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

Ph.D. Thesis

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

I. **Maráz A.**, Furák J., Pálföldi R., Eller J., Szántó E., Kahán Z., Thurzó L., Molnár J., Tiszlavicz L., Hideghéty K.

Roles of BCL-2 and MDR1 expression in the efficacy of paclitaxel-based lung cancer chemoradiation.

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II. **Maráz A.**, Furák J., Varga Z., Fodor E, Együd Zs, Borzási E, Kahán Zs, Pálföldi R, Tiszlavicz L, Hideghéty K.

Acute esophageal toxicity related to paclitaxel-based concurrent chemoradiotherapy for non-small cell lung cancer

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III. **Maráz A.**, Furák J., Varga Z., Kahán Zs, Tiszlavicz L, Hideghéty K.

Thrombocytosis has a negative prognostic value in lung cancer

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List of publications related to the subject of the thesis

IV. Szántó Erika, Ruskó László, Cserháti Adrienn, Maráz Anikó, Varga Zoltán, Thurzó László, Hideghéty Katalin
Use of contrast-enhanced computed tomography (re-CT) in conformal radiotherapy treatment planning.
RADIOTHERAPY AND ONCOLOGY 99:(1) *p. 584.* (2011)

V. Hideghéty Katalin, Cserháti Adrienn, Zag Levente, Nagy Zoltán, Lengyel Zsolt, Maráz Anikó, Fazekas Olga, Pávics László
Prospective clinical investigation on the application of 18FDG–PET/CT for curative chemo-radiotherapy.
RADIOTHERAPY AND ONCOLOGY 91:(Suppl. 2) *p. 421*. (2009)

VI. K Hideghéty K, A Cserháti, O Fazekas, Z Nagy, A Maráz, A Nikolényi, V Turcsányi, E Fodor, P Russ-Gal, L Thurzó
Clinical impact of volumetric changes of macroscopic tumours during fractionated radiotherapy detected by repeated planning CT
RADIOTHERAPY AND ONCOLOGY 88:(Suppl. 2) *pp. 349-350.* (2008)

VII. Maráz Anikó, Hideghéty Katalin, Varga Zoltán, Nagy Zoltán, Thurzó László
Tüdőtumor individuális kezelése re-CT alapján.
MAGYAR ONKOLÓGIA 51:(3) *p. 2.* (2007)

VIII. Maráz Anikó, Cserhári Adrienn, Turcsányi Veronika, Fazekas Olga, Nagy Zoltán, Thurzó László, Hideghéty Katalin
(Előre?) lépés előrehaladott tüdőtumor sugárkezelésében (kezelési stratégiaváltás).
MAGYAR ONKOLÓGIA 51: *p. 268.* (2007)

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List of abbreviations

3D	three-dimensional
AET	acute esophageal toxicity
ANOVA	analysis of variance
Bcl-2	B-cell lymphoma protein-2
CDDP	cisplatinum
CI	confidence interval
CRT	chemo-radiotherapy
CT	computer tomography
D_{max}	the maximal dose of irradiated esophagus
D_{mean}	the mean dose of irradiated esophagus
DVH	dose-volume histogram
GTV	gross tumour volume
GTV1	gross tumour volume after the repeated CT
$GTV_{<40}$	gross tumour volume reduction in range <40%
GTV_{50-40}	gross tumour volume reduction in range 50-40%
$GTV_{>50}$	gross tumour volume reduction in range >50%
HR	hazard ratio
L_{50Gy}	length of the irradiated esophagus with 50 Gy
MDRp	multidrug-resistant protein
NSCLC	non-small cell lung cancer
OAR	organ at risk
OR	odds ratio
OS	overall survival
PD	progressive disease
PFS	progression-free survival
PLT	platelet count
PR	partial remission
PTV	planning target volume
RECIST	Response Evaluation Criteria In Solid Tumors
SD	stable disease
$V_{35-60Gy}$	volume of the esophagus irradiated with 35 Gy to 60 Gy

1. Introduction

Lung cancer is the most frequent tumour worldwide. Radiotherapy is one of the main treatment modalities of lung cancer and was the conventional method of treatment until the 1980s' (1). Its efficacy alone in locally advanced non-small cell lung cancer is poor (2). Strategies designed to enhance local control include improved tumour targeting (three dimensional treatment planning and increasingly more sophisticated radiotherapy techniques), escalation of thoracic radiotherapy dose (2-4) or application of different fractionations (5-8). Individualized combinations of various treatment procedures, such as combining radiotherapy with chemotherapy tend to improve local control and survival (9, 10). The most frequently applied third-generation chemotherapeuticals are the taxanes, which have been demonstrated in clinical trials to be widely effective in advanced NSCLC (9, 10). The prototype of the taxane family is paclitaxel an excellent radiosensitizer (9, 10, 11-13).

In designing the optimum individual treatment planning, including selection of an effective chemotherapeutical and the anticipation of potential radioresistance, the physician may be aided by predictive markers (14-26). 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 (27-29). 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 (14-17), but poorer survival rates of patients with a higher bcl-2 expression have also been reported (18-20). The predictive role of bcl-2 has also been examined in various tumour types. The association between the response to platinum-based chemoradiotherapy and apoptosis-related proteins is unclear. No correlation was found in cancers of the bladder (21), the esophagus (22) or the rectum (23). The overexpression of bcl-2 predicted a more favourable outcome in head and neck (24) and lung (25) cancer, but an unfavourable effect has been described in oropharyngeal (26) and lung cancer patients (18).

Radiosensitization has been reported to increase therapy efficacy, but it may also increase therapy-induced toxicity (6, 9, 30-33). The practice of advanced techniques should reduce acute and late treatment-associated toxicity (34, 35). Radiation esophagitis seems to be one of

the most common acute toxicities, especially in the setting of combined concurrent chemo-radiation (1, 2, 36, 37). This adverse treatment side effect is often a dose-limiting factor (4), which influences the treatment outcomes and patient quality of life, therefore its dose-volume relationship has been investigated in several trials (1, 2, 5-8, 31-33, 36-40). Results have differed considerably across different institutions regarding which dosimetric factors are more critical than others. Rose and his colleagues performed a systematic literature review of published studies addressing radiation esophagitis of the thoracic radiotherapy in 2009 (37). Statistically significant relationships between specific dose-volume parameters (V_{20Gy}, V_{35Gy}, V_{60Gy}, maximal and mean esophageal dose) with or without chemotherapy and clinically significant acute esophagitis risk were identified based on the analysed studies. They found various dosimetric correlations in the literature of esophageal toxicity regarding the seriousness of the swallowing complaints. The identification of the risk factors for acute esophagitis 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 chemoradiation – 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 (41), 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 (42), but there was no difference in terms of survival between smokers and non-smokers with advanced non-small cell lung cancer (43). Thrombocytosis has been proven to have a fundamental impact on survival in advanced cervical (44) and renal cell cancer (45). Unfavorable outcome in association with thrombocytosis has been described in patients with esophageal (46), gastric (47) and soft tissue cancer (48). There are only a few articles discussing the impact of thrombocytosis on lung cancer survival, or in resected cases. Previous studies have reported mainly unfavorable (49, 50, 51-53) or no impact (54) on survival of a high thrombocyte count among patients with non-small cell lung cancer. In several studies, thrombocytosis was detected as an independent prognostic factor (50, 51, 53).

2. Aims

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

To that aim research on predictive molecular markers on the outcome of chemoradiation for better patient selection, furthermore investigation on optimisation of radiation parameters of the binary system (paclitaxel-based chemoradiotherapy) in order to reduce radiation toxity was performed prospectively. In the retrospective analysis we have searched for factors influencing the outcome of surgery applied as primery procedure or after the operable stage could be achieved by chemoradiotherapy for locally advanced NSCLC. In details:

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

2.2. A prospective investigation to the dosimetric correlations of acute esophageal toxicity during neoadjuvant and definitive paclitaxel-based three-dimensional conformal chemo-radiotherapy 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 thrombocytosis 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 lung cancer chemoradiation

3.1.1. Patients

Patients receiving chemoradiotherapy 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. The staging procedures were based on the conventional protocol and on induction chemotherapy. For each patient, the treatment plan was designed by a multidisciplinary oncoteam.

3.1.2. Methods

Systemic treatment and radiotherapy. During the radiotherapy all the patients received concomitant taxane-based chemotherapy (weekly paclitaxel 100 mg/m^2 in 4-6 cycles, depending on toxicity). Of the 19 patients (stage III.B) who completed induction chemotherapy (1 or 2 cycles), 17 (89.5%) received a taxane-based chemotherapy regimen (paclitaxel 175 mg/m^2, carboplatin 400 mg/m^2 or docetaxel 75 mg/m^2, cisplatin 75 mg/m^2, at 3-week intervals), while 2 patients received a gemcitabine-based regimen (gemcitabine 1250 mg/m^2 on days 1 and 8, CDDP 70 mg/m^2 on day 1, and then at 3-week intervals) for at least 4 weeks prior to the concomitant chemoradiotherapy. After completion of the chemoradiotherapy, additional consolidation chemotherapy was administered in 18 cases (paclitaxel 175 mg/m^2, carboplatin 400 mg/m^2 or docetaxel 75 mg/m^2, CDDP 75 mg/m^2, at 3-week intervals).

CT-based three-dimensional treatment planning and conformal radiotherapy were performed in all cases, with use of an individual immobilization system. 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, radiotherapy of the reduced volume was continued at an average dose of 22-26 Gy (with the exception of neoadjuvant cases) (Figure 1).

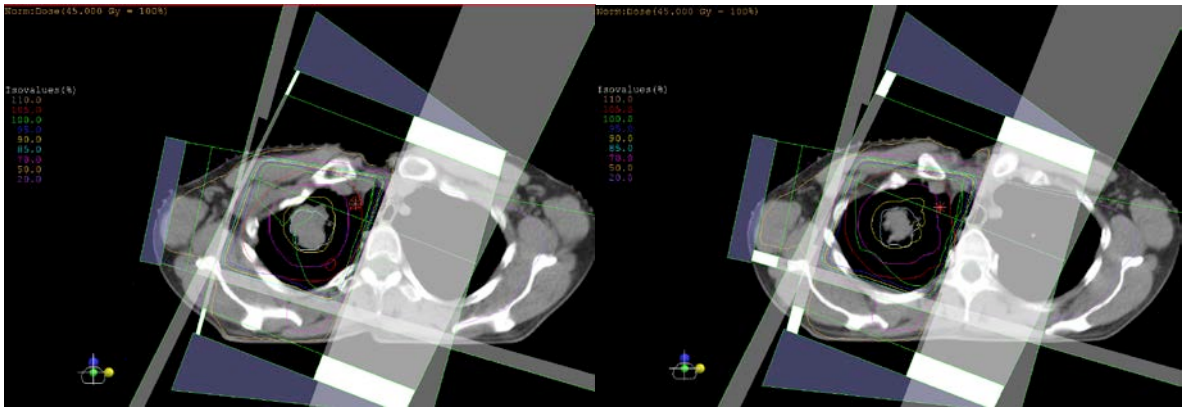


Figure 1. *Planning CT images with contoured macroscopic lung cancer as GTV (yellow line), the planning target volume as PTV (red line) before the RT (left picture); the shrinked tumour volume as GTV1 (blue line), the new PTV as PTV1 (pink line) after 45 Gy irradiation (right picture), therapeutic beams and isodose curves.*

Response analysis. During the treatment, following the administration of a 45 Gy dose, and 4-6 weeks after the completion of the chemoradiotherapy regimen, clinical and diagnostic CT examinations were performed. The CT scans were compared with the pretreatment scans provided for radiotherapy-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 $GTV_{>50,}$ GTV_{50-40} and $GTV_{<40}$, respectively), additionally, the CT scans were analysed according to the RECIST criteria system.

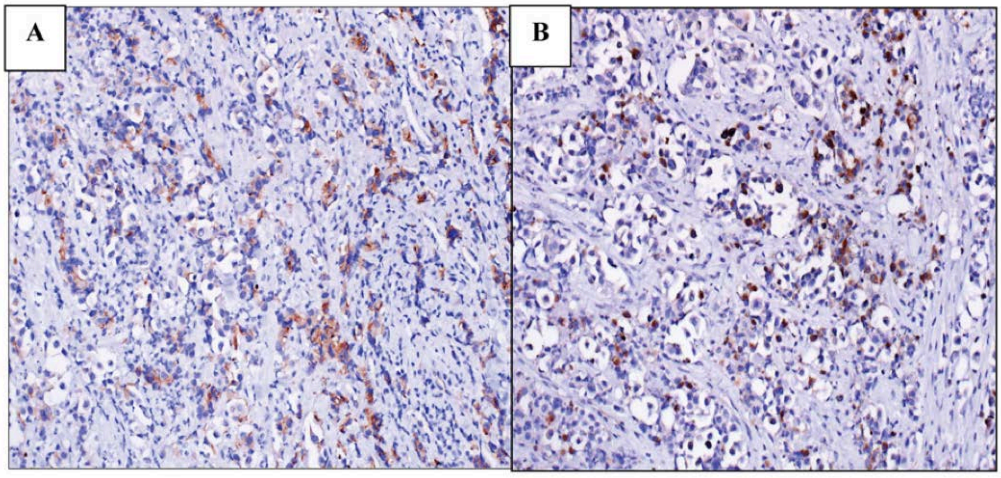


Figure 2. *Immunohistochemistry of biopsy specimens. A: Moderate cytoplasmic positivity for MDRp (original magnification x224); B: moderate nuclear and cytoplasmic positivity for bcl-2 (original magnification x224).*

Immunohistochemistry. Before the chemoradiotherapy, immunohistochemical staining was performed to quantify the bcl-2 and MDRp expressions in the biopsy samples. Histological samples from 28 of the 32 evaluated patients were examined prospectively as concerns the bcl-2 and MDRp expressions. (The histological samples from 4 patients were used for K-ras analysis and the remaining material was not sufficient for further immunohistochemical analysis.) Immunohistochemical studies were carried out on paraffin sections by an indirect peroxidase method. Sections were cut 4 μm thick. Deparaffinizing, rehydrating and antigen

retrieval were performed in a PT (Dako, Denmark) module (20 min, 99 Co), using the 3in1 (pH 6.0) solution produced by Labvision. The endogenous peroxidase activity was blocked with hydrogen peroxide (3%, 10 min) and a solution of milk powder (in 1% phosphate-buffered saline, 10 min) was used as protein block. The Real-Envision (DAB) kit (Dako, Denmark) was used as labelling system. A semiquantitative 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 (Figure 2).

Statistical analysis. All analyses were carried out using SPSS version 15.0 for Windows (SPSS Inc., Chicago, IL). The main outcome measures were the tumour response and the time to progression. The associations between the molecular marker expression and clinical factors, tumour response, local relapse and distant metastases within 6 months were evaluated with the chi-square test, while that with age was assessed with the one-way ANOVA test. In terms of both progression-free and overall survival, the outcome was analysed by Kaplan-Meier analysis (pairwise comparisons – Breslow test).

3.2. The relation of acute esophageal toxicity to paclitaxel-based concurrent chemoradiotherapy for NSCLC

3.2.1. Patients

Patients receiving chemoradiotherapy for primary unresectable or potentially operable non-small cell lung cancer at the Department of Oncotherapy between December 2006 and June 2011 were eligible for participation in this study. Histological examination was performed before the therapy in all cases. Staging examinations were based on conventional protocols (chest computed tomography, abdominal ultrasound/CT, brain CT, bone scan, bronchoscopy). For each patient the multimodal treatment strategy was designed by a multidisciplinary team.

3.2.2. Methods

Chemo- and radiotherapy, supportive therapy. During the radiotherapy all the patients received concomitant taxane-based chemotherapy (weekly paclitaxel 100 mg/m^2 in 4-6 cycles, depending on toxicity). Of the 40 patients (stage IIIB) who completed induction chemotherapy (1 or 2 cycles), 38 (95%) received a taxane-based chemotherapy regimen (mainly paclitaxel 175 mg/m^2, carboplatin 400 mg/m^2 or docetaxel 75 mg/m^2, cisplatin 75 mg/m^2, at 3-week intervals), while 2 patients received a gemcitabine-based regimen (gemcitabine 1250 mg/m^2 on days 1 and 8, CDDP 70 mg/m^2 on day 1, and then at 3-week intervals) for at least 4 weeks prior to the concomitant CRT. All patients were irradiated in supine position, with both arms elevated above the head on the thorax set of the AIO Solution^{TM} (ORFIT, Antwerpen Belgium). CT-based three-dimensional treatment planning and conformal radiotherapy were performed in all cases, with use of an individual immobilization system with thermoplastic masks. The gross tumor volume (GTV), the macroscopic lung cancer, the involved mediastinal and hilar lymph nodes was defined on [^{18}F]fluoro-2-deoxy-d-glucose positron emission tomography-CT (^{18}FDG-PET-CT) images (Figure 3). 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 25×1.8 Gy (and the total dose of neoadjuvant cases); then, after a repeated CT scan, depending on the tumour response, radiotherapy 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 (Figure 4). 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.

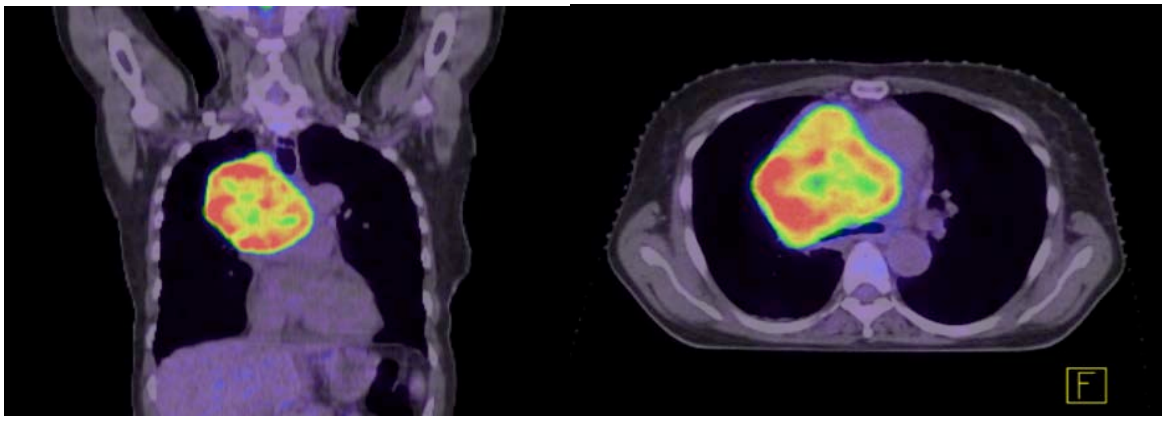


Figure 3. *^{18}FDG-PET-CT images before the KRT.*

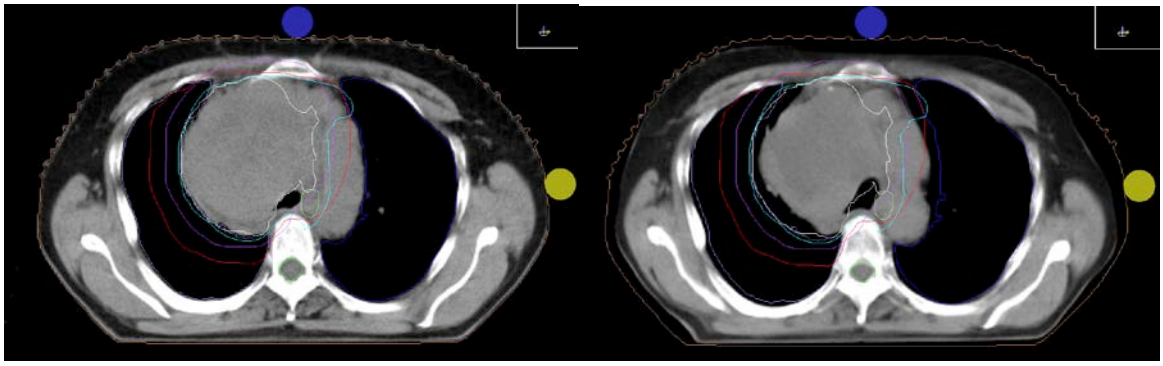


Figure 4. *Planning CT images with contoured macroscopic lung cancer and involved lymph nodes as GTV (blue line) and the planning target volume as PTV (red line) before the RT (left picture) and the schrinked tumour volume as GTV1, after 45 Gy irradiation (right picture).*

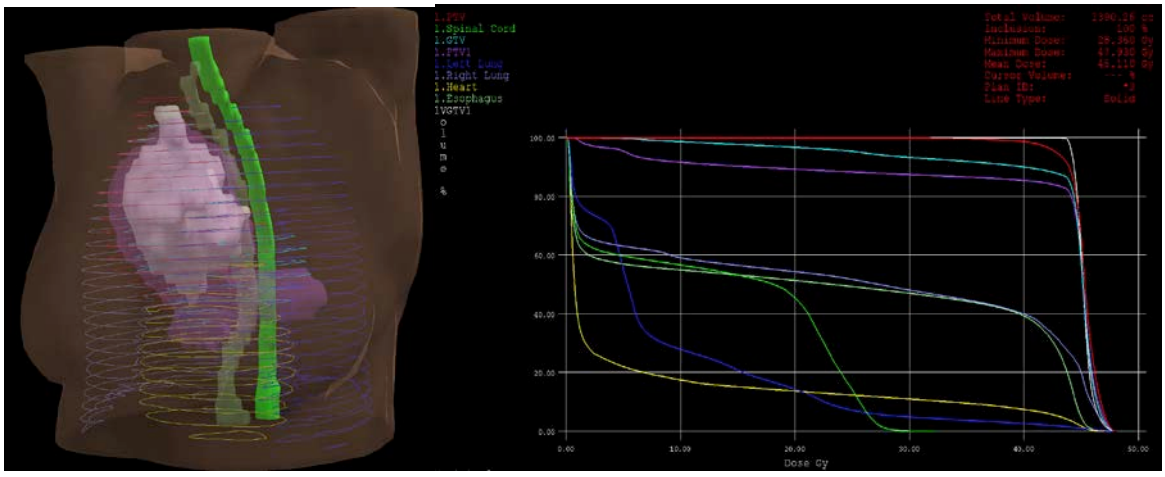


Figure 5. *Three dimensional view of GTV, PTV and organs at risk, especially the oesophagus, on the left picture; the dose-volume histograms on the right picture.*

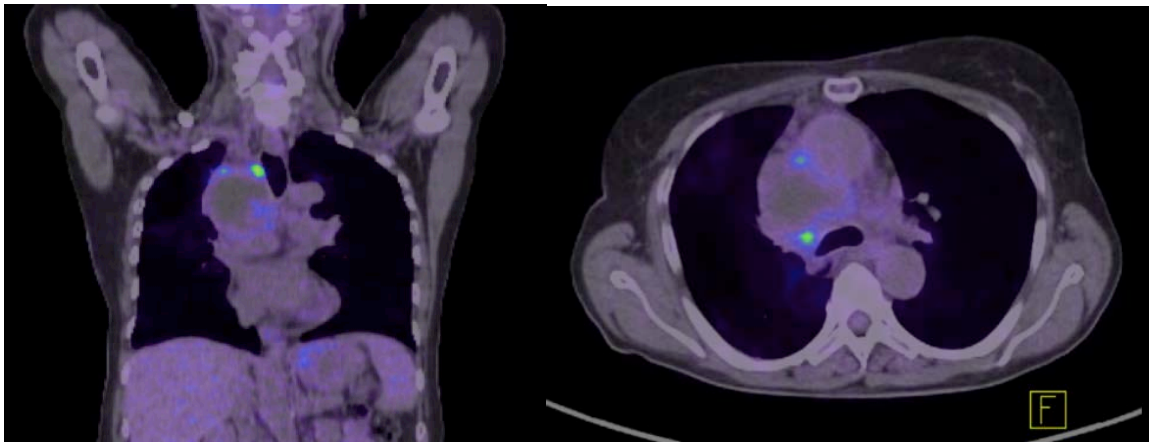


Figure 6. *^{18}FDG-PET-CT images after the successful KRT.*

Evaluation of acute esophageal toxicity. The whole esophagus was contoured from the anular cartilage to gastroesophageal junction prior to radiation planning (Figure 5). 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 ($V_{35\text{-}60\ Gy}$). AET as dysphagia was evaluated prospectively based on Common Terminology Criteria for Adverse Events, version 3.0 issued by National Cancer Institute (Table I) (55). 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.

Table I. *Common Terminology Criteria for Adverse Events, Version 3.0 - Dysphagia (difficulty of swallowing)*

Grade 1	Symptomatic, able to eat regular diet
Grade 2	Symptomatic and altered eating/swallowing (*e.g.* altered dietary habits, oral supplements); i.v. fluids indicated <24 hrs
Grade 3	Symptomatic and severely altered eating/swallowing (e.g., inadequate oral caloric or fluid intake); i.v. fluids, tube feedings, or TPN indicated ≥24 hrs
Grade 4	Life-threatening consequences (e.g., obstruction, perforation)
Grade 5	Death

TPN: total parenteral nutrition.

Statistical analysis. All statistical analyses were carried out using SPSS version 20.0 for Windows (Chicago, IL, USA). The relations between the AET, age, gender, smoking habits and the dosimetric data were evaluated. Age was assessed with t-test, gender and smoking habits were analysed with chi-square test, dose and volume were assessed with t-test. Relationship between dose-volume parameters and severity of AET was analysed with logistic regression. Reciever operating characteristic (ROC) analysis was used to find the cut-off point for $V_{45Gy.}$

3.3. Thrombocytosis as a negative prognostic value in lung cancer

3.3.1. Patients

Patients operated on for lung cancer at the Department of Surgery in a 5-year period each patient, the treatment plan was designed by a multidisciplinary onco-team.

3.3.2. Methods

Surgical, histological and staging procedures. 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 (41). Patient files were reviewed, and relevant data were collected.

Definition of thrombocytosis and smoking habits. 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 $400x10^3/\mu l$, in agreement with other studies (44, 47, 49, 50, 56), thrombocytosis was diagnosed. Based on this data, the 398 patients were divided into two groups as to whether they had normal platelet counts or thrombocytosis 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.

Statistical analysis. All analyses were carried out using SPSS version 20.0 for Windows (SPSS Inc., Chicago, IL, USA). The associations between thrombocytosis and clinical factors (stage, histology, gender) were evaluated with the chi-square test, and the correlation between thrombocytosis and age were tested with independent samples *t*-test. Overall survival was analyzed by Kaplan-Meier analysis. 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.

3. Results

4.1. The roles of bcl-2 and MDRp expression in the efficacy of paclitaxel-based lung cancer chemoradiation

Patient characteristics, response and survival. Thirty-two patients received paclitaxel-based chemoradiotherapy, 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 (±SD) of the patients was 58.9 (±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%). After the chemoradiotherapy, surgical treatment was possible in 10 cases (31%) and 18 patients (56%) received consolidation chemotherapy. 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 ($GTV_{>50}$, GTV_{50-40} 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; GTV_{50-40} 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; GTV_{50-40} 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. The difference between the surgical and non-surgical populations was not significant from the point of view of the survival (29.1 vs. 22.1 months, p=0.119), but there was a more favourable trend in the outcome after the operations. The OS rate correlated significantly only with consolidation chemotherapy (survival with or without chemotherapy, 13.4 vs. 4.8 months, p<0.001), and the duration of PFS was also longer (29.4 vs. 11.3 months, p<0.001).

4.1.2. Association of expression of drug resistance- and apoptosis-related proteins with clinicopathologic characteristics. 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 (Table II). 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).

Table II. *Proportions of bcl-2 and multi-drug resistance protein (MDRp) expression.*

n=28	**bcl-2**			**MDRp**			**bcl-2 and MDRp**		
	Negative	1+	2+/3+	Negative	1+	2+/3+	Both low/neg	Mixed expression	Both 2+/3+
n	16	6	6	19	3	6	19	6	3
%	57.2	21.4	21.4	67.8	10.7	21.4	67.9	21.4	10.7

4.1.3. Association of expression of drug resistance- and apoptosis-related proteins with response and outcome of patients. 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) (Table III). 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).

Table III. *Association of molecular marker expression (bcl-2, MDRp) with clinical outcome.*

n=28	Response (RECIST)*				Volume change (%)*				PFS**	
	PR	SD	PD	p	>50	50-40	<40	p	Month (med±S.D.)	P
bcl-2 neg / low	16	5	1		13	3	6		13.4±2.5	
bcl-2 high	2	2	2	0.082	1	1	4	0.151	4.2±0.6	0.025
MDRp neg / low	17	4	1		11	4	4		13.4±2.2	
MDRp high	1	3	2	0.016	3	-	-	0.001	1.63±1.1	<0.001
Both neg / low	15	3	1		13	3	3		13.4±0.2	
Mixed expr.	3	3	-		1	1	4	0.019	4.1 ±1.9	<0.001
Both high	0	1	2	0.005	0	0	3		3.1±1.4	

*Chi-square test, ** Kaplan-Meier, PR-partial remission, SD-stable disease, PD-progression, PFS - progression-free survival

4.2. Relation of acute esophageal toxicity to paclitaxel-based concurrent chemoradiotherapy for NSCLC

4.2.1. Patient characteristics. Altogether, 50 patients' data were analysed. Thirty-two (64%) were men, 18 (36%) were women. The mean±SD age was 59.8±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. These stages were determined according to the 6th edition of TNM system. Twenty-nine (58%) patients were smokers and 21 (42%) were non-smokers. Twelve (24%) patients underwent operation, in one case, despite remission only exploration was performed due to inoperable conditions.

4.2.2. Dose parameters. The mean±SD dose of planning target volume was 60.7±9.8 Gy in the whole investigated population, while 64.7±5.5 Gy in the definitively treated, irresectable patients. The preoperative given dose was 45.0 Gy in all 10 cases. Irradiation doses to spinal cord, ipsilateral and contralateral lung are shown in table IV.

Table IV. *Radiation dose (Gy) to critical organs (except esophagus)*

Spinal cord	Mean dose ±SD	12.1±4.5
	Maximal dose ±SD	36±6.7
Ipsilateral lung	Mean dose ±SD	26.6±7.8
	V_{20Gy} ±SD (%)	54.1±13.5
Contralateral lung	Mean dose ±SD	10.6±3.6
	V_{20Gy} ±SD (%)	14.3±9.1
Heart	Mean dose ±SD	12.5±4.6
	V_{30Gy} ±SD (%)	12.1±3.8

Maximal dose (D_{max}) to the esophagus was 57±10.8 Gy, the mean dose (D_{mean}) ±SD was 24.9±9 Gy. The mean length of the irradiated esophagus with 50 Gy was 6.99±6.7 cm.

4.2.3. Toxicity. Among the 50 participants, esophageal toxicity 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 (Table V). 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 acute esophagitis 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 Suspensio anaesthetica 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 esophageal toxicity and gender (p=0.584), age (p=0.271) or smoking habits (p=0.196) of the patients.

Table V. *Incidence of acute esophageal toxicity (AET)*

Severity of AET n=50	Grade 0	Grade 1	Grade 2	Grade 3	Grade 4-5
Patients (%)	17 (34%)	16 (32%)	14 (28%)	3 (6%)	0

4.2.4. Correlations of the dose- and volume data with AET. The maximum and mean dose to the esophagus well correlated with moderate and severe swallowing toxicity. The D_{max} ±SD to the esophagus in case of grade 0-1 and grade 2-3 toxicity was 56±11.45 and 64.07±5.55 Gy, respectively ($p<0.001$). The average D_{mean} ±SD in the cases with AET of grade 0-1 and grade 2-3 was 21.87±8.24 and 30.98±7.57 Gy, respectively ($p<0.001$). The average mean dose ±SD to the esophagus among the three grade 3 AET patients was 34.46±5.58 Gy. The length of the irradiated esophagus with 50 Gy (L_{50Gy}) was also in connection with the symptoms ($p<0.001$). In case of grade 0-1 and grade 2-3 AET the L_{50Gy} ±SD was 5.10±5.66 and 10.54±3.83 cm, respectively ($p<0.001$). $D_{30\%}$ ±SD was 39.82±14.17 and 53.74±7.21 Gy in grade 0-1 and grade 2-3 esophagitis, respectively ($p<0.001$). The volume of the esophagus irradiated with 35-60 Gy ($V_{35\text{-}60\ Gy}$) in relation with toxicity is shown in Table VI.

Table VI. *Dosimetric parameters of acute esophageal toxicity. Data are means ±SD.*

	Grade 0-1	Grade 2-3	*p*–Value (t-test)
n	33 (66%)	17 (34%)	
D_{max} (Gy)	54.56±11.45	64.07±5.55	<0.001
D_{mean} (Gy)	21.87±8.24	30.98±7.57	<0.001
V_{35Gy} (%)	34.87±17.71	49.23±11.55	0.004
V_{40Gy} (%)	30.93±17.67	46.41±12.04	0.002
V_{45Gy} (%)	20.15±18.71	43.23±12.20	<0.001
V_{50Gy} (%)	15.90±18.06	37.88±11.45	<0.001
V_{55Gy} (%)	13±15.85	29.29±16.36	0.001
V_{60Gy} (%)	8.03±12.31	19.23±16.22	0.009
Length $_{50Gy}$ (cm)	5.10±5.66	10.54±3.83	<0.001

Examining the relationship between esophageal toxicity and dose-volume parameters with logistic regression, we found that V_{45Gy} 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

4.3.1. Patient characteristics. 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 (±SD) of 58.3 (±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.

4.3.2. Association of thrombocytosis and smoking habits with clinicopathological characteristics. Of the 398 patients operated on for lung cancer, 86 (21.6%) were determined to have thrombocytosis. The incidence of thrombocytosis gradually elevated according to

increesing cancer stage. In stage I, 18.6% of cases had thrombocytosis, 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. The distribution of thrombocytosis and smoking habit according to the pathological stage of all resected lung carcinomas is described in Table VII; no significant differences were found.

Table VII. *Distribution of thrombocytosis and smoking habits according to the pathological stage in the entire population.*

		All patients, N=398		**Patients with known smoking history, N=348**	
Stage	**All patients n=398**	**PLT <400x10^3/µl n=312**	**PLT >400x10^3/µl n=86**	**Non-smokers n=99**	**Smokers n=249**
IA	18.6%	20%	14%	21%	16%
IB	28.6%	29.2%	26.7%	31%	28.9%
IIA	1.8%	1.9%	1.2%	1%	2%
IIB	20.4%	20.8%	18.6%	18%	19.7%
IIIA	19.8%	17.9%	26.7%	15%	22.1%
IIIB	7.3%	7.1%	8.1%	7%	8.4%
IV	3.5%	3.2%	4.7%	6%	2.8%

PLT, Platelet count.

Thrombocytosis was significantly more frequent in smokers (26.1%) than in non-smokers (10.1%) (p=0.001) (Table VIII). 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 thrombocytosis was also higher in the squamous cell subgroup, in which 94.9% of patients with thrombocytosis were smokers. The data for smoking habits and thrombocytosis in the squamous cell and other histological subtypes are detailed in Table IX.

Table VIII. *The association between thrombocytosis and smoking habit (p=0.001).*

	Non-smokers **n=99**	**Smokers** **n=249**
Normal platelet count **(<400x10^3/µl)**	89 (89.9%)	184 (73.9%)
Thrombocytosis **(>400x10^3/µl)**	10 (10.1%)	65 (26.1%)

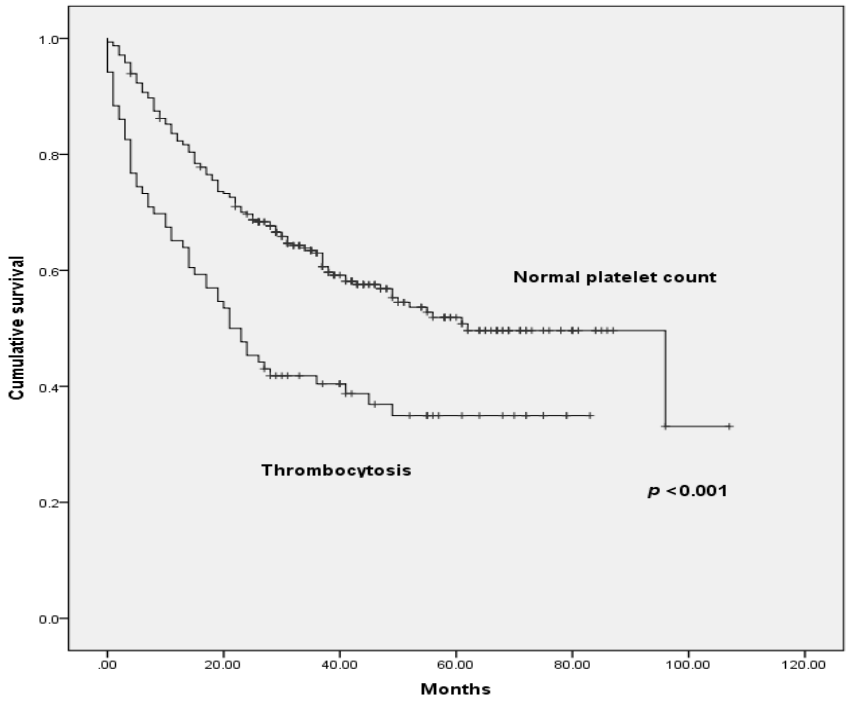
Table IX. *The distribution of thrombocytosis and smoking habit according to squamous cell and other histological subtypes.*

	All patients, N=398		**Patients with known smoking history, N=348**	
	PLT <400x10^3/µl **n=312**	**PLT >400x10^3/µl** **n=86**	**Non-smokers** **n=99**	**Smokers** **n=249**
Squamous cell lung cancer	130 (74.3%)	45 (25.7%)	35 (21.5%)	128 (78.5%)
Other histology	182 (81.6%)	41 (18.4%)	64 (34.6%)	121 (65.4%)
***p*-Value (Chi-square test)**	0.001		0.007	

PLT, Platelet count.

4.3.3. Association of thrombocytosis and smoking habit with outcome of patients. 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 thrombocytosis, and 50.8% among patients with a normal thrombocyte count (p<0.001). The overall survival time was 63.1 months in the group without and 38 months in the group with thrombocytosis (p<0.001) (Figure 7).

Figure 7. *Kaplan-Meier survival curves for patients with lung cancer patients according to platelet count.*



Patients at risk.

	0 year	1 year	2 years	3 years	4 years	5 years
G I	**312**	**258**	**214**	**139**	**75**	**47**
G II	**86**	**56**	**40**	**29**	**19**	**12**

Group I: Patients with normal platelet count, Group II: patients with thrombocytosis.

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 (thrombocytosis *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 normal platelet counts (p<0.001), but there was no difference among the patients with thrombocytosis (p=0.13), presumably because of the relatively small number of cases (Table X).

Table X. *The association between 5-year survival, platelet count and stage.*

Stage	**PLT <400x10^3/µl (n=312)**	**PLT >400x10^3/µl (n=86)**	**All**
IA, n=78	81%	44%	75%
IB, n=110	62%	48%	59%
IIA, n=7	50%	0%	43%
IIB, n=81	46%	31%	43%
IIIA, n=80	25%	26%	25%
IIIB, n=28	15%	28%	20%
IV, n=14	44%	25%	34%
***p*-Value**	<0.001	0.130	<0.001

PLT, Platelet count.

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 thrombocytosis (p<0.001) were significantly associated with a decreased survival. By multivariate analysis, the presence of thrombocytosis, greater tumor size and lymph node involvement were all independent factors related to poorer survival (Table XI).

Table XI. *Multivariate analysis of survival.*

	***p*-Value**	**HR**	**95% CI**	
			Lower	**Upper**
Thrombocytosis	**0.006**	**1.576**	**1.141**	**2.176**
T status	**0.001**	**1.341**	**1.129**	**1.594**
N status	**<0.001**	**1.726**	**1.474**	**2.020**

CI: Confidence interval; HR: Hazard ratio.

5. **Discussion**

5.1. The roles of bcl-2 and MDRp expression in the efficacy of paclitaxel-based lung cancer chemoradiation

The present study demonstrated that overexpression of the evaluated anti-apoptotic and cell membrane proteins can help predict the effectivity of paclitaxel-based chemoradiotherapy. 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 radiotherapy in relation to the expressions of the above markers, on which few data have been published. We mainly used paclitaxel with radiotherapy or paclitaxel in combination with carboplatin in the course of induction and consolidation therapy. Paclitaxel stabilizes microtubules, which blocks cell cycle progression in its most radiosensitive G2-M phase, and also induces bcl-2 hyperphosphorylation, resulting in its inactivation, thereby facilitating apoptosis (57, 58, 59). In our cohort, those demonstrating no response to paclitaxel-based chemoradiotherapy 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 (27-29). The prognostic value of bcl-2 positivity has been widely studied. Several papers have confirmed a more favourable progression in bcl-2-positive patients with either surgically resected (14) or locally advanced (17) NSCLC, or even irrespective of the stage (15, 16). However, some studies did not find a significant association between the bcl-2 expression and the survival (61, 62), or even reported a worse survival in the event of a high bcl-2 expression (18-20). 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 (21-26). Jeong et al. treated NSCLC patients with cisplatin-based chemoradiotherapy and observed that a high expression of bcl-2 was significantly associated with a longer survival and a better response to the treatment (25). The findings of Fokkema et al. indicated a more favourable PFS of patients with an overexpression of bcl-2 following radiotherapy with or without carboplatin-based chemoradiotherapy, though they did not analyse the radiotherapy and chemoradiotherapy cohorts separately (21). Hwang et al. reported that bcl-2 expression predicted a poor outcome for radiation-treated NSCLC patients (18). 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 chemoradiotherapy and in the event of the overexpression of both MDRp and bcl-2, the tumour response was significantly poorer. Bcl-2 inhibits programmed cell death, and can be associated with a more aggressive tumour cell phenotype, with resistance to treatment protocols based on microtubule damage. The efficacy of radiotherapy-induced apoptosis, and therefore the whole of the treatment, may be reduced in tumours exhibiting an overexpression of bcl-2 (18-20). 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 chemotherapeutical. A high bcl-2 expression, as an anti-apoptotic mechanism, may have inhibited the radiotherapy-induced programmed cell death. This hypothesis is supported by the finding that both markers were negative in patients demonstrating complete pathological remission. The overexpression of MDRp in our cohort

was associated with a poorer tumour response. However, patients with an overexpression of bcl-2, but not of MDRp, there was a slightly, though not significantly better therapeutic efficacy as compared with patients displaying an overexpression of both markers. We assume that paclitaxel terminated the inhibition of apoptosis by hyperphosphorylating and inactivating bcl-2, and thereby partially restored the radiosensitivity (57, 59). Our findings demonstrate that the concomitant application of paclitaxel and radiotherapy 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. The findings suggest that paclitaxel-based chemoradiotherapy is questionable in this group of patients, who may benefit more from the combination of other drugs with radiotherapy, which may be favourable even in the case of an overexpression of bcl-2 and since platina derivatives are not substrates of the multidrug efflux pump (25).

5.2. Relation of acute esophageal toxicity to paclitaxel-based concurrent chemoradiotherapy for NSCLC

In our prospective study, the occurrence of acute esophageal toxicity during paclitaxel-based chemo-radiotherapy for patients with non-small cell lung cancer was analysed in relation to patient- and dosimetric parameters. Combination of the radiotherapy with chemotherapy is directed to improve local control and survival of lung cancer patients (9, 10). Several studies have shown that, compared to radiotherapy alone, the concurrent chemo-radiotherapy appears to lower esophageal radiation tolerance (40). The acute esophageal toxicity is often a dose-limiting factor that influences the treatment efficacy (4). In our study dose reduction or permanent interruption of therapy was not necessary due to esophageal toxicity. 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%) (1, 5, 32, 38, 40). These mild side effects could be easily managed but the grade 2 or higher dysphagia causes clinically relevant symptoms (55) 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 (38), but lower than in the Rodriguez study, in which patients with lung cancer were treated with 3D-CRT technique (1). 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 (1, 2, 5-8, 31-33, 36-40). 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≤). In corcordance 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 (1, 8, 32, 38, 39). Many researchers have found association between AET and mean or maximal dose to the esophagus. In the study of Qiao, during the concurrent platinum-based chemotherapy, mean and maximal dose (above 60 Gy) to the oesophagus were in connection with grade 3≤ esophageal toxicity (36). Singh has had similar results, and found that mean and maximal dose (higher than 58 Gy) associated with grade 3 or more severe AET (31). In the Ozgen paper the mean dose of esophagus ≥28 Gy correlated with grade 2 or worse toxicity (38). Other authors evaluated the correlation between AET and V_{dose}. It shows the percentage of esophagus receiving specific dose (V_{20Gy}, V_{30Gy}, $V_{40Gy,}$ etc.). In Takeda's study the incidence of grade 1, acute esophageal toxicity increased if more than 30% (V_{35Gy}>30%) of the esophageal volume received 35 Gy (40). In the Rodriguez paper 30% of esophageal volume receiving ≥50 Gy was the most statistically significant factor associated with AET grade 1≤ (1). Belderbos and Bradley have found correlation with grade 2 or worse dysphagia and $V100\%_{20\text{-}60Gy,}$ or $V_{5\text{-}70Gy}$, respectively (32, 39). In our results, the parameter that mostly correlated with grade 2≤ 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 ($LETT_{>40\text{-}50Gy}$) and acute esophageal adverse events were also detected in relation to grade 2≤ or 3≤ swallowing toxicity in the literature (5, 32). Elevated radiation dose and combining radiotherapy with chemotherapy in the hope of better survival may increase the incidence of esophagitis. Development of acute esophageal toxicity is the most important limiting factor in the radiotherapy 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 thrombocytosis and the 5-year survival of the patients after surgery. Thrombocytosis was significantly more frequent among smokers than non-smokers. The novelty of our study lies in the analysis of a high platelet 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 thrombocytosis 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 thrombocytosis (infection or neoplasia) in patients. Thrombocytosis 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 thrombocytosis in the study of Pedersen and Milman (20% in stage I and 30% in stage IIIA) (49). The study by Hamilton et al. presented comparable data, as thrombocytosis occurred in 26% of lung cancer cases (60). In our study, thrombocytosis 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 (49). 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 (42). Among the smokers participating in our study, the incidence of squamous cell lung cancer was also the most frequent, in addition, we discovered that thrombocytosis 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. (43), but Nakamura et al. presented smoking as being significantly predictive of a poor prognosis after resection of different stages of lung cancer (42). The impact of thrombocytosis on the survival was analyzed from different aspects. Using the current TNM lung cancer classification (41), 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 thrombocytosis 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. Thus the survival rates among the different stages in patients with thrombocytosis did not show a wide range. According to Mountain (41), the 5-year survival in pathological stage IA and IB cases was 67% and 57%, close to the 5-year survival for our patients overall (75 and 59%, respectively). The survival rate was significantly reduced in patients with preoperative thrombocytosis according to Pedersen and Hamilton (49, 60), and in the evaluated patients in the trial of Aoe et al. (50), 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 platelet 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 (49), and also of Aoe et al. (50) among patients treated with resection or conservative treatments for lung cancer. To sum up, thrombocytosis 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 thrombocytosis during the perioperative period compared to patients with normal platelet counts. Thrombocytosis 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 chemo-radiotherapy 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.

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Appendix

ANTICANCER RESEARCH *31*: 1431-1436 (2011)

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Please mark the appropriate section for this paper
☐ Experimental
☑ Clinical
☐ Epidemiological

Roles of BCL-2 and MDR1 Expression in the Efficacy of Paclitaxel-based Lung Cancer Chemoradiation

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Abstract. *Background: The associations between B-cell lymphoma 2 (BCL-2) and multi-drug resistance associated P-glycoprotein (MDR1) expressions and chemoradiotherapy outcome of patients with non-small cell lung cancer (NSCLC) were analysed. Patients and Methods: Thirty-two NSCLC patients were treated with paclitaxel-based chemoradiotherapy. The tumour expressions of BCL-2 and MDR1 were analysed by means of immunohistochemistry with regard to the clinical response and survival data. Results: Partial remission and stable disease were achieved in 19 (59%) and 10 (31%) cases, respectively. Significant differences in progression-free survival were observed between responders and non-responders (13.7 vs. 6.0 months, p=0.028), and between patients with or without a gross tumour volume (GTV) shrinkage ($GTV_{>50}$ 13.7 vs. 6.0 months, p=0.009). Overexpression of BCL-2 and of MDR1 was observed in 6 (21.4%) cases each. Overexpression of both markers together was associated with poor response (GTV reduction: p=0.005; RECIST: p=0.023) and lower progression-free survival (overexpression of both, low expression of both, mixed: 3.1, 13.4, 4.1 months, respectively, p<0.001). Conclusion: BCL-2 and MDR1 overexpression may predict the inefficacy of paclitaxel-based chemoradiotherapy.*

Lung cancer is the most frequent tumour worldwide. Molecular markers may be of prognostic value, especially in the early stages. The identification of specific genes that may be targeted by new therapies appears to be a potentially rewarding approach.

Individualized combinations of various treatment procedures for locally advanced lung cancer tend to improve local control and survival. Such favourable results may be achieved through the application of increasingly more sophisticated chemotherapeuticals and their combinations with radiotherapy (1, 2). Radiosensitization has been reported to increase therapy efficacy, but it may also increase therapy-induced toxicity (1, 3).

The most frequently applied third-generation chemotherapeuticals are the taxanes, which have been demonstrated in clinical trials to be widely effective in advanced non-small cell lung cancer (NSCLC) (1, 2). The prototype of the taxane family is paclitaxel, an excellent radiosensitizer (1, 2, 4-6). In designing the optimum individual treatment planning, including selection of an effective chemotherapeutical and the anticipation of potential radioresistance, the physician may be aided by predictive markers (7-20).

The multi-drug resistance associated P-glycoprotein (MDR1, P-gp170, ABCB1 available at the homepage of the *HUGO Gene Nomenclature Committee at the European Bioinformatics Institute*) is a well-known plasma membrane drug efflux pump that is associated with resistance to a wide range of anticancer drugs. Paclitaxel is a substrate of this transporter system. The overexpression of MDR1 may play an important role in the paclitaxel-resistance of lung cancer (21-23).

The oncogenic protein B-cell lymphoma 2 (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 (7-10), but poorer survival rates of patients with higher BCL-2 expression have also been reported (11-13).

The predictive role of BCL-2 has also been examined in various tumour types. The association between the response to platinum-based chemoradiotherapy and apoptosis-related proteins is unclear. No correlation was found in cancer of the bladder (14), the oesophagus (15) or the rectum (16). The overexpression of BCL-2 predicted a more favourable outcome in head and neck (17) and lung (18) cancer, but an unfavourable effect has been described for oropharyngeal (19) and lung cancer patients (11).

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Key Words: Lung cancer, paclitaxel-based chemoradiotherapy, BCL-2, MDR1, radiosensitivity.

The aim of this study was to analyse the associations between the expressions of BCL-2 and MDR1 with the clinical outcome of paclitaxel-based chemoradiotherapy in patients with NSCLC.

Patients and Methods

The study was conducted in full accordance with the institutional regulations and all the patients gave their written informed consent for participation in the chemotherapy and radiotherapy.

Study population. Patients receiving chemoradiotherapy for primary unresectable or 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. The staging procedures were based on the conventional protocol (chest computer tomography (CT), abdominal ultrasound/CT, brain CT, bone scan, bronchoscopy) and on induction chemotherapy. For each patient, the treatment plan was designed by a multidisciplinary team.

Systemic treatment and radiotherapy. During the radiotherapy, all the patients received concomitant taxane-based chemotherapy (weekly paclitaxel 100 mg/m^2 in 4-6 cycles, depending on toxicity). Of the 19 patients (stage IIIB) who completed induction chemotherapy (1 or 2 cycles), 17 (89.5%) received a taxane-based chemotherapy regimen (paclitaxel 175 mg/m^2 with carboplatin 400 mg/m^2 or docetaxel 75 mg/m^2 with cisplatin (CDDP) 75 mg/m^2, at 3-week intervals), while 2 patients received a gemcitabine-based regimen (gemcitabine 1250 mg/m^2 on days 1 and 8, CDDP 70 mg/m^2 on day 1, and then at 3-week intervals) for at least 4 weeks prior to the concomitant chemoradiotherapy. After completion of the chemoradiotherapy, additional consolidation chemotherapy was administered in 18 cases (paclitaxel 175 mg/m^2 with carboplatin 400 mg/m^2 or docetaxel 75 mg/m^2 with CDDP 75 mg/m^2, at 3-week intervals).

CT-based three-dimensional treatment planning and conformal radiotherapy were performed in all cases, with use of an individual immobilization system. 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 25×1.8 Gy; then, after a repeated CT scan, depending on the tumour response, radiotherapy of the reduced volume was continued at an average dose of 22-26 Gy (with the exception of neoadjuvant therapy).

Response analysis. During the treatment, following the administration of a 45 Gy dose, and 4-6 weeks after the completion of the chemoradiotherapy regimen, clinical and diagnostic CT examinations were performed. The CT scans were compared with the pretreatment scans provided for radiotherapy-planning purposes. Response analysis was carried out by means of two methods: the exact values of gross tumor volume (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 $GTV_{>50}$, GTV_{50-40} and $GTV_{<40}$, respectively), additionally, the CT scans were analysed according to the RECIST criteria system (24).

Immunohistochemistry. Before the chemoradiotherapy, immunohistochemical staining of the biopsy samples was performed to quantify the BCL-2 and MDR1 expressions. Histological samples from 28 out of the 32 evaluated patients were examined prospectively for BCL-2 and P-glycoprotein 170 (clone 494) expressions. (The histological samples from 4 patients were used for K-ras analysis and the remaining material was not sufficient for further immunohistochemical analysis.) Immunohistochemical studies were carried out on paraffin sections by an indirect peroxidase method. Sections were cut 4-μm-thick. Deparaffinizing, rehydrating and antigen retrieval were performed in a PraeTreatment module (Dako, Glostrup, Denmark) (20 min, 98 Co), using the 3in (pH 6.0) solution produced by LabVision (Fremont, CA, US). The endogenous peroxidase activity was blocked with hydrogen peroxide (3%, 10 min) and a solution of milk powder (in 1% phosphate-buffered saline, 10 min) was used as protein block. The Real-Envision (DAB) kit (Dako) was used as the labelling system. A semiquantitative scoring method was used to rate immunohistochemical staining: 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 and MDR1 expression at a level of 2-3+ was classified as high expression (Figure 1).

Statistical analysis. All analyses were carried out using SPSS version 15.0 for Windows (SPSS Inc., Chicago, IL, USA). The main outcome measures were the tumour response and the time to progression. The associations between the molecular marker expressions and clinical factors, tumour response, local relapse and presence of distant metastases within 6 months were evaluated with the chi-square test, while those with age were assessed with the one-way ANOVA test. In terms of both progression-free (PFS) and overall survival (OS), the outcome was analysed by Kaplan-Meier analysis (pairwise comparisons – Breslow test).

Results

Patient characteristics, response and survival. Thirty-two patients received paclitaxel-based chemoradiotherapy, 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 (±SD) of the patients was 58.9 (±6.2) years; 21 (66%) were men. Most of the patients had stage IIIB cancer (75%). Neoadjuvant treatment was administered to 5 patients with stage IIIA and 3 with stage IIB sulcus superior (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%).

After the chemoradiotherapy, surgical treatment was possible in 10 cases (31%) and 18 patients (56%) received consolidation chemotherapy. At the time of the last follow-up (median 17 months), 14 (44%) patients 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 IIIB. 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 three patients (10%) with progressive disease

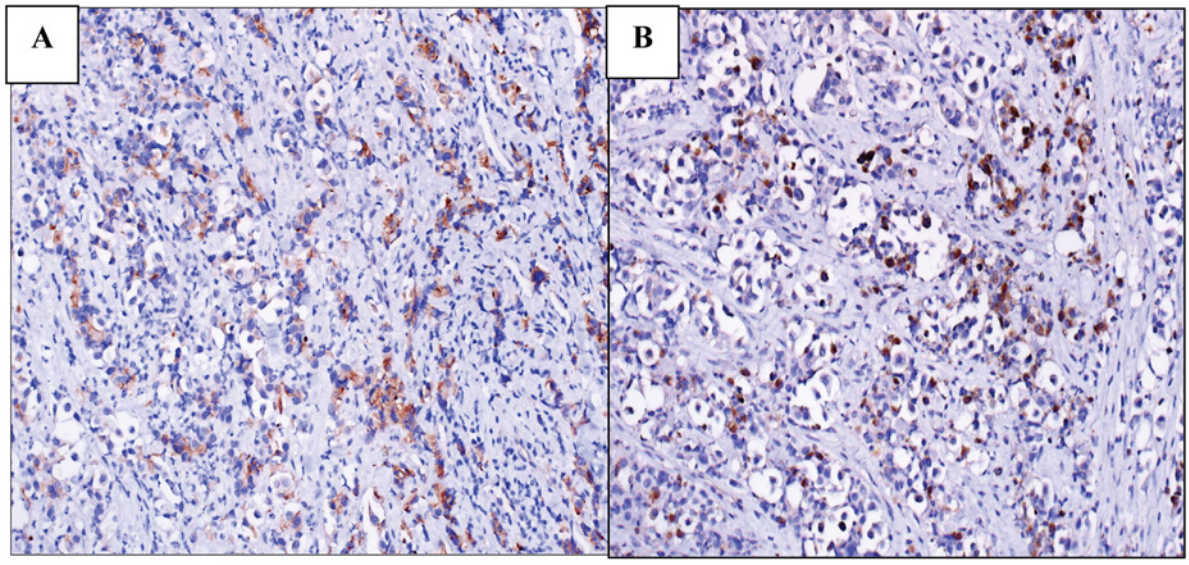

Figure 1. *Immunohistochemistry of biopsy specimens. A: Moderate cytoplasmic positivity for MDR1 (original magnification ×224); B: moderate nuclear and cytoplasmic positivity for BCL-2 (original magnification ×224).*

Table I. *Distribution of BCL-2 and multi-drug resistance associated P-glycoprotein (MDR1) expression.*

n=28	BCL-2			MDR1			BCL-2 and MDR1		
	Negative	1+	2+/3+	Negative	1+	2+/3+	Both low/neg	Mixed expression	Both 2+/3+
n	16	6	6	19	3	6	19	6	3
%	57.2	21.4	21.4	67.8	10.7	21.4	67.9	21.4	10.7

(PD; 2 locoregional and 1 distant metastasis) worsened during the treatment. There was a significant difference in the duration of 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 OS duration (29.1 *vs*. 15.7 months, p=0.256). Our analysis of the relationship between PFS and OS and the tumour volume shrinkage ($GTV_{>50}$, GTV_{50-40} and $GTV_{40>}$) indicated that the PFS results were more favourable in patients with than in those without tumour shrinkage ($GTV_{>50}$ 13.7 *vs*. 6.0 months, p=0.009; GTV_{50-40} 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; GTV_{50-40} 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 surgery, 5 (50%) exhibited pathologically complete remission and the other 5 a partial pathological response. The difference between the surgical and non-surgical populations was not significant from the point of view of their survival (29.1 *vs*. 22.1 months, p=0.119), but there was a more favourable trend in the outcome after surgery. The OS rate correlated significantly only with consolidation chemotherapy (survival with or without chemotherapy, 13.4 *vs*. 4.8 months, p<0.001), and the duration of PFS was also longer (29.4 *vs*. 11.3 months, p<0.001).

Association of expression of drug resistance- and apoptosis-related proteins with clinicopathological characteristics. There were 16 (57.2%) BCL-2-negative and 19 (67.8%) MDR1-negative cases. A low expression of BCL-2 and of MDR1 was observed in 6 (21.4%) and 3 (10.7%) cases, respectively. A high expression of BCL-2 and of MDR1 was observed in 6 (21.4%) cases each. The tumour in three (10.7%) patients displayed high expression of both markers (Table I). There were no significant correlations between a high expression of BCL-2 or MDR1 and other characteristics of the patients (age, gender, stage or histology).

Association of expression of drug resistance- and apoptosis-related proteins with response and outcome of patients. A high expression of both markers simultaneously was significantly associated with a poor response to paclitaxel-based chemoradiotherapy, when evaluated against either a GTV reduction during therapy (p=0.019), or a tumour response according to RECIST (p=0.005) (Table II). A high

Table II. *Association of molecular marker expression (BCL-2, MDR1) with clinical outcome.*

n=28	Response (RECIST)				Volume change (%)				PFS	
Expression	PR	SD	PD	*p*-value*	>50	50-40	<40	*p*-value*	Months (med±S.D.)	*p*-value**
BCL-2										
Neg/low	16	5	1	0.082	13	3	6	0.151	13.4±2.5	0.025
High	2	2	2		1	1	4		4.2±0.6	
MDR1										
Neg/low	17	4	1	0.016	11	4	4	0.001	13.4±2.2	<0.001
High	1	3	2		3	-	-		1.63±1.1	
Both neg/low	15	3	1	0.005	13	3	3	0.019	13.4±0.2	<0.001
Mixed	3	3	-		1	1	4		4.1±1.9	
Both high	0	1	2		0	0	3		3.1±1.4	

*Chi-square test; **Kaplan-Meier; PR, partial remission; SD, stable disease; PD, progressive disease; PFS, progression-free survival.

expression of BCL-2 or of MDR1 was significantly associated with a lower duration of PFS (BCL-2 high *vs.* low/negative, 4.2 *vs.* 13.4 months $p=0.025$; MDR1 high *vs.* low/negative, 1.63 *vs.* 13.4 months, respectively, $p<0.001$), whether evaluated separately or together (BCL-2 and MDR1 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 MDR1-positive tumours than in those with MDR1-negative ones (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 MDR1. Local recurrence within 6 months was more frequent in patients with overexpression of BCL-2 ($p=0.0023$) or of MDR1 ($p=0.007$).

Discussion

The present study demonstrated that overexpression of the evaluated anti-apoptotic and cell membrane proteins can help predict the efficacy of paclitaxel-based chemoradiotherapy. We observed a strong association between the concurrent overexpression of BCL-2 and MDR1, 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 radiotherapy in relation to the expressions of the above markers.

We mainly used paclitaxel with radiotherapy or paclitaxel in combination with carboplatin in the course of induction and consolidation therapy. Paclitaxel stabilizes microtubules, which blocks cell cycle progression in the most radiosensitive G_2-M phase, and also induces BCL-2 hyperphosphorylation, resulting in its inactivation, thereby facilitating apoptosis (25, 26). In our cohort, those patients demonstrating no response to paclitaxel-based chemoradiotherapy had a significantly more unfavourable PFS, and a worse (although 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 MDR1. Preclinical data demonstrated that the increased activity of the signalling pathway in paclitaxel-resistant cell lines was directly attributable to the overexpression of MDR1. It was reported that MDR1 may contribute to the multidrug resistance of lung cancer (21-23).

The prognostic value of BCL-2 positivity has been widely studied. Several papers have confirmed a more favourable disease progression in patients with BCL-2-positive tumours with either surgically resected (7) or locally advanced (10) NSCLC, or even irrespective of the stage (8, 9). However, some studies did not find a significant association between BCL-2 expression and survival (28, 29), or even reported a worse survival in the event of high BCL-2 expression (11-13).

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 (14-19). Jeong *et al.* treated NSCLC patients with cisplatin-based chemoradiotherapy and observed that a high expression of BCL-2 was significantly associated with a longer survival and a better response to the treatment (18). The findings of Fokkema *et al.* indicated a more favourable PFS of patients with an overexpression of BCL-2 following radiotherapy with or without carboplatin-based chemoradiotherapy, although they did not analyse the radiotherapy and chemoradiotherapy cohorts separately (10). Hwang *et al.* reported that BCL-2 expression predicted a poor outcome for radiation-treated NSCLC patients (11). 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

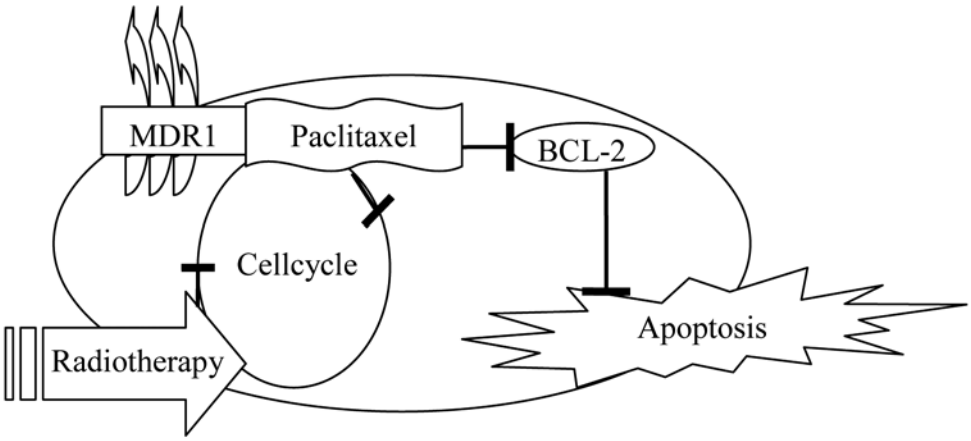

Figure 2. *The role of BCL-2 and MDR1 in paclitaxel-based chemoradioresistance. MDR1 expression indicates enhanced drug efflux activity. BCL-2, as an anti-apoptosis factor may inhibit radiotherapy-induced programmed cell death.*

chemoradiotherapy, and in the event of the overexpression of both MDR1 and BCL-2, the tumour response was significantly poorer.

BCL-2 inhibits programmed cell death, and can be associated with a more aggressive tumour cell phenotype, with conseqvent resistance to treatment protocols based on microtubule damage. The efficacy of radiotherapy-induced apoptosis, and therefore the whole of the treatment, may be reduced in tumours exhibiting an overexpression of BCL-2 (11-13). 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 MDR1 expression indicates an enhanced drug efflux activity, leaving the cell without an adequate amount of chemotherapeutical. A high BCL-2 expression, as an antiapoptotic mechanism, may inhibit the radiotherapy-induced programmed cell death (Figure 2). This hypothesis is supported by the finding that both markers were negative in tumours from patients demonstrating complete pathological remission.

The overexpression of MDR1 in our cohort was associated with a poorer tumour response. However, for patients with an overexpression of BCL-2, but not of MDR1, there was a slightly, although not significantly better therapeutic efficacy as compared with patients displaying an overexpression of both markers. We assume that paclitaxel terminated the inhibition of apoptosis by hyperphosphorylating and inactivating BCL-2, and thereby partially restoring radiosensitivity (25, 27) (Figure 2).

Our findings demonstrate that the concomitant application of paclitaxel and radiotherapy is potentially ineffective in the treatment of NSCLC, leading to a shorter PFS and more frequent local remission if the tumour indicates the overexpression of both MDR1 and BCL-2. The findings suggest that paclitaxel-based chemoradiotherapy is questionable in this group of patients, who may benefit more from the combination of other drugs with radiotherapy, which may be favourable even in the case of an overexpression of BCL-2 and since platinum derivatives are not substrates of the multidrug efflux pump (18, 30).

In conclusion, the present study has revealed that the overexpression of BCL-2 and MDR1 is of potential predictive value as regards the inefficacy of paclitaxel-based chemoradiotherapy in NSCLC.

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Acute Oesophageal Toxicity Related to Paclitaxel-based Concurrent Chemoradiotherapy for Non-small Cell Lung Cancer

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Abstract. *Background: Dosimetric data and acute oesophageal toxicity (AET) during chemoradiotherapy (CRT) were evaluated in patients with non-small cell lung cancer (NSCLC). Patients and Methods: Fifty patients were treated with paclitaxel-based conformal CRT with a mean±SD dose of 60.7±9.8 Gy. The oesophageal toxicity was prospectively registered and evaluated in relation to maximal dose (D_{max}), mean dose (D_{mean}), length and volume of oesophagus irradiated with 35-60 Gy ($V_{35\text{-}60Gy}$), and according to the seriousness of AET. Results: D_{max} and D_{mean} to the oesophagus were 57.0±10.8 Gy and 24.9±9.0 Gy, respectively. AET of grade 1, 2 and 3 developed in 16 (32%), 14 (28%) and three (6%) cases, respectively. D_{max}, the D_{mean}, the length and the $V_{35\text{-}60Gy}$ were all related to dysphagia ($p<0.001$). V_{45Gy} was the most reliable predictor of AET of grade 2 or more. Conclusion: Our results indicate that keeping oesophageal V_{45Gy} below 32.5% can prevent severe AET during CRT of NSCLC.*

Lung cancer is the most frequent tumour worldwide. Radiotherapy is one of the main treatment modalities of lung cancer and was the conventional method of treatment until the 1980s (1). Its efficacy alone in locally advanced non-small cell lung cancer is poor (2). Strategies designed to enhance local control include improved tumour targeting (three-dimensional treatment planning and increasingly more sophisticated radiotherapy techniques), escalation of thoracic radiotherapy dose (2-4), and application of different fractionations (5-8). Individualized combinations of various treatment procedures, such as combining radiotherapy with chemotherapy tend to improve local control and survival (9, 10). Radiosensitization has been reported to increase therapy efficacy, but it may also increase therapy-induced toxicity (6, 9, 11-14). The practice of advanced techniques should reduce acute and late treatment-associated toxicity (15, 16). Radiation oesophagitis seems to be one of the most common acute toxicities, especially in the setting of combined concurrent chemoradiation (CRT) (1, 2, 17, 18). This adverse treatment side-effect is often a dose-limiting factor (4), which influences the treatment outcomes and patient quality of life, therefore its dose volume relationship has been investigated in several trials (1, 2, 5-8, 12-14, 17-21). Results have differed considerably across different institutions regarding which dosimetric factors are more critical than others. Jim Rose and colleagues performed a systematic literature review of published studies addressing radiation oesophagitis after thoracic radiotherapy in 2009 (18). Statistically significant relationships between specific dose volume parameters [V_{20Gy}, $V_{35Gy,}$ V_{60Gy}, maximal and mean oesophageal dose] with or without chemotherapy and clinically significant acute oesophagitis risk were identified based on the analysed studies. They found various dosimetric correlations in the literature on oesophageal toxicity regarding the seriousness of the swallowing complaints. Identification of the risk factors for acute oesophagitis in lung cancer is important for optimizing the most effective and favourably tolerated treatment plan. Our aim was to investigate prospectively the dosimetric correlations of acute oesophageal toxicity (AET) during neoadjuvant and definitive paclitaxel-based three-dimensional conformal CRT in patients with non-small cell lung cancer.

Patients and Methods

The study was conducted in full accordance with the institutional regulations and all the patients gave their written informed consent to participate in the chemotherapy and radiotherapy.

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Key Words: Oesophageal toxicity, lung cancer, paclitaxel-based chemoradiotherapy.

Table I. *Common Terminology Criteria for Adverse Events, Version 3.0 Dysphagia: (diff*iculty of swallowing).

Grade 1	Symptomatic, able to eat regular diet
Grade 2	Symptomatic and altered eating/swallowing (*e.g.* altered dietary habits, oral supplements); *i.v.* fluids indicated <24 h
Grade 3	Symptomatic and severely altered eating/swallowing (*e.g.*, inadequate oral caloric or fluid intake); *i.v.* fluids, tube feedings, or TPN indicated ≥24 h
Grade 4	Life-threatening consequences (*e.g.* obstruction, perforation)
Grade 5	Death

TPN: total parenteral nutrition.

Study population. Patients receiving CRT for primary unresectable or potentially operable non-small cell lung cancer at the Department of Oncotherapy between December 2006 and June 2011 were eligible for participation in this study. Histological examination was performed before the therapy in all cases. Staging examinations were based on conventional protocols [chest computed tomography (CT), abdominal ultrasound/CT, brain CT, bone scan, bronchoscopy]. For each patient the multimodal treatment strategy was designed by a multidisciplinary team.

Chemo- and radiotherapy, supportive therapy. During the radiotherapy all the patients received concomitant taxane-based chemotherapy (weekly paclitaxel 100 mg/m^2 in 4-6 cycles, depending on toxicity). Of the 40 patients (stage IIIB) who completed induction chemotherapy (one or two cycles), 38 (95%) received a taxane-based chemotherapy regimen (mainly paclitaxel 175 mg/m^2, carboplatin 400 mg/m^2 or docetaxel 75 mg/m^2, cisplatin 75 mg/m^2, at 3-week intervals), while two patients received a gemcitabine-based regimen (gemcitabine 1250 mg/m^2 on days 1 and 8, cisplatin 70 mg/m^2 on day 1, and then at 3-week intervals) for at least four weeks prior to the concomitant CRT. All patients were irradiated in the supine position, with both arms elevated above the head, on the thorax set of the AIO SolutionTM (ORFIT, Antwerpen Belgium). CT-based three-dimensional treatment planning and conformal radiotherapy were performed in all cases, with use of an individual immobilization system with thermoplastic masks. The gross tumor volume (GTV), macroscopic lung cancer, the involved mediastinal and hilar lymph nodes was defined on [^{18}F]fluoro-2-deoxy-d-glucose positron emission tomography-CT images. The delineation of organs at risk (spinal cord, ipsilateral and contralateral lung, heart and oesophagus) 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 25×1.8 Gy (and the total dose for neoadjuvant cases); after a repeated CT scan, depending on the tumour response, radiotherapy of the reduced volume was then 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 consumption of hot and spicy food, chopped food was recommended in order to prevent AET. 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.

Evaluation of AET. The whole oesophagus was contoured from the anular cartilage to the 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 oesophagus with 50 Gy (L_{50Gy}) and the volume of the oesophagus irradiated with 35 Gy to 60 Gy ($V_{35\text{-}60\ Gy}$). AET as dysphagia was evaluated prospectively based on Common Terminology Criteria for Adverse Events, version 3.0 issued by the National Cancer Institute (Table I) (22). 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 defined as smokers.

Table II. *Radiation dose (Gy) to critical organs (except the oesophagus)*.

Spinal cord	Mean±SD	12.1±4.5
	Maximal±SD	36±6.7
Ipsilateral lung	Mean±SD	26.6±7.8
	V_{20Gy}±SD (%)	54.1±13.5
Contralateral lung	Mean±SD	10.6±3.6
	V_{20Gy}±SD (%)	14.3±9.1
Heart	Mean±SD	12.5±4.6
	V_{30Gy}±SD (%)	12.1±3.8

Statistical analysis. All statistical analyses were carried out using SPSS version 20.0 for Windows (SPSS Inc., Chicago, IL, USA). The relations between AET, age, gender, smoking habits and the dosimetric data were evaluated. Age, dose and volume were assessed with *t*-test, gender and smoking habits were analysed with chi-square test. The 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}.

Results

Patient characteristics. Altogether, data for 50 patients were analysed. Thirty-two (64%) patients were men, 18 (36%) were women. The mean±SD age was 59.8±8 (range=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 six (12%) patients had stage III/A carcinoma. Forty (80%) participants had stage III/B carcinoma. These stages were determined according to the sixth edition of the TNM system. Twenty-nine (58%) patients were smokers and 21 (42%) were non-smokers. Twelve (24%) patients underwent operation, in one case, despite remission only exploration was performed due to inoperable conditions.

Table III. *Incidence of acute oesophageal toxicity (AET).*

Severity of AET n=50	Grade 0	Grade 1	Grade 2	Grade 3	Grade 4-5
Patients on (%)	17 (34%)	16 (32%)	14 (28%)	3 (6%)	0

Dose parameters. The mean dose to the planning target volume was 60.7±9.8 Gy for the whole investigated population, while it was 64.7±5.5 Gy in the definitively treated patients with irresectable disease. The preoperative dose given was 45.0 Gy in all 10 cases. Irradiation doses to spinal cord, heart, ipsilateral and contralateral lung are shown in table II. The D_{max} to the oesophagus was 57±10.8 Gy, and the D_{mean} was 24.9±9 Gy. The mean L_{50Gy} was 6.99±6.7 cm.

Toxicity. Among the 50 participants, oesophageal toxicity 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 (Table III). Grade 3 toxicity occurred in three (6%) cases. Life-threatening, grade 4 or 5 AETs were not seen. Temporary interruption due to vomiting, fever, neutropenia and acute oesophagitis 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 oesophageal toxicity in 12 (24%) cases. Complaints were treated with local anaesthetics in all cases with dysphagia. Use of drinkable nutrients was also indicated for all patients, while tramadol treatment was needed in eight (16%) cases. No association was found between oesophageal toxicity and gender (p=0.584), age (p=0.271) or smoking habit (p=0.196) of the patients.

Correlations of the dose- and volume data with AET. The maximum and mean dose to the oesophagus correlated well with moderate and severe swallowing toxicity. The D_{max} to the esophagus in cases of grade 0-1 and grade 2-3 toxicity was 56±11.45 and 64.07±5.55 Gy, respectively (p<0.001). The average D_{mean} for the cases with AET of grade 0-1 and grade 2-3 was 21.87±8.24 and 30.98±7.57 Gy, respectively (p<0.001). The average mean dose to the oesophagus among the three patients with grade 3 AET was 34.46±5.58 Gy. The L_{50Gy} was also related to the symptoms (p<0.001). In cases of grade 0-1 and grade 2-3 AET, the L50Gy was 5.10±5.66 and 10.54±3.83 cm, respectively (p<0.001). $D_{30\%}$ ±SD was 39.82±14.17 and 53.74±7.21 Gy in grade 0-1 and grade 2-3 esophagitis, respectively (p<0.001). The $V_{35\text{-}60Gy}$ in relation to toxicity is shown in Table IV. Examining the relationship between oesophageal toxicity and dose volume parameters with logistic regression, we found that $V4_{5Gy}$ predicts most reliably the development of grade 2 or higher AET (odds ratio=1.089, 95%confidence interval: 1.033-1.148, p=0.001). A one percent increase of V_{45Gy} elevates the risk of grade 2 or higher AET by 8.9%. The risk of the development of AET of grade 2-3 was the highest above a cut-off value for V_{45Gy}≥32.5% according to ROC analysis.

Table IV. *Dosimetric parameters of acute esophageal toxicity. Data are means ±SD.*

	Grade 0-1	Grade 2-3	p-Value (t-test)
n	33 (66%)	17 (34%)	
D_{max} (Gy)	54.56±11.45	64.07±5.55	<0.001
D_{mean} (Gy)	21.87±8.24	30.98±7.57	<0.001
V_{35Gy} (%)	34.87±17.71	49.23±11.55	0.004
V_{40Gy} (%)	30.93±17.67	46.41±12.04	0.002
V_{45Gy} (%)	20.15±18.71	43.23±12.20	<0.001
V_{50Gy} (%)	15.90±18.06	37.88±11.45	<0.001
V_{55Gy} (%)	13±15.85	29.29±16.36	0.001
V_{60Gy} (%)	8.03±12.31	19.23±16.22	0.009
Length $_{50Gy}$ (cm)	5.10±5.66	10.54±3.83	<0.001

Discussion

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 radiotherapy with chemotherapy is directed to improve local control and survival of patients with lung cancer patients (9, 10). Several studies have shown that compared to radiotherapy alone, concurrent CRT appears to lower oesophageal radiation tolerance (21). AET is often a dose-limiting factor that influences the treatment efficacy (4). In our study, dose reduction or permanent interruption of therapy were not necessary due to oesophageal toxicity. No association was found between oesophageal toxicity and gender, age or smoking habit 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%) (1, 5, 13, 19, 21). These mild side-effects were easily managed but grade 2 or higher dysphagia causes clinically relevant symptoms (22) and remarkably influences the patient's quality of life. The incidence of grade 2 or more severe oesophagitis was slightly higher in our cohort than in Ozgen

et al.'s trial (19), but lower than that in the study of Rodriguez *et al*. (1), in which patients with lung cancer were treated with 3D-CRT technique. Definitive differences were 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 AETs. The incidence of AET and its dose volume relationship has been investigated in several trials (1, 2, 5-8, 12-14, 17-21). Although dose volume parameters are commonly used to analyse the risk of acute oesophagitis, there are large differences in the results, and in which of these parameters have the most dominant effect on the risk of AET due to the different approaches for 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 or more). In corcordance with numerous other studies, grade 2 or higher AET strongly correlated with the mean and the maximal dose, and the length and volume of the irradiated oesophagus (1, 8, 13, 19, 20). Many researchers have found association between AET and mean or maximal dose to the oesophagus. In the study of Qiao *et al*., during concurrent platinum-based chemotherapy, mean and maximal dose (above 60 Gy) to the oesophagus were related to grade 3 or more oesophageal toxicity (17). Singh *et al*. had similar results, and found the mean and maximal dose (higher than 58 Gy) to be associated with grade 3 or more severe AET (12). In the study of Ozgen *et al*., the mean dose to the oesophagus of 28 Gy or more correlated with grade 2 or worse toxicity (19). Other authors evaluated the correlation between AET and V_{dose}, which describes the percentage of the oesophagus receiving specific dose (V_{20Gy}, V_{30Gy}, V_{40Gy}, *etc*.). In Takeda *et al*.'s study, the incidence of grade 1 AET increased if more than 30% (V_{35Gy}>30%) of the oesophageal volume received 35 Gy (21). By Rodriguez *et al*., V_{50Gy}>30% was the most statistically significant factor associated with AET of grade 1 or more (1). Belderbos and Bradley found correlation between grade 2 or worse dysphagia and $V100\%_{20\text{-}60Gy}$, or $V_{5\text{-}70Gy}$, respectively (13, 20). From our results, the parameter that mostly correlated with grade 2 or more swallowing toxicity was a mean dose of 45 Gy to the oesophagus with 32.5% as cut-off value. A one percent increase elevated the risk of swallowing toxicity by 8.9%. L50Gy was also related to symptoms. Association between length of irradiated oesophagus with 40-50 Gy or more and AET were also detected in relation to grade 2 or 3 or more swallowing toxicity in the literature (5, 13). Elevated radiation dose and combining radiotherapy with chemotherapy in the hope of better survival may increase the incidence of oesophagitis. Development of AET is the most important limiting factor in the radiotherapy of chest tumours, therefore during treatment planning, a significant aim is to reduce the oesophageal volume irradiated and dose to protect patients from serious events. Our results indicate that keeping oesophageal V_{45Gy} lower than 32.5% during paclitaxel-based CRT for non-small cell lung carcinoma helps to avoid moderate and severe swallowing toxicity in patients.

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Thrombocytosis Has a Negative Prognostic Value in Lung Cancer

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Abstract. *Background: Solid tumours have worse prognosis when associated with thrombocytosis. Our study assessed the prognostic value of thrombocytosis, and its relation with smoking habits in lung cancer. Patients and Methods: A total of 398 patients were operated on then divided into two groups, those with normal platelet counts (n=312), and those with thrombocytosis (n=86); 348 out of 398 patients had data for smoking habits (99 non-smokers; 249 smokers). Results: The frequency of thrombocytosis was 18.6%, 19.3%, 27.5 and 28.6% in patients with tumor stages I to IV, respectively. Thrombocytosis appeared most frequently in patients with squamous cell lung cancer, and among smokers. The overall 5-year survival was worse in patients with thrombocytosis (p<0.001). By uni- and multivariate analyses, platelet count, and T and N status were found to be independent prognostic factors. Conclusion: Our study indicates that the presence of perioperative thrombocytosis in patients undergoing surgery should be considered as an independent prognostic factor of poor survival, and should be taken into account in regard to therapy.*

Survival after lung cancer resection is mainly dependent on the tumor stage (1), but other factors are also known to influence it. In resected stage I lung cancer cases, the tumor size, smoking index, and number of dissected mediastinal lymph nodes are all prognostic factors for overall and disease-free survival (2). Poor tumor differentiation is a risk factor for recurrence and carries an unfavorable prognosis (3). The impact of smoking on survival is widely discussed. Smoking was significantly predictive of a poor prognosis after resection of different stages of lung cancer (4) and in stage I adenocarcinoma (5), but there was no difference in terms of survival between smokers and non-smokers with advanced non-small cell lung cancer (6). Thrombocytosis has been proven to have a fundamental impact on survival in advanced cervical (7) and renal cell cancer (8). Unfavorable outcome in association with thrombocytosis has been described in patients with esophageal (9), gastric (10) and soft tissue cancer (11). There are only a few articles discussing the impact of thrombocytosis on lung cancer survival, or in resected cases. Previous studies have reported mainly unfavorable (12, 14, 16-18) or no impact (19) on survival of a high thrombocyte count among patients with non-small cell lung cancer. In several studies, thrombocytosis was detected as an independent prognostic factor (14, 16, 18). In this retrospective study, we evaluated the incidence and potential impact of thrombocytosis on outcome, also analyzing the smoking habits of patients who underwent lung cancer resection.

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Key Words: Lung cancer, prognosis, thrombocytosis, smoking.

Patients and Methods

The study was conducted in full accordance with the institutional regulations and all the patients gave their written informed consent prior to participation in the surgery.

Study population. 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. For each patient, the treatment plan was designed by a multidisciplinary onco-team.

Surgical, histological and staging procedures. 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 (1). Patient files were reviewed, and relevant data were collected.

Definition of thrombocytosis and smoking habits. The platelet counts were assessed three times during the perioperative period: just before surgery, and on the first and seventh postoperative days. If

Table I. *Distribution of thrombocytosis and smoking habits according to the pathological stage in the entire population.*

Stage	All patients n=398	All patients, N=398		Patients with known smoking history, N=348	
		PLT <400×10^3/μl n=312	PLT >400×10^3/μl n=86	Non-smokers n=99	Smokers n=249
IA	18.6%	20%	14%	21%	16%
IB	28.6%	29.2%	26.7%	31%	28.9%
IIA	1.8%	1.9%	1.2%	1%	2%
IIB	20.4%	20.8%	18.6%	18%	19.7%
IIIA	19.8%	17.9%	26.7%	15%	22.1%
IIIB	7.3%	7.1%	8.1%	7%	8.4%
IV	3.5%	3.2%	4.7%	6%	2.8%

PLT, Platelet count.

all three samples were evaluated to be higher than 400×10^3/μl, in agreement with other studies (7, 10, 12, 14, 15), thrombocytosis was diagnosed. Based on this data, the 398 patients were divided into two groups as to whether they had normal platelet counts or thrombocytosis 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.

Statistical analysis. All analyses were carried out using SPSS version 20.0 for Windows (SPSS Inc., Chicago, IL, USA). The associations between thrombocytosis and clinical factors (stage, histology, gender) were evaluated with the chi-square test, and the correlation between thrombocytosis and age were tested with independent samples *t*-test. Overall survival was analyzed by Kaplan-Meier analysis. 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.

Results

Patient characteristics. 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 (±SD) of 58.3 (±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.

Association of thrombocytosis and smoking habits with clinicopathological characteristics. Of the 398 patients operated on for lung cancer, 86 (21.6%) were determined to have thrombocytosis. The incidence of thrombocytosis gradually elevated according to increesing cancer stage. In stage I, 18.6% of cases had thrombocytosis, 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. The distribution of thrombocytosis and smoking habit according to the pathological stage of all resected lung carcinomas is described in Table I; no significant differences were found. Thrombocytosis was significantly more frequent in smokers (26.1%) than in non-smokers (10.1%) (p=0.001) (Table II). 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 thrombocytosis was also higher in the squamous cell subgroup, in which 94.9% of patients with thrombocytosis were smokers. The data for smoking habits and thrombocytosis in the squamous cell and other histological subtypes are detailed in Table III.

Table II. *The association between thrombocytosis and smoking habit (p=0.001).*

	Non-smokers n=99	Smokers n=249
Normal platelet count (<400×10^3/μl)	89 (89.9%)	184 (73.9%)
Thrombocytosis (>400×10^3/μl)	10 (10.1%)	65 (26.1%)

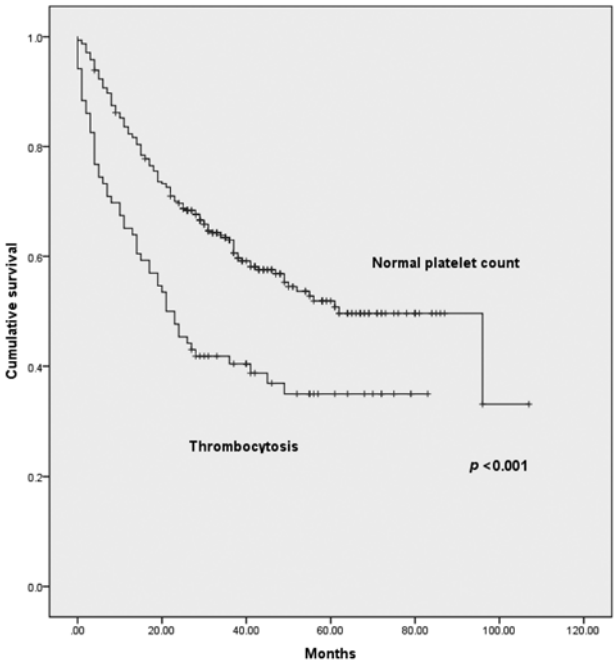
Association of thrombocytosis and smoking habit with outcome of patients. The median follow-up time of the entire

Table III. *The distribution of thrombocytosis and smoking habit according to squamous cell and other histological subtypes.*

	All patients, N=398		Patients with known smoking history, N=348	
	PLT <400×10^3/μl n=312	PLT >400×10^3/μl n=86	Non-smokers n=99	Smokers n=249
Squamous cell lung cancer	130 (74.3%)	45 (25.7%)	35 (21.5%)	128 (78.5%)
Other histology	182 (81.6%)	41 (18.4%)	64 (34.6%)	121 (65.4%)
p-Value (Chi-square test)	0.001		0.007	

PLT, Platelet count.

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 thrombocytosis, and 50.8% among patients with a normal thrombocyte count ($p<0.001$). The overall survival time was 63.1 months in the group without and 38 months in the group with thrombocytosis ($p<0.001$) (Figure 1). 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 (thrombocytosis *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 normal platelet counts ($p<0.001$), but there was no difference among the patients with thrombocytosis ($p=0.13$), presumably because of the relatively small number of cases (Table IV). 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 thrombocytosis ($p<0.001$) were significantly associated with a decreased survival. By multivariate analysis, the presence of thrombocytosis, greater tumor size and lymph node involvement were all independent factors related to poorer survival (Table V).



Patients at risk.

	0 year	1 year	2 years	3 years	4 years	5 years
G I	312	258	214	139	75	47
G II	86	56	40	29	19	12

Group I: Patients with normal platelet count, Group II: patients with thrombocytosis.

Figure 1. *Kaplan-Meier survival curves for patients with lung cancer patients according to platelet count.*

Discussion

The present study demonstrated that an 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 thrombocytosis and the 5-year survival of the patients after surgery. Thrombocytosis was significantly more frequent among smokers than non-smokers. The novelty of our study lies in the analysis of a high platelet 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 thrombocytosis 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 thrombocytosis (infection or neoplasia) in patients. Thrombocytosis has a prevalence as high as 30% in patients with lung cancer, and has been associated with extensive and/or metastatic disease

Table IV. *The association between 5-year survival, platelet count and stage.*

Stage	PLT <400×10^3/μl n=312	PLT >400×10^3/μl n=86	All
IA, n=78	81%	44%	75%
IB, n=110	62%	48%	59%
IIA, n=7	50%	0%	43%
IIB, n=81	46%	31%	43%
IIIA, n=80	25%	26%	25%
IIIB, n=28	15%	28%	20%
IV, n=14	44%	25%	34%
p-Value	<0.001	0.130	<0.001

PLT, Platelet count.

Table V. *Multivariate analysis of survival.*

	p-Value	HR	95% CI	
			Lower	Upper
Thrombocytosis	0.006	1.576	1.141	2.176
T status	0.001	1.341	1.129	1.594
N status	<0.001	1.726	1.474	2.020

CI: Confidence interval; HR: Hazard ratio.

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 thrombocytosis in the study of Pedersen and Milman (20% in stage I and 30% in stage IIIA) (12). The study by Hamilton *et al.* presented comparable data, as thrombocytosis occurred in 26% of lung cancer cases (13). In our study, thrombocytosis 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 (12).

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 (4). Among the smokers participating in our study, the incidence of squamous cell lung cancer was also the most frequent, in addition, we discovered that thrombocytosis 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.* (6), but Nakamura *et al.* presented smoking as being significantly predictive of a poor prognosis after resection of different stages of lung cancer (4). The impact of thrombocytosis on the survival was analyzed from different aspects. Using the current TNM lung cancer classification (1), 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 thrombocytosis 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. Thus the survival rates among the different stages in patients with thrombocytosis did not show a wide range. According to Mountain (1), the 5-year survival in pathological stage IA and IB cases was 67% and 57%, close to the 5-year survival for our patients overall (75 and 59%, respectively). The survival rate was significantly reduced in patients with preoperative thrombocytosis according to Pedersen and Hamilton (12), and in the evaluated patients in the trial of Aoe *et al.* (14), 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 platelet 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 (12), and also of Aoe *et al.* (14) among patients treated with resection or conservative treatments for lung cancer. To sum up, thrombocytosis 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 thrombocytosis during the perioperative period compared to patients with normal platelet counts. Thrombocytosis was evidently more frequent in smokers. In conclusion, the present study has revealed that 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.

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