histopathological types. On immunostaining expression pattern of CK in large cell carcinoma was similar to that of adenocarcinoma.

**Conclusion:** The investigations of CK expression give us additional information concerning histopathological differentiation of primary lung cancer. Though more investigations are needed, there is a strong possibility that classification based upon proteomic analysis, as well as morphological features, may reflect the biological characteristics of tumor cells.

### P2-060 BSTB: Others Posters, Tue, Sept 4
**Altered iron metabolism, inflammation, transferrin receptors and ferritin expression in lung cancer**

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**Introduction:** Alterations in whole-body iron metabolism are known to occur in patients with cancer. Iron could participate in carcinogenesis and overabundance of iron is associated with increased risk of neoplasia at the site of metal deposition.

**Aim:** The relationship between the iron status and survival of lung cancer patients and the expression of transferrin receptors 1 (TfR1) and ferritin in tumor tissue, tumor stroma and normal lung were studied.

**Patients and Methods:** These findings were correlated with tumor type and clinical outcome in 111 male patients. Iron metabolism and inflammation parameters were determined by automated laboratory measurements at the time of diagnosis. TfR1 and ferritin expression were determined by immuno-histochemical methods on cancer tissue, tumor stroma and on the surrounding normal lung tissue.

**Results:** More than fifty percentages of patients survived less than 12 months. At the time of diagnosis approximately a half of the patients had mild anemia of chronic disease and significantly elevated serum ferritin. Non-specific laboratory markers of inflammation were present. Tumor tissue expressed much more TfR1 and ferritin than the tumor stroma and normal lung tissue. The expression of TfR1 and the ferritin content in tumor tissue depended on the carcinoma type. TfR1 and the ferritin content in tumor tissue did not show correlation with systemic parameters of most of iron metabolism parameters. Strong ferritin expression in tumor tissue correlates only with lower transferrin saturation.

**Conclusion:** Higher expression of ferritin in tumor tissue is not the result of higher body iron accumulation. Elevated serum ferritin in lung cancer patients is results of inflammation and oxidative stress rather than body iron overload.

### P2-061 BSTB: Others Posters, Tue, Sept 4
**ProGRP and NSE for follow-up of small cell lung cancer patients with limited disease**

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**Introduction:** For some years, NSE has been known as a marker of choice for small cell lung cancer. However, its diagnostic sensitivity and specificity are not completely satisfactory, due to relatively high false negative rate in SCLC patients with limited disease and false positive rate in patients with non-malignant lung disease and non-small cell lung cancer. Recently, the usefulness of pro-gastrin-releasing peptide (ProGRP) as a tumor marker for SCLC has been investigated. Although the diagnostic sensitivity and specificity of ProGRP was found to be higher than serum NSE, only small number of data concern its utility in disease therapy monitoring and its value in prediction of response to treatment. The aim of the study was the evaluation of ProGRP and NSE levels at the time of diagnosis and during chemo- and radiotherapy of SCLC patients with limited disease in respect to their prognosis.

**Material and Methods:** Studies of NSE and ProGRP were performed in a group of 52 SCLC-LD. Patients with SCLC with limited disease were treated simultaneously with chemo and radiotherapy. All of them also received prophylactic cranial irradiation between fourth and fifth course of chemotherapy. The increment ratio of tumor markers was calculated as serum concentration divided by the cut off, for assessment of prognostic value of these markers.

**Results:** ROC curve analysis confirmed that ProGRP was a better than NSE tumor marker for diagnostics of SCLC-LD patients (Area under curves ROC: 0.935 vs. 0.789, p = 0.000).

There were observed significant differences in the frequency of elevated NSE and ProGRP levels before each course of chemotherapy and 3 months after its finishing, respectively: 1st 57.6% vs. 78.8%, 2nd 5.8% vs. 67.3%, 3th 0% vs. 36.5%, 4th 1.9% vs. 21.2%, in restaging 6.7% vs. 15.7%. Changes in NSE levels during therapy were more intensive than for ProGRP what was reflected in tumor markers half-life (NSE: 4.6 - 11 days; ProGRP: 19-28 days) as well as in the frequency of increment of tumor markers ratio values. Patients with tumor marker levels 2 times exceeding NSE cut off and 12.5-times ProGRP cut off before treatment, and those with NSE and ProGRP having these ratio values higher than 0.4 and 0.65 during restaging 3 months after finishing therapy has shown worse prognosis.

Multivariate analysis confirmed that independent prognostic factors in SCLC with limited disease were: NSE level exceeding 2-times cut off value before treatment as well as NSE threshold 0.4 cut off and 0.65 ProGRP cut off value 3 months after therapy.

**Conclusions:**

1. Changes of ProGRP during combined therapy of SCLC-LD seem to be more adequate to actual clinical status of patients than NSE
2. NSE before treatment is a better than ProGRP prognostic factor in SCLC-LD patients however after finishing therapy both markers have similar predictive value.

### P2-062 BSTB: Others Posters, Tue, Sept 4
**Optimizing the yield of circulating DNA from plasma and serum**

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**Background:** Low levels of circulating cell-free DNA are present in normal individuals. In cancer patients, much higher levels of circulating DNA are found. Importantly, circulating DNA in lung cancer patients demonstrates genetic alterations typical of the tumour, leading to interest in plasma and serum DNA for early clinical diagnosis, prognosis and disease monitoring. There is considerable variation among studies in the reported levels of circulating DNA and its characteristics, which may be attributable to differences in selection of the patient and control groups, and the methods used for DNA extraction and estimation of circulating DNA concentrations. Here, we compare the efficiency of different methods for extracting low-level circulating DNA from blood samples.
Methods: Blood, plasma and serum samples were spiked with known quantities of human DNA. To optimize the yield of DNA, we investigated the effects of changes in the blood collection and processing, storage and DNA extraction protocols. We compared two DNA purification methods, the QIAamp blood DNA kit (QIAGEN) (with and without protease K pre-digestion) and phenol/chloroform DNA extraction after heat denaturation (5 minutes at 95°C). Extracted DNA was quantified using the PicoGreen assay and its quality was checked by conventional PCR analysis for the p53 gene.

Results: Using the standard QIAamp protocol, the efficiency of DNA extraction from serum was 13.8 ± 5.9% (Mean ± SD). Pre-incubation with protease K (0.4 mg/ml, QIAGEN) at 37°C for 2 hours significantly increased the DNA recovery (p<0.0001, unpaired t-test), but longer incubation time and incubation at 50°C did not improve the DNA yield further. Further improvements in the efficiency of the QIAamp protocol were obtained by increasing the volume of elution buffer, but reloading the columns and using different buffers did not improve the yield. Using the phenol/chloroform extraction method after heat denaturation, the efficiency of DNA extraction was 52.7 ± 4.6% (Mean ± SD), which is significantly better than the QIAamp protocol (p<0.0001, unpaired t-test). Delays in processing blood samples reduced the DNA yield. Importantly, all the recovered DNA was of quality suitable for PCR analysis.

Conclusions: We recommend that blood samples are held on ice for no more than 1 hour before plasma/serum separation by double spin. Because of its higher efficiency, low-cost and good quality products for PCR analysis, we prefer the phenol/chloroform circulating DNA isolation method with heat denaturation.

BSTB: Prognostic Factors

P2-063

Clinical and prognostic significance of serum carcinoembryonic antigen level in patients with IIIB and IV stage non-small cell lung cancer: A prospective study

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Background: Previous studies have shown pre and post-operative serum carcinoembryonic antigen (CEA) levels are a prognostic factor for survival. We prospectively investigated the clinical and prognostic significance of serum CEA levels in patients with non-small cell lung cancer (NSCLC). We prospectively investigated the clinical and pathological characteristics in patients with stage IIIB and IV NSCLC.

Methods: From March 2005 to January 2007, 308 patients with IIIB and IV stage NSCLC were included. Several chemotherapy protocols including gemcitabine, vinorelbine or paclitaxel combined with cisplatin, carboplatin, or erlotinib were used. Serum CEA levels were obtained before and after 2 cycles of treatment. Stage was determined by CT and bone scans.

Results: One hundred twenty patients had brain CT scan at diagnosis due to neurological symptoms. Twenty eight per cent had stage IIIB, and 61.4% stage IV disease. Eighteen per cent had CNS metastases at the time of diagnosis. Histological type was adenocarcinoma in 69.1% and squamous in 30.9%. Mean serum CEA concentration was 11.3 ng/ml ± 24.5 with a median of 6.7 (range 0.2 to 4578). Patients with CEA levels > 10, 20, 50 and 100 were 43.6, 32.5, 24 and 17.9%; respectively. Associated factors with CEA >20 were age >62 (p=0.02), stage IV (p=0.05), CNS metastases (p=0.0001) and histotype (p=0.01). However, logistic regression analysis showed the only associated factors were histotype (p=0.0001) and CNS metastases (p=0.0001). Patients with CEA elevation >20 had a hazard ratio of 26.3 (CI 11.2 to 61.6) for the development of brain metastases. Overall survival associated factors included poor status performance (p=0.006), tobacco use (p=0.05), CEA elevation (p=0.0022), CNS metastases (p=0.0157), and male gender (p=0.08). There were no differences between stages (IIIB vs. IV) and hepatic metastases. The associated factors with survival by Cox proportional hazards regression multivariate analysis were poor performance status (p=0.05) and CEA elevation (p=0.008). The independent factors associated with CEA elevation were adenocarcinoma histotype and brain metastases.

Conclusions: Patients with stage IIIB and IV NSCLC and with serum CEA >20 without neurological symptoms could benefit from early CT brain scan. In this group of patients, the most important independent factors for survival were poor performance status and CEA elevation.

P2-064

The impact on survival of high baseline Cyfra 21-1 in non-small-cell-lung cancer (NSCLC) submitted to surgery: a 15-years study including 829 patients (pts). The role of adjuvant and neo-adjuvant chemotherapy

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The aim of this study was to assess the prognostic impact on survival of Cyfra 21-1, CEA and NSE in NSCLC pts receiving complete resection. Eight-hundred and twenty nine pts with histologically proven NSCLC stage I-III A receiving complete resection were included (stage I- 381; stage II- 212; stage IIIA - 227). The serum levels of all markers were measured using commercially available immunoassays and were obtained immediately before surgery. With a medium follow-up of 88 months, patients with initial Cyfra 21-1 higher than 3.3 ng/ml had a very significant worse prognosis (p<.00001). Higher than 9.8 ng/ml baseline CEA showed also a bad prognostic impact, but only for adenocarcinoma (p=0.0023). NSE showed no prognostic importance. In a Cox regression model, Cyfra 21-1 proved to be, among 25 other usual independent factors, the strongest independent prognostic factor for both overall survival and disease-free interval. In fact, the overall 5-years survival-rate for all pts in stage I was 54.3%, but increase to 75.7% in the 268 pts (70.3%) with normal baseline Cyfra 21-1 and strongly decrease to less than 15% for the 113 pts (29.7%) with higher than 3.3 ng/ml baseline Cyfra 21-1 levels. Very similar results were obtained with stage II and IIIA pts and were presented in our communication. For those pts with higher Cyfra 21-1 values adjuvant (used in 248 pts) and particularly neo-adjuvant chemotherapy (used in 88 pts) seems to reduce clearly these critical survival results. Survival comparisons will be presented in detail.