Ex-vivo volatilomics profiling of gastric cancer and non-cancerous tissue

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BACKGROUND

The human body releases numerous volatile organic compounds (VOCs) through various body fluids and tissues. These compounds create a specific chemical profile, which may subsequently be employed for the detection of metabolic changes in the human body caused by cancer. A number of recent studies involving various analytical devices have provided sufficient evidence that breath analysis can pinpoint novel biomarkers for the diagnosis of gastric cancer. Unfortunately, the chemical identity of the gastric cancer markers in breath is still unclear. In this context, the analysis of volatilome in gastric cancer tissue can suport the biomarker discovery. Here, we identify VOCs emitted from gastric cancer and non-cancerous tissues, and determine differences in this emission. VOCs released by tissue samples were preconcentrated using **headspace solid phase microextraction (HS-SPME)** and then analysed using **gas chromatography with mass spectrometric detection (GC-MS)**. A total of 44 patients (32 males and 12 females) were enrolled in the study.

TISSUE SAMPLES

44 patients — 32 males and 12 females

Paired tissue samples — ca. 100 mg

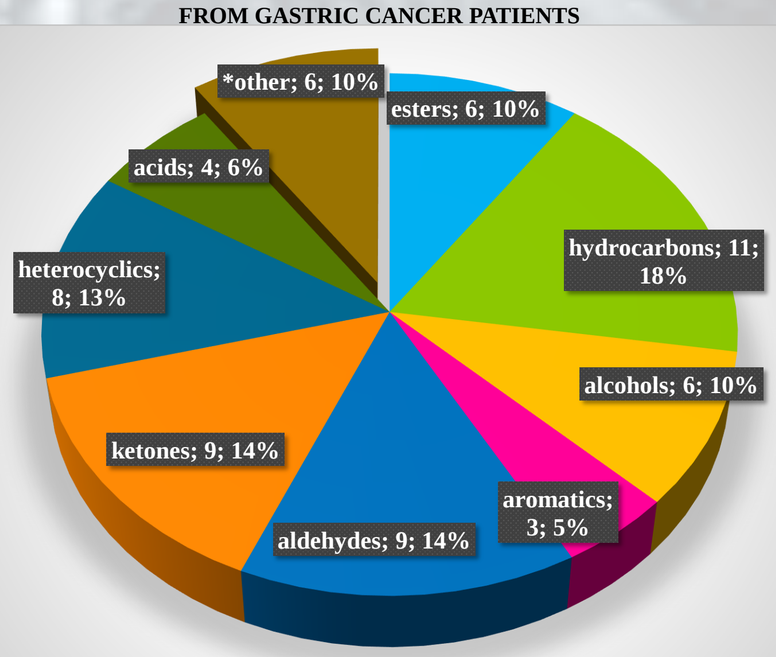
HS-SPME-GC-MS

62 compounds identified

RESULTS

Sixty-two VOCs were reliably identified through a comparison of peak spectra with the NIST mass spectral library and corresponding retention indices obtained from reference standards. The predominant chemical classes were: ketones, aldehydes, hydrocarbons and heterocyclic compounds. The most ubiquitous compounds were 2-butanone, 2-pentanone, ethyl acetate, n-pentane, isoprene, butyrolactone, n-octane, pyrrole, dicyclopentadiene, 2-methyl-2-propanol, and 6-methyl-5-hepten-2-one. Based on preliminary analyses, differences in emission between normal and tumor tissue were found for **thirty-two** of the identified compounds. Further analyses are required to perform more robust statistical analysis and pinpoint potential markers of gastric cancer. Moreover, several compounds, e.g. ethanol, 1-propanol, acetone, propofol, sevoflurane and dodecane, are not emitted by tissue samples. Their presence is the result of sampling site conditions or they are contaminants originating from the measurement environment. Some of the identified compounds were present singularly in tissue samples or for some of them additional analysis is required to confirm their presence in the analysed samples.

CLASSIFICATION OF VOCS FOUND IN GASTRIC TISSUE SAMPLES OBTAINED FROM GASTRIC CANCER PATIENTS



*** nitrogen and sulphur containing compounds, propofol, sevoflurane**

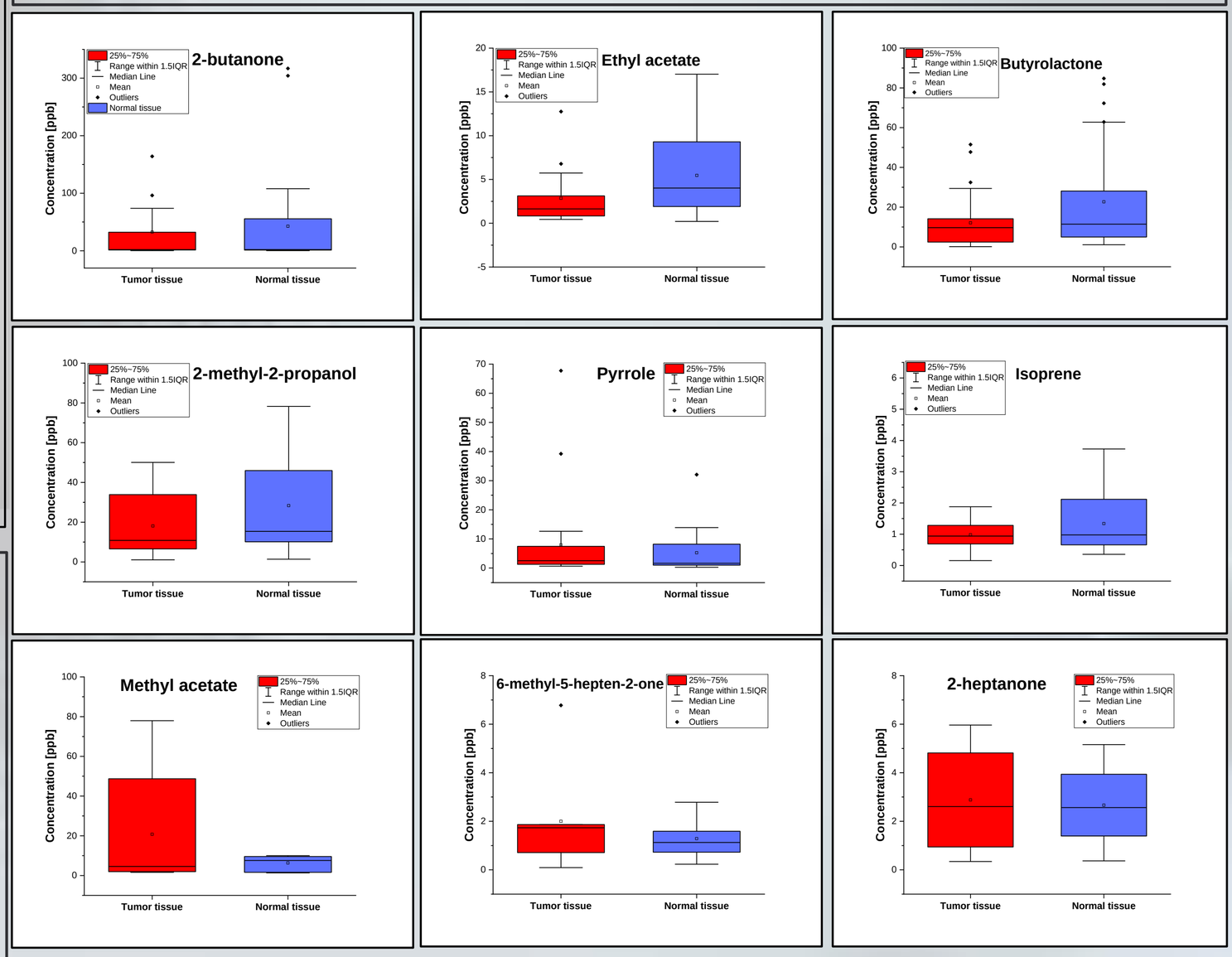


Figure 1. Concentrations [ppb] of 2-butanone, ethyl acetate, butyrolactone, 2-methyl-2-propanol, pyrrole, isoprene, methyl acetate, 6-methyl-5-hepten-2-one and 2-heptanone in the HS of tested tissues

CONCLUSIONS

1. HS-SPME-GC-MS analysis of gastric cancer tissue samples resulted in the identification and quantification of sixty-two VOCs.
2. Thirty-two coumponds were found to be emitted.
3. The chemical signatures of gastric cancer non-cancerous tissues exhibit differences that can be employed for biomarker discovery.
4. Further analyzes involving different clinical centers from different geographical regions are needed to perform more robust statistical analysis and pinpoint these differences
5. Differences between the chemical patterns can assist in the identification of potential markers of gastric cancer helping to gain better insight into their origin in the human body.

Table 1. The most common compounds in normal and cancerous tissue samples obtained from gastric cancer patients with concentration ranges, median, coefficients of variation (R^2), frequency of occurrence and the outcome of a Wilcoxon signed rank test, n.s. – no significant.

R_t, min	VOC	CAS number	Tumor tissue		Normal tissue		R^2	N valid	Frequency of occurrence [%]	p-value Wilcoxon test ($p < 0.05$)
			Concentartion ranges, ppb	Median, ppb	Concentartion ranges, ppb	Median, ppb				
3.06	**n-pentane**	109-66-0	0.196 – 4,239	0.966	0.091 – 8.279	1.023	0.999	41	**93**	n.s.
3.38	**isoprene**	78-79-5	0.156 – 1.878	0.646	0.185 – 6.119	0.981	0.992	41	**93**	2.7×10^{-6}
4.30	**methyl acetate**	79-20-9	1.052 – 77.952	3.160	1.319 – 20.614	1.389	0.995	28	63	n.s.
4.80	**2-methyl-2-propanol**	75-65-0	1.050 – 96.273	27.866	1.394 – 121.493	20.086	0.992	31	70	n.s.
7.60	**2-butanone**	78-93-3	0.259 – 269.425	1.818	0.219 – 316.873	1.871	0.993	44	**100**	n.s.
7.83	**ethyl acetate**	141-78-6	0.197 – 12.754	1.617	0.177 – 17.025	2.590	0.993	43	**98**	1.4×10^{-3}
11.45	**3-methylbutanal**	590-86-3	0.363 – 16.989	0.739	0.302 – 7.658	0.483	0.996	27	61	n.s.
13,97	**2-pentanone**	107-87-9	0.184 – 4.561	0.788	0.191 – 5.474	0.841	0.989	44	**100**	n.s.
18.19	**n-octane**	111-65-9	0.031 - 3.022	0.622	0.084 - 4.002	0.506	0.996	34	77	n.s.
19.96	**pyrrole**	109-97-7	0.578 – 67.761	2.055	0.285 – 32.076	1.321	0.999	33	75	n.s.
22.55	***p*-xylene**	106-42-3	0.021 – 0.557	0.181	0.010 – 0.823	0.212	0.998	25	57	n.s.
23.02	**1-methoxy-2-propyl acetate**	108-65-6	0.081 – 1.165	0.163	0.074 – 0.870	0.185	0.996	26	59	n.s.
24.29	**2-heptanone**	110-43-0	0.128 – 5.964	0.738	0.219 – 5.160	0.470	0.958	23	52	n.s.
27.52	**butyrolactone**	96-48-0	1.036 – 51.494	3.495	1.059 – 84.760	8.864	0.997	35	**80**	1.25×10^{-3}
27.91	**6-methyl-5-hepten-2-one**	110-93-0	0.093 – 6.777	1.111	0.233 – 2.784	0.624	0.997	30	68	0.025
28.52	***D* - limonene**	5989-27-5	0.166 – 49.914	0.807	0.116– 163.243	1.071	0.992	30	68	n.s.
28.64	**dicyclopentadiene**	77-73-6	0.676 – 3.191	1.545	0.653 – 4.632	1.364	0.960	32	73	n.s.
28.94	**eucalyptol**	470-82-6	0.089 – 156.114	0.233	0.084 – 228.478	0.220	0.997	27	61	n.s.

ACKNOWLEDGEMENTS

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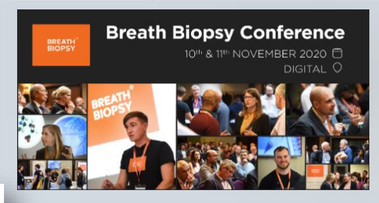

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