METABOLIC PHENOTYPE BY NMR SPECTROSCOPY: A BIOMARKER FOR LUNG CANCER

E Louis[1], K Baeten[1], K Vandeurzen[2], K Vanhove[3], K Darquennes[4], L Mesotten[5], M Thomeer[5], E de Jonge[5], K van der Speeten[5], J Mebis[6], P Bulens[6], P Adriaensens[7]

[1]Biomedical Research Institute, Hasselt University, Diepenbeek, Belgium, [2]Mariaziekenhuis Noord-Limburg, Overpelt, Belgium, [3]Ziekenhuis Maas en Kempen, Maaseik, Belgium, [4]AZ Vesalius, Tongeren, Belgium, [5]Ziekenhuis Oost-Limburg, Genk, Belgium, [6]Jessa ziekenhuis, Hasselt, Belgium, [7]Institute of Material Research, Hasselt University, Diepenbeek, Belgium

INTRODUCTION

• Lung cancer is the second most common cancer in men and the third in women. Moreover, lung cancer is the leading cause of cancer death
• Screening for lung cancer remains a challenge. Consequently, lung cancer is often diagnosed at a metastatic stage
• Recent studies indicate that metabolic fingerprinting of a blood sample by means of nuclear magnetic resonance (NMR) spectroscopy has the potential for early disease detection. For several diseases, changes in the metabolic fingerprint have been shown to correlate with the presence of a certain pathology. Identifying which changes in the metabolic fingerprint correlate with the presence of lung cancer could allow us on the long-term to easily detect the presence of lung cancer in a simple blood sample
• 18-fluorodeoxyglucose-positron emission tomography-computed tomography (^{18}F-FDG-PET-CT) also allows early disease detection based on metabolic changes that occur *in vivo*. This technique is non-invasive and determines the degree of glucose metabolism in the tumor. Previous studies have shown that the uptake of ^{18}F-FDG is substantially increased in most types of cancer, reflecting an elevated glucose consumption by tumor cells

HYPOTHESES

1) Metabolic fingerprint analyses by NMR spectroscopy allow the detection of lung cancer

2) Metabolic changes, detected in the blood of lung cancer patients, correlate with metabolic alterations at the tumor site

METHODOLOGY

NMR spectroscopy

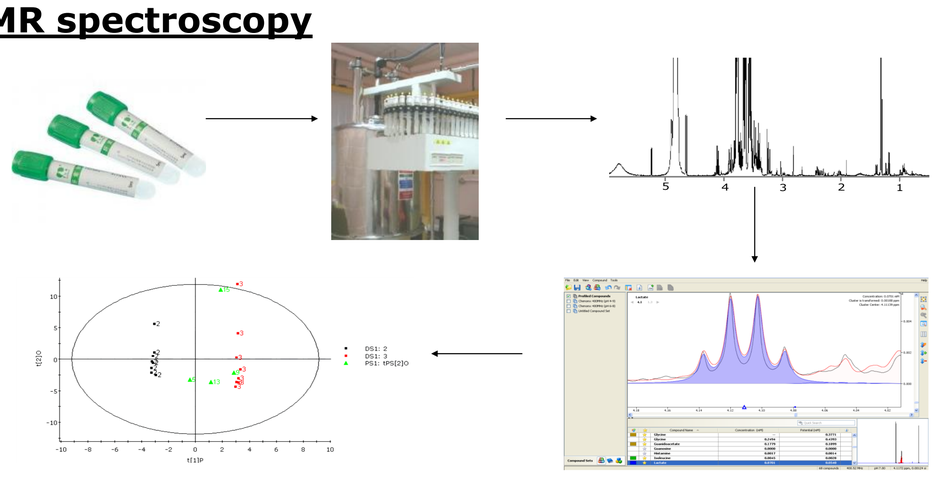


Figure 1: Measurement procedure

• Venous blood samples of patients with confirmed lung cancer (before treatment or surgery) and control subjects were collected
• After sample preparation, NMR spectra were obtained using a 400 MHz Varian NMR spectrometer
• To reduce the complexity of the NMR data and to facilitate statistical analyses, the obtained spectra were reduced to 96 integral segments
• At first, the metabolic differences between lung cancer patients and control subjects were investigated by student T-tests. Subsequently, supervised orthogonal partial least squares-discriminant analyses (OPLS-DA) were applied

^{18}F-FDG-PET-CT

• Patients were fasted for minimally 6 hours and their blood glucose levels were lower than 200 mg/dl
• The radioactive tracer ^{18}F-FDG was administered intravenously with a dose of 4 megabecquerel (MBq)/kg. The exact dose and time of injection were recorded to permit correction for radioactive decay
• One hour after injection, a whole body scan was performed (Gemini TF Big Bore PET/CT, Philips). Initially, a CT-scan was performed. Subsequently, a PET-scan, which covered the same axial field, was carried out.
•The reconstructed image was visually evaluated by the local Nuclear Medicine Physician

•The degree of tumoral ^{18}F-FDG uptake was measured by the standardized uptake value (SUV). This SUV was normalized for lean body mass (SUL). Moreover, SUL peak was determined. This is the largest possible mean value of a 1cm^3 spherical volume centering around the hottest point in the tumor

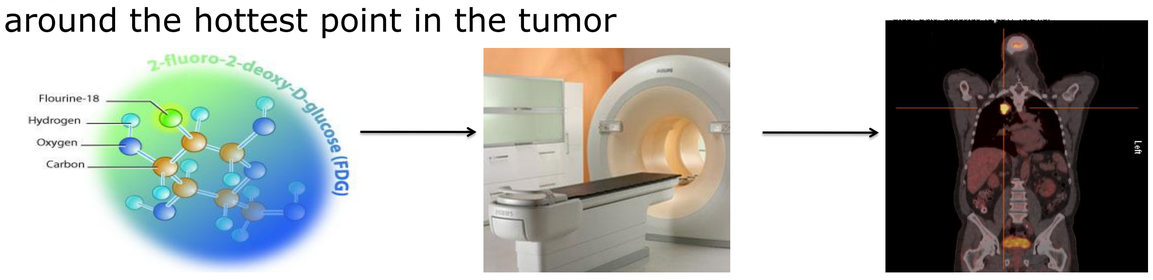
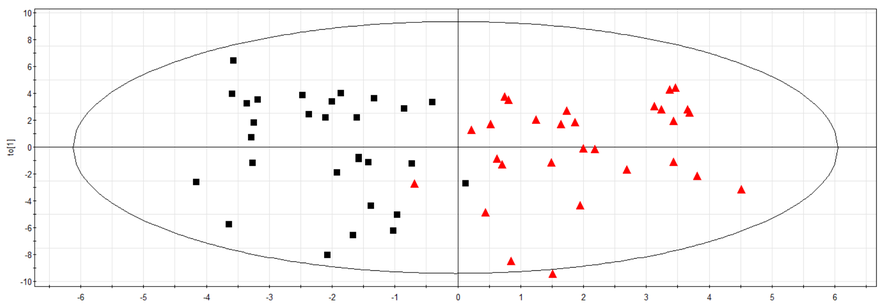


Figure 2: Obtaining an image with ^{18}F-FDG-PET-CT

PRELIMINARY DATA

Discrimination of lung cancer patients from healthy controls based on their metabolic profile



Specificity 96%
Sensitivity 93%

Figure 3: OPLS-DA analyses of plasma samples distinguish between lung cancer patients and healthy controls

• After collecting plasma samples from 29 lung cancer patients (▲) and 29 healthy controls (■) metabolic fingerprinting was performed by NMR spectroscopy
• By means of OPLS-DA analyses on a well-defined and selected panel of metabolites present, a high degree of discrimination between control subjects and patients with lung cancer was achieved with a specificity of 96% and a sensitivity of 93%

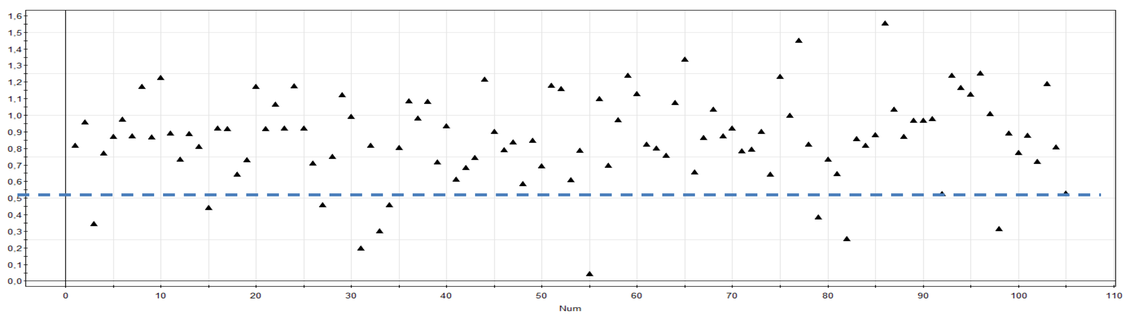
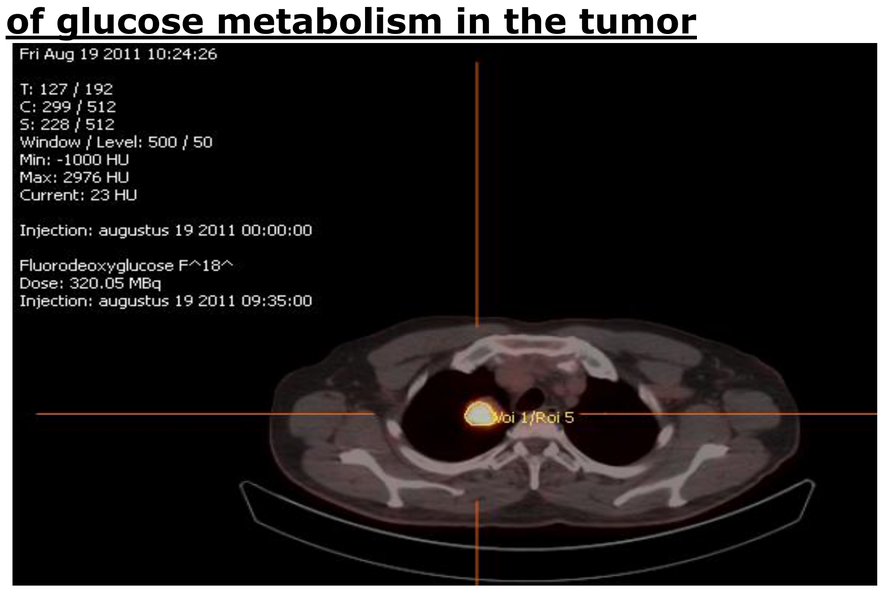


Figure 4: OPLS-DA analyses classify 90% of all the patients in the right group

• Upon validation, the clinical status of 105 new samples (all control) was predicted using the above created model. Ninety-five out of hundred and five patients (90%) were correctly indicated as having no lung cancer. However, further validation with additional samples of cancer patients and controls is necessary.

Correlation between the metabolic profile and the degree of glucose metabolism in the tumor



SUL peak = 4,62

Figure 5: Calculation of SUL peak on a reconstructed image

FUTURE RESEARCH

• The samples of a second cohort of lung cancer patients and controls will be analyzed by means of NMR spectroscopy and statistical analyses will be performed
• The SUL peak of all patients will be calculated. Furthermore, regression analysis between the SUL peak and all the metabolites present in the metabolic fingerprint will be performed to determine the correlation between the metabolic changes at the tumor site and changes in the blood

Evelyne.louis@uhasselt.be