Validation of ^{1}H-NMR-based metabolomics as a new, complementary tool for the detection of lung cancer via human blood plasma

Evelyne Louis[1], Karolien Vanhove[1,2], Kirsten Stinkens[1,3], Liesbet Mesotten[1,3], Gunter Reekmans[4], Wanda Guedens[4], Kurt Vandeurzen[5], Anna Sadowska[6], Johan Vansteenkiste[7], Christophe Dooms[7], Michiel Thomeer[1,3], Peter Adriaensens[4]

[1]Faculty of Medicine and Life Sciences, Hasselt University, Hasselt, Belgium, [2]Algemeen Ziekenhuis Vesalius, Tongeren, Belgium, [3]Ziekenhuis Oost-Limburg, Genk, Belgium, [4]Institute for Materials Research, Hasselt University, Hasselt, Belgium, [5]Mariaziekenhuis Noord-Limburg, Overpelt, Belgium, [6]Ziekenhuis Maas en Kempen, Maaseik, Belgium, [7]Universitaire Ziekenhuizen Leuven, Leuven, Belgium

INTRODUCTION

- Lung cancer is the leading cause of cancer death worldwide.
- It is often diagnosed owing to symptoms of advanced disease. This underscores the importance of a screening tool which allows to detect early stage lung cancer.
- A promising tool for lung cancer screening is low-dose computed tomography, which has recently been shown to reduce lung cancer mortality by 20% compared with screening using chest radiography. However, it has several limitations, such as a low positive predictive value and high costs.
- These limitations encourage the search for complementary, non-invasive tools which enable to detect early stage lung cancer.
- Accumulating evidence has shown that the metabolism of cancer cells differs from that of normal cells. More specifically, the entire metabolism of cancer cells is reorganized to favor 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 a fast, non-invasive identification and quantification of complex mixtures of metabolites, as in blood plasma, in a single run and without extended sample preparation.

OBJECTIVES

1) Investigate whether the metabolic phenotype of blood plasma determined by ^{1}H-NMR spectroscopy allows to discriminate between 233 lung cancer patients and 226 controls (**training cohort**)
2) Examine the predictive accuracy of the metabolic phenotype by external validation in an independent **validation cohort** of 98 lung cancer patients and 89 controls
3) Explain the disturbed biochemical pathways in lung cancer

SUBJECTS AND METHODS

Study population

The lung cancer patients from both cohorts were included in the Limburg Positron Emission Tomography center (Hasselt, Belgium) and at the Department of Respiratory Medicine of University Hospitals Leuven (Leuven, Belgium). The diagnosis of lung cancer was confirmed by means of an pathological biopsy or by a medical doctor with expertise in radiological or clinical data. The control groups of both cohorts consist of patients with non-cancer diseases who were referred to the department of Nuclear Medicine in Ziekenhuis Oost-Limburg (Genk, Belgium), Hasselt University (Hasselt, Belgium) and University Hospitals Leuven (Leuven, Belgium).

^{1}H-NMR spectroscopy

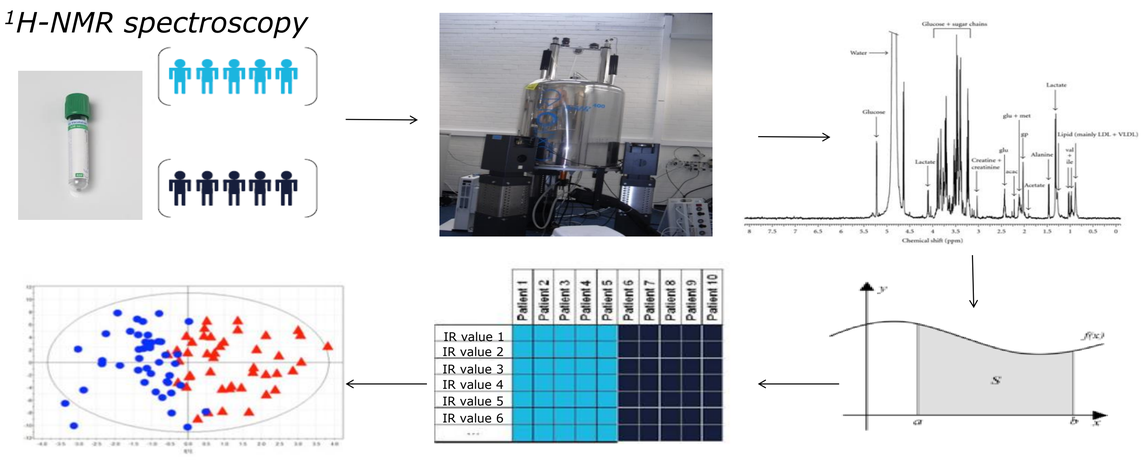

Figure 1. Determination of the metabolic phenotype by ^{1}H-NMR-based metabolomics.

- 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 NMR spectrometer.
- The ^{1}H-NMR spectra were segmented into 112 variable-sized spectral regions based on spiking experiments with known metabolites. After excluding water and TSP, the remaining 110 regions were integrated and normalized relatively to the total integrated area of all regions, resulting in 110 normalized integration values, being the variables for multivariate statistics.

Multivariate statistics

- Orthogonal partial least squares discriminant analysis (OPLS-DA) was used to construct a statistical classifier (model) to discriminate between lung cancer patients and controls.
- The predictive accuracy of the model was assessed by means of external validation in an independent cohort. Furthermore, model robustness was evaluated by means of a receiver operating characteristic (ROC) curve.
- To identify the most differential variables, the S-plot and variable importance for the projection (VIP) values of the constructed model were studied in detail. Variables with a VIP value exceeding 0.5 were considered in order to explain the disturbed biochemical pathways in lung cancer.

RESULTS

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

	Training cohort		Validation cohort	
	Lung cancer patients	Controls	Lung cancer patients	Controls
Number of subjects	233	226	98	89
Gender	M: 160 (69%)	M: 119 (53%)	M: 66 (67%)	M: 44 (49%)
	F: 73 (31%)	F: 107 (47%)	F: 32 (33%)	F: 45 (51%)
Age	68 ± 10	67 ± 11	64 ± 9	69 ± 10
BMI	25.8 ± 4.5	28.3 ± 5.0	26.2 ± 4.7	28.4 ± 5.7
Smoking habits	Active: 113 (49%)	Active: 47 (21%)	Active: 48 (49%)	Active: 15 (17%)
	Stopped>6m: 100 (47%)	Stopped>6m: 102 (45%)	Stopped>6m: 46 (47%)	Stopped>6m: 36 (40%)
	Never: 10 (4%)	Never: 77 (34%)	Never: 4 (4%)	Never: 38 (43%)

Table 2. Pathological diagnosis and stage of the lung tumors.

		Training cohort	Validation cohort
Total number of tumors		239	102
Histology	Adenocarcinoma	91 (38%)	46 (45%)
	Adenosquamous carcinoma	5 (2%)	1 (1%)
	Spinocellular carcinoma	66 (28%)	29 (28%)
	Not otherwise specified	8 (3%)	6 (6%)
	Carcinoid	5 (2%)	0 (0%)
	Oatcell carcinoma	30 (13%)	15 (15%)
	Unknown	34 (14%)	5 (5%)
Stage	I	76 (32%)	17 (17%)
	II	26 (11%)	11 (11%)
	III	74 (31%)	29 (28%)
	IV	63 (26%)	45 (44%)

Discrimination between lung cancer patients and controls based on the metabolic phenotype

- The metabolic phenotype allows to classify 78% of the lung cancer patients and 92% of the controls correctly with a positive predictive value of 91%, a negative predictive value of 80% and an AUC of 0.881.
- Furthermore, it classifies 71% of the lung cancer patients and 81% of the controls from an independent cohort correctly with a positive predictive value of 80%, a negative predictive value of 72% and an AUC of 0.843.

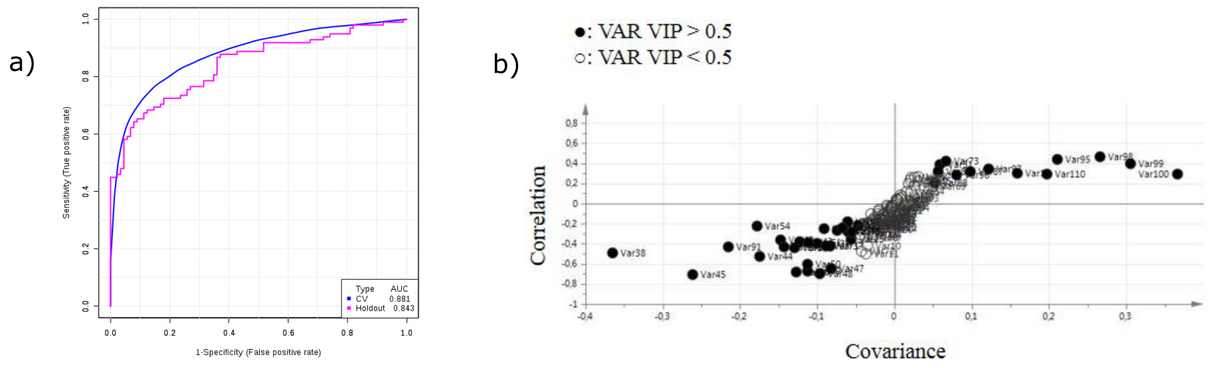

Figure 2. a) ROC curves showing for the cross-validation (CV) as well as for the independent validation (hold-out) a high predictive accuracy of the OPLS-DA model, b) S-plot of the complete OPLS-DA model showing the variables contributing most to the group differentiation. Variables which are used to explain the disturbed biochemical pathways in lung cancer are marked (●).

Unraveling the disturbed metabolism in lung cancer

- The disturbed metabolism in lung cancer is depicted in **Figure 3**. The metabolic changes can be mainly linked to a counteraction of the body in response to the well-known Warburg effect in cancer cells. In order to compensate for the lack of glucose as an energy source for normal cells, liver glycogen will be degraded and glucose will be synthesized via the gluconeogenic pathway. Consequently, ketone bodies and fatty acids are formed and the Krebs cycle is impaired in cancer cells. The produced fatty acids are used by the cancer cells to form lipids which are necessary for phospholipid membrane synthesis.

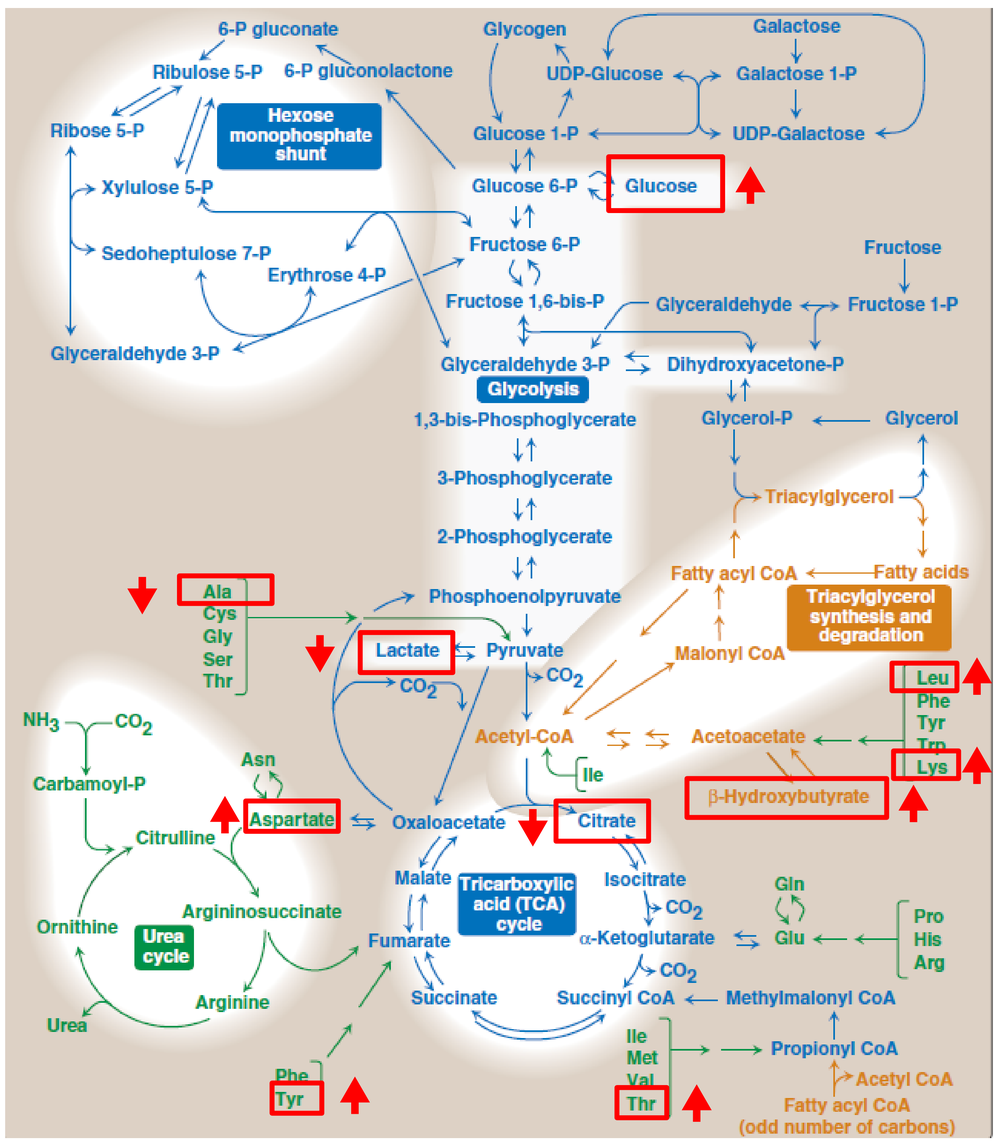

Figure 3. The disturbed biochemical pathways in lung cancer.

CONCLUSION

- Metabolic phenotyping of blood plasma by means of ^{1}H-NMR spectroscopy can become an important tool to stratify high-risk individuals before subsequent screening with low-dose computed tomography.
- A large prospective trial which evaluates the added value of metabolic phenotyping of blood plasma in screening individuals at risk for lung cancer will need to confirm its clinical utility.

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Contact information: evelyne.louis@uhasselt.be