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Title: Aberrant methylation in lung cancer identified by EBUS-TBNA as a marker of advanced staging

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Body: Introduction Aberrant methylation of DNA results in gene silencing and is frequently observed in tumours from lung cancer patients. Methylation aberrancies can also be detected in lymph nodes, but it is unknown if the assessment of the nodal methylation status may identify more advanced stages of the disease Objective To determine the relationship between the methylation status of 5 genes in metastatic lymph node and tumour samples obtained by EBUS-TBNA and the presence of an advanced stage of lung cancer Methods Nodal and tumour samples positive for lung cancer were obtained with EBUS-TBNA. The methylation status of DAPK, p16, RASSF1, APC and CDH13 genes was determined by methylation-sensitive high resolution melting Results 23 samples were analysed, 15 samples (12 mediastinal nodes, 1 lobar node and 2 tumour) were early cases of lung cancer (T1/T2) and 8 (4 mediastinal nodes, 3 lobar nodes and 1 tumour) more advanced lung cancer (T3/T4). Percentages of methylated samples for each gene according to T staging are shown in the figure. The samples from patients with a more advanced stage of lung cancer had significantly more genes methylated ($p=0.043$) than the samples from patients with early lung cancer.

Conclusion The level of methylation in tumour cells obtained from lymph nodes and/or tumours accessible to EBUS-TBNA is higher in patients with more advanced stages of lung cancer, identified by a higher T staging Funded FIS09/01612.