



NEW APPROACHES IN THE TREATMENT OF LOCALLY ADVANCED NON-SMALL CELL LUNG CANCER

Ph.D. Thesis

/Short version/

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1. Introduction

Lung cancer is the most frequent tumour worldwide. Radiotherapy (RT) is one of the main treatment modalities of lung cancer, its efficacy alone in locally advanced non-small cell lung cancer (NSCLC) is poor. Strategies designed to enhance local control include improved tumour targeting, escalation of thoracic RT dose or application of different fractionations. Individualized combinations of various treatment procedures, such as combining RT with chemotherapy tend to improve local control and survival. The most frequently applied third-generation chemotherapeutics are the taxanes, which have been demonstrated in clinical trials to be widely effective in advanced NSCLC. The prototype of the taxane family is paclitaxel an excellent radiosensitizer. In designing the optimum individual treatment planning, including selection of an effective chemotherapeutic and the anticipation of potential radioresistance, the physician may be aided by predictive markers. The multidrug-resistant protein (MDRp) is a well-known plasma membrane drug efflux pump that is associated with resistance to a wide range of anti-cancer drugs. Paclitaxel is a substrate of this transporter system. The overexpression of MDRp may play an important role in the paclitaxel resistance of lung cancer. The oncogenic protein bcl-2, which plays a central role in apoptosis, has been found to correlate with the prognosis of NSCLC. Most studies have suggested a more favourable prognosis in bcl-2-positive cases, but poorer survival rates of patients with a higher bcl-2 expression have also been reported. The predictive role of bcl-2 has also been examined in various tumour types. The association between the response to platinum-based chemoradiotherapy (CRT) and apoptosis-related proteins is unclear.

Radiation esophagitis seems to be one of the most common acute toxicities, especially in the setting of combined concurrent CRT. This adverse treatment side effect is often a dose-limiting factor, which influences the treatment outcomes and patient quality of life, therefore its dose-volume relationship has been investigated in several trials. Results have differed considerably across different institutions regarding which dosimetric factors are more critical than others. The identification of the risk factors for acute esophageal toxicity (AET) in lung cancer is important for optimizing the most effective and favourably tolerated treatment plan. In the case of locally advanced NSCLC the introduction of neoadjuvant CRT – particularly if good tumor response could be achieved - lead to better outcome of the surgery, the third main pillar of the complex management of lung cancer.

Survival after lung cancer resection is mainly dependent on the tumor stage, but other factors are also known to influence it. Smoking was significantly predictive of a poor prognosis after resection of different stages of lung cancer, but there was no difference in terms of survival between smokers and non-smokers with advanced NSCLC. There are only a few articles discussing the impact of thrombocytosis (TCTs) on lung cancer survival, or in resected cases. Previous studies have reported mainly unfavorable or no impact on survival of a high thrombocyte (TCT) count among patients with NSCLC. In several studies, TCTs was detected as an independent prognostic factor.

2. Aims

General aim of this thesis was to seek for strategies resulting in improved therapeutic index for locally advanced NSCLC.

2.1. A prospective analysis of the associations between the expressions of bcl-2 and MDRp and the clinical outcome of paclitaxel-based CRT in patients with NSCLC in order to predict the tumor response.

2.2. A prospective investigation to the dosimetric correlations of AET during neoadjuvant and definitive paclitaxel-based three-dimensional conformal CRT in patients with NSCLC, with the aim of optimization of the radiation treatment plan.

2.3. A retrospective study to evaluate the incidence and potential impact of TCTs on the outcome and survival, also analyzing the smoking habits of patients who underwent lung cancer resection.

3. Patients and methods

3.1. The roles of bcl-2 and MDRp expression in the efficacy of paclitaxel-based CRT

Patients receiving CRT for primary irresectable or with potentially resectable NSCLC at the Department of Oncotherapy in the period between December 2006 and June 2010 were eligible for participation in this study. All tumours were proven by histological verification. During the RT all the patients received concomitant taxane-based chemotherapy (weekly paclitaxel 100 mg/m² in 4-6 cycles, depending on toxicity). After completion of the CRT, additional consolidation chemotherapy was administered in 18 cases (paclitaxel 175 mg/m², carboplatin 400 mg/m² or docetaxel 75 mg/m², CDDP 75 mg/m², at 3-week intervals). CT-based three-dimensional treatment planning and

conformal RT were performed. The planning target volume encompassed the macroscopic lung cancer, the involved mediastinal and ipsilateral hilar lymph node regions and the safety zone, according to the local protocol. The initial radiation dose was 25x1.8 Gy; then, after a repeated CT scan, depending on the tumour response, RT of the reduced volume was continued at an average dose of 22-26 Gy (with the exception of neoadjuvant cases). During the treatment, following the administration of a 45 Gy dose, and 4-6 weeks after the completion of the RT regimen, clinical and diagnostic CT examinations were performed. The CT scans were compared with the pretreatment scans provided for RT-planning purposes. Response analysis was carried out by means of two methods: the exact values of the GTV and GTV1 were determined and the tumour volume reduction was calculated (reductions in the ranges >50%, 50-40% and <40% are referred to as GTV_{>50}, GTV₅₀₋₄₀ and GTV_{<40}, respectively), additionally, the CT scans were analysed according to the RECIST criteria system. Before the CRT, immunohistochemical (IHC) staining was performed to quantify the bcl-2 and MDRp expressions in the biopsy samples. Semiquantitative IHC scoring method was used: positive cell rates in the ranges 0-1%, 2-33%, 34-66% and >66% were scored as 0, 1+, 2+ and 3+, respectively. The intensity staining score was 2+ (moderate) in all cases. Any cytoplasmic staining with Bcl-2 was considered positive. Bcl-2 or MDRp expression at a level of 2-3+ was classified as a high expression.

3.2. The relation of AET to paclitaxel-based concurrent CRT for NSCLC

Patients receiving CRT for primary unresectable or potentially operable NSCLC at the Department of Oncotherapy between December 2006 and June 2011 were eligible for participation in this study. During the RT all the patients received concomitant taxane-based chemotherapy (weekly paclitaxel 100 mg/m² in 4-6 cycles, depending on toxicity). CT-based three-dimensional treatment planning and conformal RT were performed in all cases. The gross tumor volume (GTV), the macroscopic lung cancer, the involved mediastinal and hilar lymph nodes was defined on [¹⁸F]fluoro-2-deoxy-d-glucose positron emission tomography-CT images. The delineation of organs at risk (spinal cord, ipsilateral and contralateral lung, heart and esophagus) was conducted according to the local protocol. The planning target volume encompassed the GTV, the involved lymph node regions (clinical target volume) and the safety margins. The initial radiation dose was 25x1.8 Gy (and the total dose of neoadjuvant cases); then, after a repeated CT scan,

depending on the tumour response, RT of the reduced volume was continued based on a new three-dimensional plan, to an additional average dose of 22-26 Gy, resulting in a total dose of 67-72 Gy. Avoiding smoking and the consumption of hot and spicy eating, chopped food was recommended in order to prevent acute esophageal toxicity. Symptoms were alleviated based on protocols with local anaesthetics, liquid, mushy food, antihistamines, when required with mucosal coating, proton-pump inhibitors, tramadol derivatives, systemic non-steroids or calcium. The whole esophagus was contoured from the anular cartilage to gastroesophageal junction prior to radiation planning. The following dosimetric data were analysed in relation to dysphagia: the maximal dose (D_{max}), the mean dose (D_{mean}), the length of the irradiated esophagus with 50 Gy (L_{50Gy}) and the volume of the esophagus irradiated with 35 Gy to 60 Gy ($V_{35-60 Gy}$). AET as dysphagia was evaluated prospectively based on Common Terminology Criteria for Adverse Events, version 3.0 issued by National Cancer Institute. The worst grade of toxicity was taken into account. Follow-up visits with the evaluation of swallowing complaints were performed weekly. Patients who smoked during the CRT were called smokers.

3.3. TCTs as a negative prognostic value in lung cancer

Patients operated on for lung cancer at the Department of Surgery in a 5-year period between January 2003 and December 2007 were eligible for analysis in this study. Resections performed for the 398 lung cancer cases were as follows: 124 pneumonectomies, 214 lobectomies, 6 bi-lobectomies, 27 atypical resections and 27 explorations. In all cases systematic mediastinal lymphadenectomy was performed. Preoperative staging examinations routinely included a chest X-ray, chest CT, bone scintigraphy, brain CT, abdominal ultrasound, bronchoscopy and spirometry based on the conventional protocol. The primary tumor and the mediastinal lymph nodes were histologically analyzed by the use of a standard pathological local protocol and AJCC TNM classification, sixth edition. Patient files were reviewed, and relevant data were collected. The platelet counts were assessed three times during the perioperative period: just before surgery, and on the first and seventh postoperative days. If all three samples were evaluated to be higher than $400 \times 10^3/\mu\text{l}$, in agreement with other studies (44, 47, 49, 50, 56), TCTs was diagnosed. Based on this data, the 398 patients were divided into two groups as to whether they had normal platelet counts or TCTs in the perioperative period.

Among all patients, two subgroups were formed regarding their smoking habits. A total of 348 out of the 398 patients had smoking habit data. Non-smokers either never smoked or smoked little in the past, but stopped 10 years or more prior to lung surgery, and smokers smoked at the time of surgery or had smoked in the past 10 years.

All statistical analyses were carried out using SPSS (3.1. method with version 15.0, 3.2. and 3.3. methods with version 20.0) for Windows (SPSS Inc., Chicago, IL). The associations between the molecular marker expression and clinical factors, tumour response, local relapse and distant metastases within 6 months; gender and smoking habits; the associations between TCTs and clinical factors (stage, histology, gender) were evaluated with the chi-square test. The molecular marker expression with age was assessed with the one-way ANOVA test. The dose and volume and the correlation between TCTs and age were tested with independent samples *t*-test. Relationship between dose-volume parameters and severity of AET was analysed with logistic regression. Receiver operating characteristic (ROC) analysis was used to find the cut-off point for V_{45Gy} . In terms of both progression-free and overall survival, the outcome was analysed by Kaplan-Meier analysis (pairwise comparisons – Breslow test). The univariate- and multivariate analysis of the platelet count, T status, N status, stage and their impact on survival were evaluated with Cox regression test.

4. Results

4.1. *The roles of bcl-2 and MDRp expression in the efficacy of paclitaxel-based CRT*

Thirty-two patients received paclitaxel-based CRT, at a mean dose of 64.0 (45.0-70.0) Gy, in combination with a mean of 5 (4-6) cycles of chemotherapy. The mean age (\pm SD) of the patients was 58.9 (\pm 6.2) years; 21 (66%) were men. Most of the patients had stage III.B cancer (75%). Neoadjuvant treatment was administered to 5 patients with stage III.A and 3 with stage II.B Pancoast tumours, respectively. The performance status of the patients was good (ECOG 0 and 1, 44% and 56%). The histological type was adenocarcinoma in 20 (62.5%) and squamous cell carcinoma in 12 cases (37.5%). At the time of the last follow-up (median 17 months), 14 (44%) subjects had died, 12 due to lung cancer, 1 following the surgical procedure, due to pulmonary embolization, and another after the chemoradiotherapy, due to pneumonitis, all in stage III.B. Fifteen patients (47%) developed local or distant recurrence. Of the 32 lung cancer patients, 19 (59%) exhibited

partial remission (PR), while 10 (31%) had stable disease (SD). The condition of all 3 patients (10%) with progressive disease (PD; 2 locoregional and 1 distant metastasis) worsened during the treatment. There was a significant difference in the duration of progression-free survival (PFS) between the responders (PR) and the non-responders (SD+PD) (13.7 vs. 6.0 months, $p=0.028$), but there was no significant difference in the overall survival (OS) duration (29.1 vs. 15.7 months, $p=0.256$). Our analysis of the connections between PFS and OS and the tumour volume shrinkage (GTV_{>50}, GTV₅₀₋₄₀ and GTV_{40<}) indicated that the PFS results were more favourable with than without tumour shrinkage (GTV_{>50} 13.7 vs. 6.0 months, $p=0.009$; GTV₅₀₋₄₀ 13.43 vs. 4.8 months, $p=0.008$; GTV_{<40} 13.4 vs. 4.8 months, $p=0.008$), but no association was found between the OS and the shrinkage (GTV_{>50} 29.1 vs. 26.6 months, $p=0.979$; GTV₅₀₋₄₀ 26.6 vs. 22.0 months, $p=0.656$; GTV_{<40} 26.7 vs. 29.1 months, $p=0.846$). Of the 10 patients who underwent the operation, 5 (50%) exhibited pathologically complete remission and the other 5 a partial pathological response. There were 16 (57.2%) bcl-2-negative and 19 (67.8%) MDRp-negative patients. A low expression of bcl-2 or of MDRp was observed in 6 (21.4%) and 3 (10.7%) cases, respectively. A high expression of bcl-2 or MDRp was observed in 6 (21.4%) cases, each. The tumour in 3 (10.7%) patients displayed high expressions of both markers. There were no significant correlations between a high expression of bcl-2 or MDRp and other characteristics of the patients (age, gender, stage or histology). A high expression of both markers simultaneously was significantly associated with a poor response to paclitaxel-based chemoradiotherapy, evaluated against either a GTV reduction during therapy ($p=0.019$), or a tumour response according to RECIST ($p=0.005$). A high expression of bcl-2 or MDRp was significantly associated with a decreased duration of PFS (bcl-2 high vs. low/negative, 4.2 vs. 13.4 months $p=0.025$; MDRp high vs. low/negative, 1.63 vs. 13.4 months, respectively, $p<0.001$), evaluated either separately or together (bcl-2 and MDRp both high, both low/negative or mixed expression, 3.1, 13.4 and 4.1 months, respectively, $p<0.001$). PFS was shorter in cases with MDRp-positive than in those with MDRp-negative cancers (3.1 vs. 13.4 months $p=0.003$). In patients with pathologically complete remission, both markers were negative. No association was found between OS, the appearance of early metastases (within 6 months) and the expression of bcl-2 or MDRp. Local recurrence within 6 months was more frequent in patients with overexpression of bcl-2 ($p=0.0023$) or MDR ($p=0.007$).

4.2. Relation of AET to paclitaxel-based concurrent CRT for NSCLC

Altogether, 50 patients' data were analysed. Thirty-two (64%) were men, 18 (36%) were women. The mean \pm SD age was 59.8 \pm 8 (39-78) years. Histological examination proved squamous cell carcinoma and adenocarcinoma in 22 (44%) and 28 (56%) patients, respectively. Four (8%) patients had stage II/B and 6 (12%) patients had stage III/A carcinoma. Forty (80%) participants had stage III/B carcinoma. Twenty-nine (58%) patients were smokers and 21 (42%) were non-smokers. Twelve (24%) patients underwent operation. The mean \pm SD dose of planning target volume was 60.7 \pm 9.8 Gy in the whole investigated population, while 64.7 \pm 5.5 Gy in the definitively treated, irresectable patients. The preoperative given dose was 45.0 Gy in all 10 cases. RT doses to spinal cord, heart, ipsilateral and contralateral lung were appropriate to optimal values. The D_{\max} was 57 \pm 10.8 Gy, the $D_{\text{mean}} \pm$ SD was 24.9 \pm 9 Gy. The $L_{50\text{Gy}}$ was 6.99 \pm 6.7 cm. Among the 50 participants, AET did not develop in 17 (34%) cases, while side effects were registered in 66%. AET of grade 1 and grade 2 developed in 16 (32%) and 14 (28%) cases, respectively. Grade 3 toxicity occurred in 3 (6%) cases. Life-threatening, grade 4 or 5 AET was not seen. Temporary interruption due to vomiting, fever, neutropenia and AET was necessary in 18 (36%) patients. The mean duration of the interruption was 9.0 days. Out of 18 patients, the reason for interruption was esophageal toxicity in 12 (24%) cases. Complaints were treated with local anaesthetics in all cases with dysphagia. Use of drinkable nutrients was indicated also in all patients, while tramadol treatment was needed in 8 (16%) cases. No association was found between AET and gender ($p=0.584$), age ($p=0.271$) or smoking habits ($p=0.196$) of the patients. The D_{\max} and D_{mean} well correlated with moderate and severe swallowing toxicity. The $D_{\max} \pm$ SD to the esophagus in case of grade 0-1 and grade 2-3 toxicity was 56 \pm 11.45 and 64.07 \pm 5.55 Gy, respectively ($p<0.001$). The average $D_{\text{mean}} \pm$ SD in the cases with AET of grade 0-1 and grade 2-3 was 21.87 \pm 8.24 and 30.98 \pm 7.57 Gy, respectively ($p<0.001$). The $D_{\text{mean}} \pm$ SD among the three grade 3 AET patients was 34.46 \pm 5.58 Gy. The $L_{50\text{Gy}}$ was also in connection with the symptoms ($p<0.001$). In case of grade 0-1 and grade 2-3 AET the $L_{50\text{Gy}} \pm$ SD was 5.10 \pm 5.66 and 10.54 \pm 3.83 cm, respectively ($p<0.001$). $D_{30\%} \pm$ SD was 39.82 \pm 14.17 and 53.74 \pm 7.21 Gy in grade 0-1 and grade 2-3 esophagitis, respectively ($p<0.001$). The $V_{35-60\text{Gy}}$ all were in significant relation with toxicity. Examining the relationship between AET and dose-volume parameters with logistic regression, we found that $V_{45\text{Gy}}$ predicts most

reliably the development of grade 2 or higher AET (OR=1.089, 95%CI: 1.033-1.148, $p=0.001$). One percent increase of V_{45Gy} elevates the risk of grade 2 or higher AET with 8.9%. The risk of the development of AET of grade 2-3 was the highest above the cut-off value of $V_{45Gy} \geq 32.5\%$ according to ROC analysis.

4.3. *Thrombocytosis as a negative prognostic value in lung cancer*

Three-hundred and ninety eight consecutive patients with primary lung cancer were included in the study. There were 293 (73.6%) males and 105 (26.4%) females, with a mean age (\pm SD) of 58.3 (\pm 8.99) (range 36-79) years. Most of the patients had stage I cancer (47.2%). The performance status of the patients was good (ECOG 0 and 1, 44% and 56%, respectively). The histological type was squamous cell carcinoma in 175 (44%), adenocarcinoma in 163 (41%) and large cell, small cell lung cancer and carcinoid in 33 (8.3%), 13 (3.3%) and 14 (3.5%) cases, respectively. Of the 398 patients operated on for lung cancer, 86 (21.6%) were determined to have TCTs. The incidence of TCTs gradually elevated according to increasing cancer stage. In stage I, 18.6% of cases had TCTs, in stage II, III and IV 19.3%, 27.8% and 28.6%, respectively. There were no significant associations between stage ($p=0.074$), histology ($p=0.078$), age ($p=0.089$), gender ($p=0.516$) and platelet count values. Only 348 out of the 398 patients had data concerning their smoking habits: 260 of these patients (75%) were male and 88 patients (25%) were female. A total of 249 (71.6%) out of the 348 patients were smokers and 99 (28.4%) were non-smokers. No significant differences were found in relation to the distribution of TCTs and smoking habit according to the pathological stages of all resected lung carcinomas. TCTs was significantly more frequent in smokers (26.1%) than in non-smokers (10.1%) ($p=0.001$). This correlation was detected in the squamous cell subgroup ($p=0.004$), in contrast with patients with non-squamous cell histology ($p=0.082$). The frequency of smokers was also higher in patients who suffered from squamous cell cancer than those with other histology. The incidence of TCTs was also higher in the squamous cell subgroup ($p=0.001$), in which 94.9% of patients with TCTs were smokers ($p=0.007$). The median follow-up time of the entire population was 62.0 (range=1-103) months. The overall survival of the entire population was 31.0 months, and 14.8% of the patients were still alive after five years of follow-up. The overall 5-year survival was 35% among patients with TCTs, and 50.8% among patients with a normal TCT count ($p<0.001$). The overall survival time was 63.1 months in the group without and 38 months in the group

with TCTs ($p < 0.001$). There were no significant associations between the overall survival and gender ($p = 0.392$), smoking habit ($p = 0.724$) or histology ($p = 0.148$). A significant association was detected in the case of overall survival in the squamous histological subgroup according to the patient's platelet count (TCTs vs. normal) ($p < 0.001$), but such an association was not found in the other histological subgroups ($p = 0.916$). The survival of the patients significantly correlated with tumor stage for the whole study group ($p < 0.001$) and also in the subgroup of patients with TCT counts ($p < 0.001$), but there was no difference among the patients with TCTs ($p = 0.13$), presumably because of the relatively small number of cases. By univariate analysis, except histology ($p = 0.148$), advanced stage of lung cancer ($p < 0.001$), greater tumor size ($p < 0.001$), lymph node involvement ($p < 0.001$) and the presence of TCTs ($p < 0.001$) were significantly associated with a decreased survival. By multivariate analysis, the presence of TCTs ($p = 0.006$, HR:1.576, 95%CI:1.141-2.176), greater tumor size ($p = 0.001$, HR:1.341, 95%CI:1.129-1594) and lymph node involvement ($p < 0.001$, HR:1.726, 95%CI:1.474-2.020) were all independent factors related to poorer survival.

5. Discussion

5.1. *The roles of bcl-2 and MDRp expression in the efficacy of paclitaxel-based CRT*

The present study demonstrated that overexpression of the evaluated anti-apoptotic and cell membrane proteins can help predict the effectivity of paclitaxel-based CRT. We observed a strong association between the concurrent overexpression of bcl-2 and MDRp, and the tumour response and PFS in NSCLC patients. The novelty of our study lies in the analysis of the efficacy of paclitaxel and concomitant RT in relation to the expressions of the above markers, on which few data have been published. In our cohort, those demonstrating no response to paclitaxel-based CRT had a significantly more unfavourable PFS, and a worse (though not significantly) OS, which highlights the importance of predicting the potentially non-responsive patient population. We observed a significant association between the tumour response, the reduced PFS and the overexpression of MDRp. The preclinical data demonstrated that the increased activity of the signalling pathway in the paclitaxel-resistant cell lines was directly attributable to the overexpression of MDR-1. It was reported that MDRp may contribute to the multidrug resistance of lung cancer. The prognostic value of bcl-2 positivity has been widely

studied. Considerably fewer studies have examined the interaction between the expression of bcl-2 and the outcome of oncological treatment, i.e the role of bcl-2 in predicting the tumour response, and yielded controversial results. Jeong et al. treated NSCLC patients with cisplatin-based CRT and observed that a high expression of bcl-2 was significantly associated with a longer survival and a better response to the treatment. The findings of Fokkema et al. indicated a more favourable PFS of patients with an overexpression of bcl-2 following RT with or without carboplatin-based CRT, though they did not analyse the RT and CRT cohorts separately. Hwang et al. reported that bcl-2 expression predicted a poor outcome for radiation-treated NSCLC patients. Our own results revealed that, as compared with patients with a negative or low bcl-2 expression, patients with an overexpression of bcl-2 demonstrated a significantly worse RECIST tumour response and a poorer PFS after paclitaxel-based CRT and in the event of the overexpression of both MDRp and bcl-2, the tumour response was significantly poorer. The efficacy of RT-induced apoptosis, and therefore the whole of the treatment, may be reduced in tumours exhibiting an overexpression of bcl-2. The predictive value of bcl-2, especially for radiotherapy combined with third-generation chemotherapy, a prevalent advanced treatment procedure, is still unclear. In our study the overexpression of both biomarkers was found in the patients with the poorest tumour response and PFS. We hypothesize that a high MDRp expression indicates an enhanced drug efflux activity, leaving the cell without an adequate amount of chemotherapeutic. A high bcl-2 expression, as an anti-apoptotic mechanism, may have inhibited the RT-induced programmed cell death. This hypothesis is supported by the finding that both markers were negative in patients demonstrating complete pathological remission. Our findings demonstrate that the concomitant application of paclitaxel and RT is potentially ineffective in the treatment of NSCLC lung cancer, leading to a shorter PFS and more frequent local remission if the tumour indicates the overexpression of both MDRp and bcl-2.

5.2. Relation of AET to paclitaxel-based concurrent chemoradiotherapy for NSCLC

In our prospective study, the occurrence of AET during paclitaxel-based CRT for patients with non-small cell lung cancer was analysed in relation to patient- and dosimetric parameters. Combination of the RT with chemotherapy is directed to improve local control and survival of lung cancer patients. Several studies have shown that, compared to RT alone, the concurrent CRT appears to lower esophageal radiation tolerance. The AET

is often a dose-limiting factor that influences the treatment efficacy. In our study dose reduction or permanent interruption of therapy was not necessary due to AET. No association was found between esophageal toxicity and gender, age or smoking habits of the patients. Similarly to the literature, mild, acute swallowing toxicity or its absence was detected in most of our cases (grade 0-1 in 66%). These mild side effects could be easily managed but the grade 2 or higher dysphagia causes clinically relevant symptoms and influences remarkably the patient's quality of life. The incidence of grade 2 or more severe esophagitis was slightly higher in our cohort than in Ozgen's trial, but lower than in the Rodriguez study, in which patients with lung cancer were treated with 3D-CRT technique. Definitive difference could be detected in the applied concomitant chemotherapeutic agents between the present and the mentioned studies. None of their results perceived life-threatening grade 4 or 5 AET. The incidence of AET and its dose-volume relationship has been investigated in several trials. Although dose-volume parameters are commonly used to analyse the risk of acute esophagitis, there are large differences in the results, and in which of the dose-volume parameters have the most dominant effect on the risk of AET due to the different approach of evaluation. We compared dosimetric parameters of the group of patients with mild swallowing toxicity or the absence of it (grade 0-1) to the group with moderate or severe dysphagia (grade $2 \leq$). In concordance of numerous other studies the grade 2 or higher AET strongly correlated with the mean and the maximal dose, also the length and volume of the irradiated esophagus. Many researchers have found association between AET and mean or maximal dose to the esophagus. In the Ozgen paper the mean dose of esophagus ≥ 28 Gy correlated with grade 2 or worse toxicity. Other authors evaluated the correlation between AET and V_{dose} . It shows the percentage of esophagus receiving specific dose ($V_{20\text{Gy}}$, $V_{30\text{Gy}}$, $V_{40\text{Gy}}$, etc.). In the Rodriguez paper 30% of esophageal volume receiving ≥ 50 Gy was the most statistically significant factor associated with AET grade $1 \leq$. Belderbos and Bradley have found correlation with grade 2 or worse dysphagia and $V100\%_{20-60\text{Gy}}$, or $V_{5-70\text{Gy}}$, respectively. In our results, the parameter that mostly correlated with grade $2 \leq$ swallowing toxicity was 45 Gy mean dose to the esophagus with 32.5% cut-off value. One percent increase elevated the swallowing toxicity of the previously mentioned grade with 8.9%. Length of irradiated esophagus with 50 Gy was also in connection with symptoms. Association between dose of esophageal length and acute esophageal adverse events were also detected in relation to grade $2 \leq$ or $3 \leq$ swallowing toxicity in the literature. Elevated

radiation dose and combining RT with chemotherapy in the hope of better survival may increase the incidence of esophagitis. Development of AET is the most important limiting factor in the RT of chest tumours, so during treatment planning a significant aim is to decrease the esophageal volume and dose to protect patients from the serious events.

5.3. *Thrombocytosis as a negative prognostic value in lung cancer*

The present study demonstrated that a perioperatively increased platelet count can help to predict unfavorable outcome in lung cancer. We observed a strong association not only between the T and N status and stages, but also between the presence of TCTs and the 5-year survival of the patients after surgery. TCTs was significantly more frequent among smokers than non-smokers. The novelty of our study lies in the analysis of a high TCT count as a potential prognostic marker in relation to the outcome of lung cancer and on which few data have been published. Secondary, or reactive TCTs is observed in a variety of underlying conditions, which may cause either an acute and transient elevation of platelet count (trauma, major surgery, acute bleeding) or more sustained TCTs (infection or neoplasia) in patients. TCTs has a prevalence as high as 30% in patients with lung cancer, and has been associated with extensive and/or metastatic disease and a worse prognosis. The percentage of patients with elevated platelet counts was 21.6% among all our resected lung cancer cases, the frequency of the incidence was higher in more advanced stages (18.6% in stage I and 27.5% in stage III), which is similar to the proportion of TCTs in the study of Pedersen and Milman (20% in stage I and 30% in stage IIIA). The study by Hamilton et al. presented comparable data, as TCTs occurred in 26% of lung cancer cases. In our study, TCTs appeared most frequently in squamous cell lung cancer (52%) than in other histological subtypes. Similar data were presented in the study by Pedersen and Milman. Smoking habit can have an impact on the type of lung cancer. Nakamura et al. reported squamous cell cancer as being most frequent among smokers. Among the smokers participating in our study, the incidence of squamous cell lung cancer was also the most frequent, in addition, we discovered that TCTs was significantly more frequent in smokers than in non-smokers. There was no difference in 5-year survival between smokers and non-smokers in patients with normal platelet counts or thrombocytosis. There was no significant difference in survival between smokers and non-smokers in advanced lung cancer cases presented by Toh et al., but Nakamura et al. presented smoking as being significantly predictive of a poor prognosis after resection of

different stages of lung cancer. The impact of TCTs on the survival was analyzed from different aspects. Using the current TNM lung cancer classification there was a significant difference in the survival based on the stages, using an overall comparison. In our study, analyzing all patient data or only data for patients with normal platelet counts, we found the same significant correlation in survival rates in the different stages. When we analyzed the survival among patients with TCTs during the perioperative period, there was no significant difference in survival among the stages, presumably, because thrombocytosis resulted in the unfavorable outcome of the whole group, or because of the relatively small number of cases. The survival rate was significantly reduced in patients with preoperative TCTs according to Pedersen and Hamilton and in the evaluated patients in the trial of Aoe et al. while in our study there was also a significant difference in the overall survival between the high and normal platelet level groups. Using univariate analysis of the gender, smoking habit, histology, T status, N status, stage and TCT count, only the latter four had a significant impact on survival. Multivariate analysis of the T status, N status and platelet count showed that all were independent factors for survival. This finding is in accord with the results of Pedersen and Milman, and also of Aoe et al. among patients treated with resection or conservative treatments for lung cancer. To sum up, TCTs had a significant negative impact on survival by both uni- and multivariate analyses, and in the separately evaluated squamous histological subgroup. Survival after lung resection was remarkably lower in patients with TCTs during the perioperative period compared to patients with normal platelet counts. TCTs was evidently more frequent in smokers.

6. Summary, conclusions

6.1. The overexpression of both bcl-2 and MDRp is a potential predictive value as regards the inefficacy of paclitaxel-based concomitant chemoradiotherapy in NSCLC.

6.2. Keeping esophageal V_{45Gy} lower than 32.5% during paclitaxel-based chemoradiotherapy of non-small cell lung carcinoma patients helps to avoid moderate and severe swallowing toxicity.

6.3. The thrombocytosis during the perioperative period in patients undergoing lung cancer resection can be considered as a potential negative independent factor for survival, and should be taken into account in the decision of the indication for adjuvant therapy.

List of full papers that served as the basis of the Ph.D. thesis

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