Validation of ^{1}H-NMR-based metabolomics as a tool to detect lung cancer in human blood plasma

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INTRODUCTION

- Lung cancer is the leading cause of cancer death worldwide.
- There is an urgent need for detection methods with an improved sensitivity and specificity because lung cancer is often only diagnosed owing to symptoms of advanced disease.
- Accumulating evidence shows that the metabolism of cancer cells differs from that of normal cells. More specifically, the entire metabolism of cancer cells is reorganized to increase anabolic reactions which induce cell growth and survival. Disturbances in biochemical pathways which occur during the development of cancer provoke changes in the metabolic phenotype.
- Nuclear magnetic resonance (NMR) spectroscopy enables the identification and the quantitative analysis of complex mixtures of metabolites, as in blood plasma, without an extended sample preparation. Recent studies have indicated the huge potential of this technique to detect different cancer types in an early stage. For several diseases, changes in the metabolic phenotype have been shown to correlate with the presence of a certain pathology. Identifying which changes in the metabolic phenotype correlate with the presence of lung cancer could allow us on the long-term to detect the presence of lung cancer via a simple blood sample.
- Recently, our research group has constructed a statistical classifier by means of multivariate orthogonal partial least squares-discriminant analysis (OPLS-DA). This classifier (constructed with 110 integration regions as variables) allows to discriminate between 209 lung cancer patients and 199 controls (constituting the **training cohort**) with a sensitivity of 81%, a specificity of 92% and an area under the curve (AUC) of 0.86. When only the 28 most discriminating variables (variable importance for the projection or VIP value>0.8) were selected to construct the classifier (i.e. regions representing glucose, lactate, myo-inositol, β-hydroxybutyrate, threonine, citrate and lipids) a sensitivity of 72%, a specificity of 88% and an AUC of 0.80 is achieved.

OBJECTIVE

We aim to investigate the predictive accuracy of these classifiers in an independent **validation cohort** of 50 lung cancer patients and 64 controls.

SUBJECTS AND METHODS

Study population

Subjects in whom lung cancer was detected by computed tomography (CT) and confirmed by positron emission tomography (PET)/CT in the Limburg PET center (Hasselt, Belgium) were included. The diagnosis of lung cancer was confirmed by means of an anatomopathological (APO) biopsy or by a medical doctor with expertise in radiological or clinical data. The control group consists of subjects, who were referred to the department of Nuclear Medicine in Ziekenhuis Oost-Limburg (ZOL) for a stress examination of the heart. This control group did not undergo a PET/CT. The study protocol was approved by the Medical Ethical Committees of ZOL and Hasselt University.

^{1}H-NMR spectroscopy

- Collection of fasting venous blood samples from lung cancer patients and controls.
- Analysis of the metabolic composition of blood plasma by means of a 400 MHz Varian Inova NMR spectrometer.
- ^{1}H-NMR spectra were divided into fixed integration regions defined on the basis of metabolite spiking. After removal the signals of water and TSP, the integration values (area under the peaks) of these regions were normalized relatively to the total integrated area of all signals, except these originating from glucose and lipids. The end result corresponds to a set of 110 normalized integration values characteristic for 110 integration regions.

Multivariate data analysis

- Classification of the independent validation cohort is accomplished by means of the constructed OPLS-DA statistical classifiers described in the introduction.
- The predictive accuracy of the constructed classifiers is further evaluated by means of a receiver operating characteristic (ROC) curve, using the independent validation cohort as a hold-out dataset.

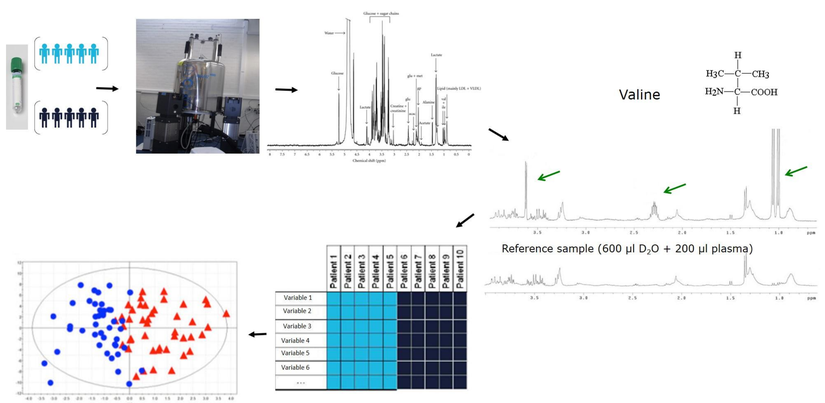

Figure 1. Determination of the metabolic phenotype of a blood plasma sample by means of ^{1}H-NMR-based metabolomics.

RESULTS

Table 1. Subject characteristics of the training cohort and the validation cohort.

	Training cohort		Validation cohort	
	Lung cancer patients	Controls	Lung cancer patients	Controls
Number of subjects	223	220	50	64
Gender	M: 157 (70%)	M: 114 (52%)	M: 29 (58%)	M: 28 (44%)
	F: 66 (30%)	F: 106 (48%)	F: 21 (42%)	F: (56%)
Age	68 ± 10	67 ± 11	67 ± 9	70 ± 10
BMI	25.8 ± 4.6	28.2 ± 5.1	25.7 ± 4.2	28.3 ± 6.1
Smoking habits	Active: 104 (47%)	Active: 47 (21%)	Active: 27 (54%)	Active: 9 (14%)
	Stopped>6m: 109 (49%)	Stopped>6m: 98 (45%)	Stopped>6m: 22 (44%)	Stopped>6m: 25 (39%)
	Never: 10 (4%)	Never: 75 (34%)	Never: 1 (2%)	Never: 30 (47%)

Table 2. Anatomopathological (APO) diagnosis of the lung tumors in the training and validation cohort.

		Training cohort	Validation cohort
Total number of tumors		223 (6 patients with 2 tumors)	53 (3 patients with 2 tumors)
Histology	Adenocarcinoma	86 (37%)	20 (38%)
	Adenosquamous carcinoma	6 (3%)	1 (2%)
	Spinocellular carcinoma	61 (27%)	14 (26%)
	Large cell carcinoma	8 (3%)	2 (4%)
	Carcinoid	5 (2%)	0 (0%)
	Oatcell carcinoma	29 (13%)	10 (19%)
	No APO	34 (15%)	6 (11%)
Stage	I	68 (30%)	17 (32%)
	II	25 (11%)	7 (13%)
	III	73 (32%)	16 (30%)
	IV	60 (26%)	13 (25%)
	?	3 (1%)	0 (0%)

Predictive accuracy of the constructed classifiers

- Applying the classifier constructed with all 110 variables on the independent validation cohort, 86% of the lung cancer patients and 72% of the controls are correctly classified, with an AUC of 0.93.
- By means of the classifier constructed with the 28 most discriminating variables (VIP value>0.8), a sensitivity of 90%, a specificity of 83% and an AUC of 0.86 is achieved.

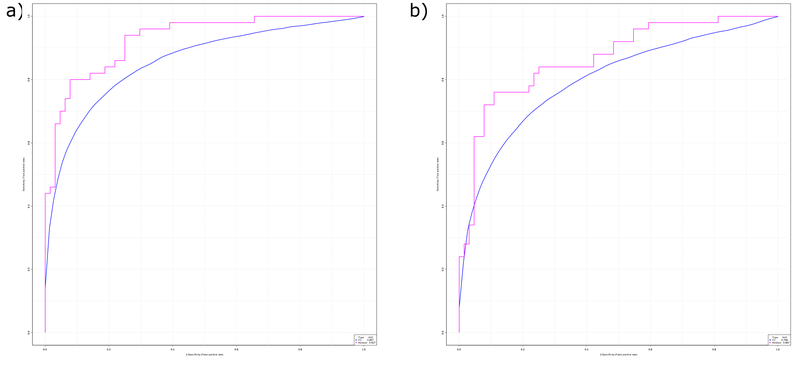

Figure 2. a) ROC curve of the classifier constructed with all 110 variables, using the data of the training cohort (blue, AUC of 0.86) and using the data of the validation cohort (pink, AUC of 0.93), b) ROC curve of the classifier constructed with the 28 most discriminating variables, using the data of the training cohort (blue, AUC of 0.80) and using the data of the validation cohort (pink, AUC of 0.86).

CONCLUSION

Both statistical classifiers (constructed with all 110 variables or with only the 28 most discriminating variables (VIP value>0.8) show a good predictive accuracy. Future experiments are ongoing to investigate whether the constructed classifiers have potential as valid screening tool.

ACKNOWLEDGEMENTS

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