# REAL-TIME PCR ASSESSMENT OF PLASMA DNA LEVELS IN NON-SMALL CELL LUNG CANCER *VS.* CHRONIC RESPIRATORY INFLAMMATORY DISEASES AND HEALTHY CONTROLS

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BACKGROUND: Free-circulating DNA is present in minute amounts in plasma of healthy individuals, whereas increased levels are found in a number of benign and malignant pathologies including non-small cell lung cancer (NSCLC). Intensified inflammatory processes, apoptosis and necrosis seem to play a crucial role in DNA release from cells to bloodstream. It is believed that quantitative real-time PCR analysis of plasma DNA in treated NSCLC patients, though cancer unspecific, might support the imaging modalities in therapy efficiency monitoring by the indication of a patient's early physiological response. Real-time PCR is regarded as the standard method currently available for DNA quantification. In the presented research we evaluated plasma DNA levels in NSCLC patients prior and after the surgery with respect to healthy controls and patients with chronic respiratory inflammatory diseases.

MATERIAL AND METHODS: Blood samples were drawn from 16 healthy volunteers, 16 patients with chronic respiratory inflammatory diseases (stable asthma, sarcoidosis), and 30 patients with resectable (I-IIIA) non-small cell lung cancer before and 1-2 week after surgery. Plasma was separated by two centrifugations and banked at -80ºC. DNA was extracted from 0.5 ml plasma aliquots with QIAmp DNA Blood Midi kit (Qiagen, Germany) and measured quantitatively by real-time PCR using human β-actin housekeeping gene as the amplifying (99 bp) target.

RESULTS: The values of plasma DNA concentration ranged from 0.9 to 7.0 ng/ml in healthy individuals, from 0.8 to 8.9 ng/ml in patients with asthma or sarcoidosis, and from 1.5 up to 50 ng/ml in NSCLC patients before treatment. Concentrations of plasma DNA in healthy controls and patients with chronic respiratory inflammatory diseases did not differ significantly (mean 2.65 and 2.87 ng/ml; p=1.00). Cancer group showed several-fold higher mean free-circulating DNA concentration with respect to the controls (mean 12.10 *vs.* 2.65 ng/ml; p=0.001). A drastic increase in plasma DNA level up to a mean 68.74 ng/ml was observed 1-2 week after the primary tumor resection (p=0.01). A greater variability of plasma DNA concentrations was observed in NSCLC patients than in controls (SD 14.50 vs. 2.02 respectively). The area under the ROC curve built preliminarily was 0.87 (95% CI, 0.744 to 0.954, p<0.0001).

CONCLUSIONS: Non-small cell lung cancer is associated with elevated levels of cell-free DNA in plasma with respect to healthy controls, while chronic respiratory inflammation do not cause the significant increase in plasma DNA concentration. Postsurgical trauma results in drastic increase in NSCLC plasma DNA level and real-time PCR method can effectively measure that phenomenon, confirming its utility to monitor early physiological events related to NSCLC therapy. The study is to be continued and the groups extended.