakdown in the drift chamber.

Taking into account, the specifics of the exhaled air analysis problems the method of spectrum analysis of m/z values in a row in a certain range is chosen. As follows from literature data [6-7], the substances can not be detected in exhaled air with concentrations greater than the PTR-MS threshold detection unit (0.5 – 1.0 ppb) for the m/z values greater than 141. Consequently, the range of the test spectrum m/z value is 21 - 141 except channels 21, 30, 32 and 37, which correspond to the concentrations of ions generated in the drift chamber. The information from the literature is confirmed by the breath sample analyze experiments. Concentrations do not exceed the device detection threshold for m/z values of 141 to 201.

The specialized software supplied by the manufacturer (the company Ionicon, Austria) controls the instrument PTR-MS. This software uses a proprietary data file format, and in addition, has a number of disadvantages in use. The specialized software tools have been developed for rapid transformation of the data file to format suitable for further analysis. These tools perform the calculation of average concentrations and standard deviations for each m / z values separately for periods of zero flow of air and test sample. The automated exception of transient analysis is carried out at switching the solenoid valve. Duration of the transients is about 1 - 2 minutes. The time has been determined experimentally.

The time of one m / z values spectrum analysis within a selected range is 1 minute. The standard concentration deviations is calculated in the range from 3 to 10% of their average values. The obtained values characterize noise of the device and, apparently, cannot be significantly improved by changing the experimental procedures in existing conditions. Recent results are presented in Table 1.

3. IMS study
It remains an open question of portable HF-PEF diagnostic system. Meanwhile dozens of diseases may be recognized by changes in the chemical composition of volatile organic compounds (VOCs) in the air exhaled by the gas chromatography - ion mobility spectrometry (GC-IMS). For instance some studies show that the mobility spectra from GC-IMS breath analysis of patients with cancer lung damage differs from spectrums of healthy peoples [9-10].

Interest in using Multi Capillary Column MCC IMS for clinical analyses has arisen through concerns regarding the integrity of samples when transported to the laboratory. It is necessary about 10 mL of breath to carry out a full analysis. For investigations of human breath at a high level of humidity a combination of a polar Multi Capillary Column (MCC) (ISC, OV-5,OOO "Siberteh", Novosibirsk, Russia) partly preseparating the analytes is used in combination with a conventional ion mobility spectrometer [9-12]. An IMS coupled to a MCC columns allows the identification and quantification of volatile metabolites occurring in human breath down to the ng/L and pg/L range of analytes within less than 12 minutes and without any preconcentration steps. The analyte passes through the exhalation of 1000 parallel capillaries, each with an internal diameter of 40 micrometers. The overall diameter of 3 mm separation columns, which allows the carrier gas supplied from the optimum for spectrometry at 300 ml/min. The IMS study of acetone, acetic acid control samples shows the sensitivity in ppb range.

It is carried out further investigations of exhaled breath samples with improved sample device.
Table 1. Recent results. Adapted from [1] with permission.

| Substance name         | Patients without CHF | Patients with CHF |
|------------------------|----------------------|-------------------|
|                        | The average concentration, ppb (pts = 16) | The average concentration, ppb (pts = 19) |
| Acetylene              | 5.9±1.63             | 5.68 (4.75-6.79)  |
| HCN                    | 15.16±1.54           | 15.46±1.8         |
| formaldehyde           | 1271.17±96.34        | 1365.93 (1294-1469.5) |
| methanol               | 261.31±60.27         | 256.3 (226.5-330.2) |
| acetonitrile           | 17.61 (8.9-46.63)    | 30.5±27.47        |
| Propylene              | 107.87±33.64         | 1.55 (1.25-3.23)  |
| Acetaldehyde           | 194.21±29.74         | 15.26 (12.43-32.3) |
| ethanol                | 10.43 (9.25-20.94)   | 15.26 (12.43-32.3) |
| 1,3-butadiene          | 1.55 (1.25-3.23)     | 1.55 (1.25-3.23)  |
| acetone                | 325.72±97.46         | 34.3±12.5         |
| acetic acid            | 22.94±4.1            | 34.3±12.5         |
| dimethyl sulphide      | 5.0±2.0              | 5.92 (3.04-8.36)  |
| isoprene               | 16.31±5.22           | 12.8±7.2          |
| methyl vinyl ketone, methacrolein | 3.04±1.5             | 4.3±3.8           |
| methyl ethyl ketone    | 1.6±0.7              | 1.93 (1.32-4.08)  |
| peroksiatsetilnitrat   | 0.66 (0.54-0.8)      | 0.65 (0.5-0.97)   |
| benzene, dimethylsulfoxide | 0.76 (0.68-2.29)    | 0.98 (0.65-1.5)   |
| terpenes               | 3.79 (2.84-6.98)     | 3.22 (1.83-4.8)   |
| 2-methyl-3-2-ol        | 0.89 (0.74-2.57)     | 1.1 (0.86-2.7)    |
| toluene                | 1.18 (0.9-3.63)      | 1.66 (1.3-3.1)    |
| phenol                 | 2.44 (1.7-10.33)     | 2.93 (2.0-10.9)   |
| dimethylfuran, dimethylpyrazole | 0.55 (0.41-1.43) | 0.69 (0.48-1.2)   |
| styrene                | 0.37 (0.29-0.54)     | 0.42 (0.34-0.7)   |
| xylene, benzene-C8     | 0.64 (0.59-1.19)     | 1.82 (0.79-4.9)   |
| propyl                 | 0.6 (0.44-1.07)      | 0.75 (0.54-1.4)   |
| NO +                   | 0.71 (0.62-1.07)     | 0.8±0.1           |
| O2 +                   | 16.35±1.5            | 16.9±1.5          |
| H2O + (H2O)            | 0.23±0.05            | 0.26±0.08         |

4. Results

The baseline characteristics of patients are in Table 2.

Table 2. The baseline statistical characteristics of patients.

| Characteristic                             | HF-PEF (pts = 21) | Control group (pts = 16) |
|-------------------------------------------|------------------|-------------------------|
| Mean age (range), years                   | 72 (60-75)       | 64 (38-70)              |
| Male sex - n, %                           | 10 (47.6%)       | 9 (56.3%)               |
| Hypertension - n, %                       | 21 (100%)        | 14 (87.6%)              |
| Diabetes mellitus - n, %                  | 8 (38.1%)        | 2 (12.5%)               |
| Previous myocardial infarction - n, %     | 10 (47.6%)       | 2 (12.5%)               |
| Atrial fibrillation - n, %                | 13 (61.9%)       | 0                       |
| Mean EF, %                                | 60±7             | 69±4                    |

In compare with control group the concentrations of several biomarkers were significantly higher in HF-PEF group. They are acetone, acetic aldehyde, ethanol, propylene. The median (interquartile range) concentration of acetone in HF-PEF group when compared to control group is 934 ppb (324-2432) versus 322 ppb (280-368), p=0,002; acetic aldehyde 297 ppb (213-441) versus 193 ppb (177-231),
p=0.004; ethanol 18 ppb (14-28) versus 10.6 ppb (9-21), p=0.018; propylene 343 ppb (122-873) versus 124 ppb (94-141), p=0.04 respectively.

The median (interquartile range) concentration of biomarkers is presented in Table 3.

| Biomarker \ Groups | HF-PEF group (n=21) concentration, ppb | Control group (n=16) concentration, ppb |
|-------------------|----------------------------------------|----------------------------------------|
| acetone           | 934                                    | 322                                    |
| acetic acid       | 297                                    | 193                                    |
| ethanol           | 18                                     | 10.6                                   |
| propylene         | 343                                    | 124                                    |

The study shows applicability of PTR-MS for HF-PEF diagnostics. Nevertheless it is necessary to confirm results by a systematic statistical study.

5. Conclusion
There is a significant difference in exhaled breath PTR-MS spectrums between patients with HF-PEF and without heart failure. In compare with control group the concentrations of acetone, acetic acid, ethanol, propylene biomarkers were significantly higher in HF-PEF group. The IMS study of acetone, acetic acid control samples shows the sensitivity in ppb range. It is carried out further investigations of exhaled breath samples with improved sample device. Further investigation is necessary to determine the correlation between these biomarkers and levels of natriuretic peptides.

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