Clinical Aspects of Inflammation in Non-small Cell Lung Cancer

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Mit dem Wissen wächst der Zweifel.

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Abstract

Lung cancer is the most common cause of cancer death worldwide, with about 1.2 million deaths every year. In Sweden, about 3500 new cases are diagnosed every year. The majority of patients presents with advanced non-small cell lung cancer (NSCLC) and is treated with palliative intent. Standard treatment in these patients in performance status 0-2 is combination chemotherapy. Radiotherapy may be added for palliative purposes. Median survival time with such treatment is 6-10 months. New treatment strategies are urgently needed. There is growing evidence for a link between cancer and inflammation and consequently, inflammation may be a possible target for the treatment of lung cancer.

The aim of this thesis was to study clinical aspects of inflammation in non-small cell lung cancer. A central issue was to adapt the projects as close to clinical routine as possible.

In a retrospective study of 289 patients (paper I), we investigated the prognostic value of C-reactive protein (CRP), a nonspecific marker of systemic inflammation, and smoking in patients with advanced NSCLC treated with palliative first-line chemotherapy. We found that patients with elevated CRP values (≥10 mg/ml) and current smokers at onset of treatment had inferior survival compared to patients with normal CRP values and patients who were not smoking. CRP and smoking status were independent prognostic factors and provided additional information to established prognostic factors such as stage of disease and performance status.

The expression of COX-2, an important enzyme involved in inflammation, was prospectively analysed in 53 patients with cytologically diagnosed lung cancer (paper II). The study showed that the analysis of COX-2 expression in cytological material is technically easy to perform with routine diagnostic methods and results in good quality slides. There was great variation in the proportion of COX-2 positive cells between the patients as well as in the intensity of staining between individual cells in many single cases.

The major project (paper III) of this thesis was the CYCLUS study, an academic, randomised, double-blind, phase III trial. The scientific question was if addition of the COX-2 inhibitor celecoxib to first-line palliative chemotherapy would prolong survival in patients with advanced NSCLC. 316 patients were included at 13 centres in Sweden. There was no survival difference between the treatment arms. Celecoxib appeared to have more favourable effect on survival in women than in men, but the differences were not significant. Small but not statistically significant differences in global quality of life and pain were seen favouring the celecoxib group. No increased incidence of cardiovascular events was observed in the celecoxib group.

Populärvetenskaplig sammanfattning på svenska

Lungcancer är den största orsaken till cancerdöd globalt och prognosen är sämre än för någon annan vanlig cancertyp. Ca 1,2 miljoner människor dör i lungcancer över hela världen varje år. Behovet av att utveckla behandlingen vid lungcancer är därför mycket stort. I Sverige insjuknar ca 3500 människor årligen och av dessa drabbas ca 1800 av så kallad avancerad icke småcellig lungcancer. Om allmäntillståndet är gott kan behandling med kemoterapi för flertalet patienter i den sistnämnda gruppen lindra symptom och förlänga överlevnad, men effekten är begränsad och övergående. De flesta patienter kan inte botas. Utvecklingen av kemoterapi har nått en platå och det krävs nya metoder för att förbättra behandlingen.

Det finns många forskningsstudier som tyder på att inflammation spelar en viktig roll vid cancersjukdom. I experimentella studier har man observerat att antiinflammatoriska läkemedel kan bromsa tumörtillväxt och öka tumörcellsdöd. Även små kliniska studier tyder på att en kombination av antiiflammatoriska läkemedel och kemoterapi kan ha bättre effekt än enbart kemterapi. Syftet med denna avhandling var att studera olika kliniska aspekter av inflammation vid icke småcellig lungcancer. Det var av stor vikt att planera och genomföra projekten så nära klinisk rutinsjukvård som möjligt.

C-reaktivt protein (CRP) är ett blodprov som används mycket inom rutinsjukvården för att påvisa inflammation. Rökning framkallar systemisk inflammation i kroppen. I den första studien (delarbete I) undersökte vi om CRP och rökstatus vid start av cytostatikabehandling har betydelse hos patienter med avancerad icke småcellig lungcancer. Vi fann att patienter med ett högt CRP-värde och patienter som rökte vid behandlingsstart hade sämre överlevnad jämfört med patienter som inte rökte och som hade normalt CRP-värde.

Cyklooxygenas-2 (COX-2) är ett enzym som har en viktig roll vid inflammation. Hämning av detta enzym med antiinflammatoriska läkemedel vid cancersjukdom är föremål för forskning. Det finns studier som tyder på att förekomst av COX-2 skulle kunna förutsäga om sådan behandling har effekt. Dock har man hittills enbart undersökt COX-2 i vävnadsprover och inte i cellprover, vilket är en vanlig metod för diagnos av lungcancer i Sverige. I delarbete II har vi visat att analys av COX-2 i cellprover med god kvalitet är fullt möjlig med metoder som används för rutindiagnostik vid lungcancer.

CYCLUS-studien (delarbete III) har varit huvudprojektet i denna avhandling. Det är en nationell klinisk studie som har undersökt om tillägg av ett antiinflammatoriskt läkemedel (celecoxib) till den behandlingen som ges idag, kemoterapi, kan förbättra överlevnaden hos patienter med avancerad icke småcellig lungcancer. Studien har koordinerats från Universitetssjukhuset i Linköping. Tretton sjukhus i Sverige har deltagit och sammanlagt blev 316 patienter inkluderade i studien. Median överlevnadstid i studien var 8,5 månader och det fanns ingen skillnad mellan patienterna som fick celecoxib och dem som fick placebo. Vad gäller livskvalitet fann vi att patienter som fick celecoxib upplevde bättre global livskvalitet och hade mindre smärta . Dessa skillnader var dock inte statistiskt signifikanta.

Preface

Lung cancer is a common disease with poor prognosis. There is a need to improve treatment outcomes. Already in the nineteenth century, Rudolf Virchow noted leucocytes in neoplastic tissues and suggested a connection between inflammation and cancer.

The overall aim of this thesis was to study various aspects of inflammation in non-small cell lung cancer. Research questions were posed and studied from the perspective of the clinician and – as far as possible – close to clinical routine.

The major project of this thesis was the performance of the CYCLUS* study, an academic, multicenter, randomised, double-blind, phase III trial to study the effect of an anti-inflammatory drug in patients with advanced non-small cell lung cancer.

*CYCLUS: Acronym for **CY**clooxygenase inhibitor, **C**hemotherapy, **LU**ng cancer, **S**urvival

List of publications

I. **Koch A,** Fohlin H, Sörenson S.
   Prognostic significance of C-reactive protein and smoking in patients with advanced non-small cell lung cancer treated with first-line palliative chemotherapy.
   J Thorac Oncol 2009, Mar; 4(3):326-32

II. **Koch A,** Gustafsson B, Fohlin H, Sörenson S.
   Cyclooxygenase-2 expression in lung cancer cells evaluated by immunocytochemistry.
   Diagn Cytopathol 2011, Mar; 39(3):188-193. doi: 10.1002/dc.21366

III. **Koch A,** Bergman B, Holmberg E, Sederholm C, Ek L, Kosieradzki J, Lamberg K, Thaning L, Ydreborg SO, Sörenson S.
   Effect of Celecoxib on Survival in Patients with Advanced Non-Small Cell Lung Cancer: a double blind randomised clinical phase III trial (CYCLUS-study) by the Swedish Lung Cancer Study Group.
   Eur J Cancer. In press.

CONTENTS

Abbreviations

A.D.	Anno Domini
ASA	Acetylsalicylic acid
ASCO	American Society of Clinical Oncology
B.C.	Before Christ
BSC	Best supportive care
CI	Confidence interval
COX	Cyclooxygenase
CRP	C-reactive protein
CTCAE	Common Terminology Criteria for Adverse Events
CVD	Cardiovascular disease
CYCLUS	Acronym for **CY**clooxygenase inhibitor, **C**hemotherapy, **LU**ng cancer, **S**urvival
EGFR	Epithelial growth factor receptor
EORTC	European Organisation for Research and Treatment of Cancer
EUCTD	European Union Clinical Trials Directive (2001/20)EC)
HR	Hazard Ratio
IHC	Immunohistochemistry
IL	Interleukin
NSAID	Non-steroidal anti-inflammatory drug
NSCLC	Non-small cell lung cancer
OS	Overall survival
PFS	Progression-free survival
PG	Prostaglandin
PS	Performance status
QoL	Quality of Life
SCLC	Small cell lung cancer
TNF	Tumour-necrosis factor
VEGF	Vascular endothelial growth factor
WHO	World Health Organization

1. Background

Lung cancer – an overview

Epidemiology

Lung cancer has been the most common cancer in the world since 1985 (1). In 2002, there were 1.35 million new cases reported. It was also the most common cause of cancer-related death with 1.18 million deaths. Almost 50% of all cases occur in the developing countries (2). In Europe, lung cancer is the third most common form of cancer with an estimated 391 000 new cases in 2008, following colorectal cancer and breast cancer (*Figure 1*). Lung cancer is the leading cause of death from cancer with about 342 000 deaths (19.9% of all cancer-related deaths) (3).

Figure 1*: Cancer in Europe 2008, new cases and deaths*
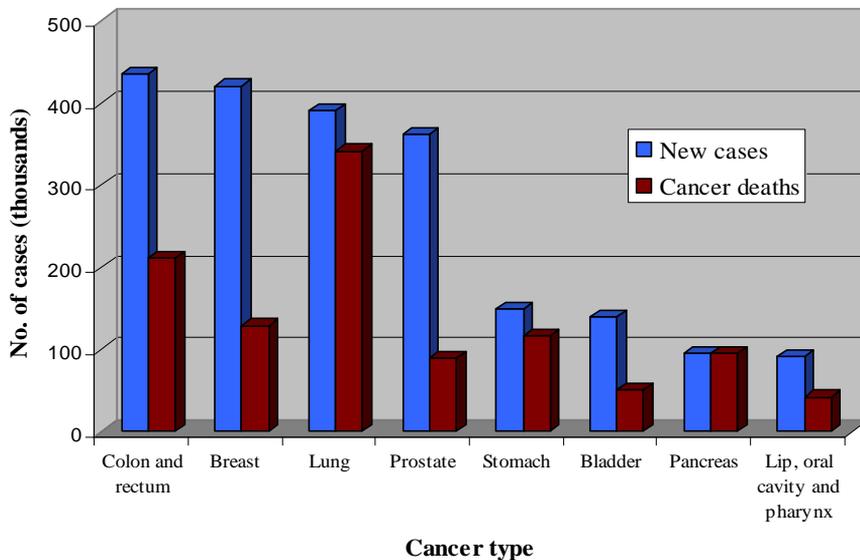
In Sweden, about 3500 new cases are diagnosed every year. Lung cancer is now as common in women as in men of all cases, with 1792 vs. 1787 cases, respectively (4). The number of cases in women has increased, while the incidence in men has decreased during the last decades (5).

Median age at diagnosis is 69 years (range, 10-97). The majority of patients presents with locally advanced or metastatic disease (stage IIIB/IV, *Figure 2*). The prognosis in lung cancer is poor. Women have better prognosis than men. In Sweden, the relative 5-year survival rates were 10.1% for men and 15.4% for women in the period 2000-2008. The 1-year relative survival rate for men increased from 30% in the early 1990s to 35% for men diagnosed 1999-2001. For women, the corresponding improvement was from 33% to 41% (6).

Figure 2: *Stage distribution in new cases of lung cancer in Sweden, percent (5)*
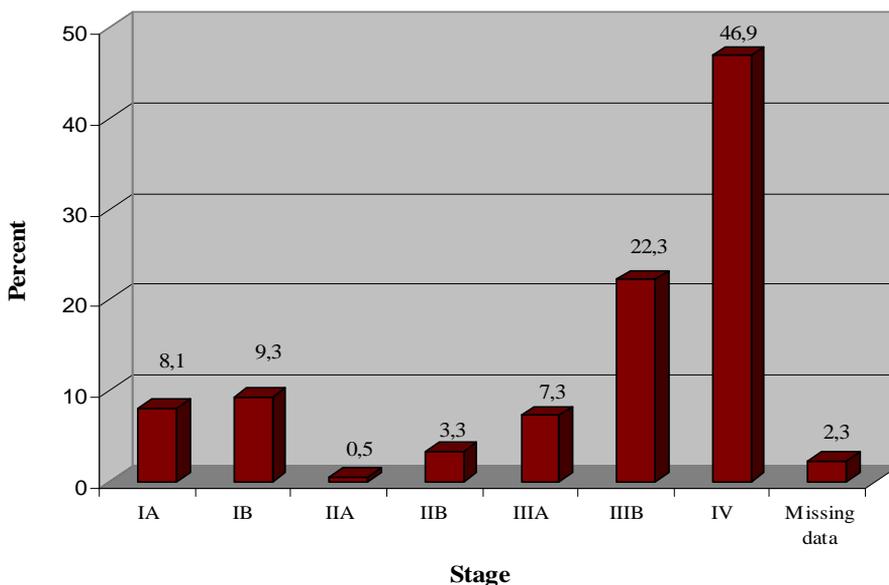
Histologic classification

Lung cancer is divided into non-small cell (NSCLC) and small cell lung cancer (SCLC). NSCLC comprises squamous cell carcinoma, adenocarcinoma, large cell carcinoma and further subtypes not specified here. An overview over the WHO classification of malignant tumours of the lung is found in *Table 1*. NSCLC accounts for around 80% of all lung cancers in Sweden. Adenocarcinoma is the most common histological type accounting for about 40% today and it is even the most common cell type in non-smokers (5).

Table 1. *Histological classification of malignant epithelial tumours of the lung. Extract from The 2004 World Health Organization/International Association for the Study of Lung Cancer (7)*

Malignant epithelial tumours
Squamous cell carcinoma
Small cell carcinoma
Adenocarcinoma
Large cell carcinoma
Adenosquamous carcinoma
Sarcomatoid carcinoma
Carcinoid tumour
Salivary gland tumours
Preinvasive lesions
Mesenchymal tumours

Staging

Staging has a key role for the decision of treatment in lung cancer patients. A system classifying lung cancer based on status of the primary tumour (T), regional lymph nodes (N) and metastases (M) was first proposed in 1946 by Denoix. The first edition of *The TNM Classification of Malignant Tumours* was published in 1968 by the Union Internationale Contre le Cancer (8). The TNM classification for lung cancer was revised in 1986, 1997 and 2009. The last version is based on a final data set involving 81 015 cases after exclusion of ineligible cases, compared to 5319 cases in the previous revision from 1997 (9). In our studies, the sixth edition of the TNM classification from 1997 was used. The TNM stages IIIB and IV are often together named as advanced NSCLC.

The main revisions in the 7th edition related to stage IIIB and IV are (se also *Table 2*):

- *Satellite nodule(s)* in the same lobe as the primary tumour will now classify the tumour as T3 (previously T4), whereas their presence in a different lobe of the same lung is classified as T4 (previously M1).

- *Redefinition of metastases (M):* subdivision of M into M1a and M1b. M1a includes both tumour nodule(s) in the contralateral lung and malignant pleural and pericardial effusions. Malignant pleural and pericardial effusions were previously classified as T4 disease.

- *Change of stage* grouping: T4N0M0 and T4N1M0 tumours are now classified as stage IIIA (previously IIIB) (10).

Table 2. *Definitions of stages III and IV, TNM 6th edition versus 7th edition*

Stage	TNM 6th edition (1997)	TNM 7th edition (2009)
IIIA	T(1-2)N2M0 T3N(1-2)M0	T(1-3)N2M0 T3N1M0 T4N(0-1)M0
IIIB	T4N(0-2)M0 T(1-4)N3M0	T4N2M0 T(1-4)N3M0
IV	Any T, any N, M1	Any T, any N, M1

The differences between the 6th and 7th edition of the TNM-classification are not relevant for the definition of the patient population in the CYCLUS study as we included both stage IIIB and IV patients treated with palliative chemotherapy.

Performance status

In medical oncology, performance status is an attempt to quantify the functional/ physical performance of a patient. Various scoring systems are in clinical use. Most commonly used are the Karnofsky score and the WHO score. In our studies, we used the latter one, defined as follows (11):

0. Able to carry out all normal activity without restriction.
1. Restricted in physically strenuous activity but ambulatory and able to carry out light work.
2. Ambulatory and capable of all self-care but unable to carry out any work; up and about more than 50% of waking hours.
3. Capable of only limited self-care; confined to bed or chair more than 50% of waking hours.
4. Completely disabled; cannot carry on any self-care; totally confined to bed or chair.

Prognostic and predictive factors

A *prognostic factor* is a characteristic of a patient that influences the prognosis regardless of the subsequent therapy. A *predictive factor* is a condition or finding that predicts effect of a specific treatment. Prognostic and predictive factors can contribute to clinical decision making and can help to individualize treatment in the heterogeneous population of lung cancer patients.

A great number of prognostic factors in lung cancer have been described. In a systematic review of the literature published between 1990 and 2001, Brundage et al. found 169 prognostic factors described in 887 articles, regarding survival of patients with NSCLC(12). Most of these factors are, however, not easily available in routine clinical practice.

Smoking

Tobacco smoking is by far the leading cause of lung cancer, accounting for about 90% of lung cancer cases in the developed countries (13). The risk of lung cancer increases with the duration of smoking and the number of cigarettes smoked (14).

In Sweden, about 90% of the patients diagnosed with lung cancer are current or former smokers. However, 6% of men and 15% of women who get lung cancer have never smoked. The predominant cell type in never smokers is adenocarcinoma, but the great majority of the patients who develop this type of cancer has smoked (5).

Other risk factors for lung cancer are environmental tobacco smoke (passive smoking) and occupational exposure to different agents, for example asbestos and residential radon (15).

Smoking has been described as a prognostic factor in lung cancer (16-18). Survival data related to smoking status were presented in a subgroup analysis of a phase III study which compared pemetrexed + cisplatin with gemcitabine + cisplatin in patients with advanced NSCLC. Former/current smokers had a significantly higher risk of death compared with never-smokers (HR=1.74, no confidence interval to be found in the publication) (19).

Treatment of non-small cell lung cancer

Current treatment

Every year, about 2600 patients in Sweden are diagnosed with NSCLC. The majority, about 2/3 of all patients, have stage IIIB/IV disease.

Surgery is the treatment of choice in early stages of NSCLC, but can be performed only in 18% of the patients (5, 20). Radiotherapy plays an important role in the management of NSCLC. In earlier stages, when a surgical resection cannot be performed because of medical contraindications, curative radiotherapy may be a treatment option. In patients with locally advanced NSCLC who are not candidates for surgery but have good prognostic factors, limited tumour extension and no major weight loss, chemoradiotherapy with curative intent can be given. In advanced disease, radiotherapy has a central role for palliation of symptoms.

Systemic chemotherapy plays a central role in the treatment of NSCLC and has become standard in advanced NSCLC (21). In patients with advanced NSCLC and PS 0-2, chemotherapy prolongs survival and leads to symptom palliation as well as improvement in quality of life (QoL) (22-24).

Systemic chemotherapy is recommended for patients in performance status 0-2 in Sweden. Internationally, there is no consensus on the role of chemotherapy in patients with a PS of 2, which is an explanation for the fact that many international trials only include patients with a PS of 0-1. However, clinically relevant improvements of quality of life in advanced NSCLC PS 2 patients have been found (25).

In 2006, at the start of the CYCLUS study, a combination of a platinum compound (cisplatin or carboplatin) with a third-generation agent (docetaxel, gemcitabine, paclitaxel or vinorelbine) was recommended. Less toxicity and easier administration compared with cisplatin are the reasons why carboplatin is preferred in Sweden in the majority of patients. Survival is, however, slightly inferior with carboplatin compared to cisplatin in combination with third generation drugs (26).

The duration of chemotherapy in advanced NSCLC has been investigated in several randomised studies. A meta-analysis of 13 randomised trials showed that chemotherapy with third-generation drugs beyond three or four cycles increased progression-free survival but not overall survival (27). In Sweden, treatment with four cycles is recommended.

Median survival time for patients with advanced NSCLC in performance status 0-2 receiving palliative first-line chemotherapy is 6-10 months (28-31).

Newer drugs and treatment strategies

Several types of targeted agents are under investigation in lung cancer. A role has been established for the oral the epithelial growth factor receptor (EGFR) tyrosine kinase inhibitors erlotinib and gefitinib.

There is also growing awareness of the fact that subgroups of patients (histology, gender, smoking status) might have better effect of a specific treatment than others.

Erlotinib is an alternative to docetaxel or pemetrexed in second-line treatment (32). In a randomized, double blind, placebo controlled phase III study of erlotinib as second or third line treatment of patients who were not suited for conventional chemotherapy (BR.21), explorative analyses showed that the effect of erlotinib was better in Asian women, patients with adenocarcinoma and lifetime non-smokers (33). Recently, Cappuzzo et al. found that maintenance therapy with erlotinib prolonged progression-free survival (PFS) in patients with NSCLC who had stable disease after four cycles of chemotherapy compared with placebo (34). Interestingly, the improvement of PFS was seen in the overall population irrespective of EGFR status (as determined by immunohistochemistry), sex, ethnic origin, histology or smoking status. Biomarker analysis of EGFR mutation status showed that the effect of erlotinib on progression-free survival was much better in patients with EGFR-activating mutations (n=49).

In patients with advanced NSCLC selected for the presence of EGFR mutation, treatment with first-line *gefitinib* is associated with longer PFS, higher objective response rate, a more favourable toxicity profile and better quality of life compared to platinum-based chemotherapy. Improvement of overall survival is, however, not documented (35).

In a meta-analysis regarding the effect and safety of *bevacizumab*, a monoclonal antibody against VEGF, Yang et al. found that neither high-dose (15 mg/kg) nor low-dose (7.5 mg/kg) increased 1-year overall survival in unresectable NSCLC. Low-dose bevacizumab may significantly improve progression-free survival, while high-dose bevacizumab may increase 2-year overall survival rates and prolong progression-free survival (36).

Pemetrexed is an inhibitor of thymidylate synthase and other folate-dependent enzymes. Scagliotti et al. showed that cisplatin/pemetrexed is not inferior to cisplatin/gemcitabine in

chemotherapy naïve patients with stage IIIB/IV NSCLC in PS 0-1. A preplanned subgroup analysis showed that OS was statistically superior for cisplatin/pemetrexed in patients with adenocarcinoma and large-cell carcinoma (19). In a study on maintenance therapy with pemetrexed in patients with advanced NSCLC (37), pemetrexed significantly improved overall survival compared with placebo (13.4 vs. 10.6 months, HR 0.79, 95% CI 0.65-0.95, p=0.012). In a subgroup analysis according to histology, improvement in overall survival was only seen in patients with non-squamous histology but not in patients with squamous cell carcinoma.

Earlier and current research on *COX-2 inhibitors* is summarized in "COX-2 and lung cancer" and "Discussion".

Cancer and inflammation

Inflammation

Inflammation (Latin, *inflammare*, to set on fire) was first described by Aulus Cornelius Celsus, a Roman physician and medical writer, who lived from about 30 B.C. to 38 A.D. He described the first four classical signs of inflammation: *calor* (heat), *dolor* (pain), *rubor* (redness) and *tumor* (swelling). The fifth one, *functio laesa* (loss of function), was added later, possibly by Virchow in the nineteenth century. There are numerous different definitions of inflammation which probably reflects the complexity of this process. Inflammation could be defined as the response of an organism's immune system to damage caused by microbial pathogens, chemical or physical insults or of unknown origin.

Cancer-related inflammation

There is growing evidence for a link between cancer and inflammation (38-39). Mantovani et al. summarize the yet unravelled molecular pathways between inflammation and cancer in a review published in 2008. In some types of cancer, inflammatory conditions can be found before a malignant change occurs. In other types of cancer, the oncogenic change induces an inflammatory microenvironment that promotes the delevopment of tumours. The hallmarks of cancer-related inflammation include the presence of inflammatory cells and mediators and are similar to those seen in chronic inflammation. Inflammation in the tumour microenvironment has tumour-promoting effects. However, there are many unanswered questions concerning cancer-related inflammation (40).

Epidemiological studies have shown that chronic inflammation is linked to various types of cancer. Many triggers of chronic inflammation seem to increase the risk of developing cancer. Examples are gastric cancer which is associated with infection of Helicobacter pylori, colon cancer which is associated with inflammatory bowel disease or the increased risk of lung cancer in never smoking individuals with asthma (38, 40-41).

Cachexia is a common phenomenon in cancer patients. Cancer cachexia, or "cancer-related anorexia/cachexia syndrome", is a multifactorial syndrome characterized by body weight loss (mainly due to loss of lean body mass), metabolic alterations, fatigue and reduced performance status. It is often accompanied by anorexia, leading to reduced food intake (42). At the time of diagnosis, 60% of patients with lung cancer have substantial weight loss (43).

Weight loss and cachexia in cancer patients are related to the presence of a systemic inflammatory response (42, 44-45).

C-reactive protein

C-reactive protein (CRP) was discovered in 1930 and is widely used as s sensitive, but non-specific, marker of systemic inflammation (46-48). Elevated levels of circulating CRP, defined as CRP >10 mg/l, have been found in 75-80% of patients with inoperable NSCLC (49-50). The significance of CRP as a negative prognostic factor has been shown in patients with several malignancies, i.e. multiple myeloma (51), melanoma (52), renal cell carcinoma (53) and gastrointestinal cancer (54-55).

CRP is an inexpensive, easily available marker which is often analysed routinely in lung cancer patients. In 203 patients who underwent curative resection of NSCLC, the preoperative serum CRP level was an independent predictor of survival (56). Forrest et al. found that CRP was a prognostic factor in 161 patients with inoperable NSCLC. The patients received chemotherapy and/or radical radiotherapy or supportive treatment (57). An increase in the magnitude of inflammation, represented by an elevation of CRP levels, was associated with shorter survival, increased weight loss and poorer quality of life in a study of 106 patients with stage III and IV NSCLC (44, 50). No information on the treatment given was available in this study.

Arachidonic acid pathway and cyclooxygenases

The arachidonic acid pathway plays an important role in inflammation. Arachidonic acid is released from phospholipids in the cell membrane and metabolized by enzymes such as cyclooxygenase (COX), lipoxygenase (LOX) and P450 epoxygenase to form eicosanoids. The definition of eicosanoids includes all products of the oxidation of arachidonic acid. The cyclooxygenases are a family of enzymes which catalyze the conversion of arachidonic acid to prostaglandins, prostacyclins and thromboxane. Traditionally, two isoforms of the COX enzyme have been described, COX-1 and COX-2, which differ in their pattern of expression (58). A third isoform, COX-3, has been identified and is mainly found in the brain and the kidney. The role of COX-3 is currently not known (59).

COX-1 is constitutively expressed in many tissues and is involved in a number of physiological functions such as the maintenance of renal blood flow and the protection of gastric lining. The inducible isoform, COX-2, is regulated by growth factors and different

cytokines such as IL1β, IL6, or TNFα. COX-2 catalyzes the production of prostaglandins mediating pain, inflammation, inhibiting apoptosis and stimulating angiogenesis. COX-2 induction is associated with an increased production of PGE2, one of the major products of COX-2 which is known to modulate cell death and tumour invasion in many types of cancer, including lung cancer. Proangiogenetic factors, such as vascular endothelial growth factor (VEGF), are induced by COX-2 (58-60).

Non-steroidal anti-inflammatory drugs and cancer

The mechanism that defines non-steroidal anti-inflammatory drugs (NSAIDs) as a class is their ability to inhibit COX. Thereby, they block the biosynthesis of prostaglandins. NSAIDs vary in their ability to inhibit COX-1 and COX-2. Acetyl salicylic acid (ASA) is a relatively selective inhibitor of COX-1 in platelets when given in doses of 50-100 mg daily (61). Many other conventional NSAIDs, such as ibuprofen and naproxen, inhibit COX-1 and COX-2 to the same extent. Coxibs, such as celecoxib and rofecoxib, selectively inhibit COX-2 (61-62).

Several reviews have summarized the accumulating evidence that NSAIDs have promise as anticancer drugs. Epidemiologic studies have found that long-term users of ASA or other NSAIDs have a lower risk of colorectal cancer than non-users (61, 63). Several studies suggest that regular medication with ASA may decrease the lung cancer risk (63-66). In a recently published analysis of randomised trials of daily ASA versus no ASA given for 4 years or longer on the risk of cancer death, benefit of ASA was confined to adenocarcinomas, for both lung and oesophageal cancer (67).

COX-2 and lung cancer

Increased expression of COX-2 has been found in a number of tumours, including lung cancer (59, 68-70). In lung cancer, a clear difference in the level of COX-2 expression between pathologic types of lung cancer has been found with a high proportion in adenocarcinomas and an almost negligible number in small cell lung cancer (58, 68, 70).

Increased expression of COX-2 has been associated with worse prognosis in lung cancer patients (70-72). Transcription of COX-2 is upregulated by tobacco smoke (73-74).

COX-2 appears to be involved in carcinogenesis by promoting cell division, inhibiting apoptosis, enhancing metastasis and stimulating angiogenesis (75-80). COX-2 inhibitors stimulate apoptosis and inhibit angiogenesis (61, 81-82). The exact mechanisms by which

COX-2 inhibitors act are not completely understood. There are also several COX-2 independent mechanisms used by celecoxib to mediate its anticarcinogenic effects (75).

Preclinical studies have shown inhibition of growth of lung cancer cells by COX-2 inhibitors, both as single agents and in combination with chemotherapy *in vitro* and *in vivo*. Clinical studies have suggested that a combination of chemotherapy with a COX-2 inhibitor might have better effect in non-small cell lung cancer than chemotherapy alone (58, 82-84). In a clinical phase II study, 29 patients with NSCLC stages IB to IIIA were treated with two preoperative cycles of paclitaxel and carboplatin, as well as daily celecoxib. A higher response rate was seen in comparison with historical response rates when patients only received neoadjuvant chemotherapy (83). In 2005, Nugent et al. published a phase II trial where patients with relapse after platinum-based chemotherapy for NSCLC were treated with a combination of docetaxel 75mg/m^2 every 21 days for a maximum of 6 weeks, and celecoxib 400 mg twice daily. Treatment with celecoxib was given until disease progression. Thirty-nine patients received at least one cycle of docetaxel and celecoxib. Median survival time was 11.3 months, progression-free survival 19.6 weeks and the response rate was 10.2% (85). Treatment with celecoxib was safe in both studies. No phase III trial was published until May 2006. For clinical studies published after start of the CYCLUS study see "Discussion".

Among the COX-2 inhibitors available for clinical use, celecoxib is the best studied in NSCLC and was therefore chosen in the CYCLUS study.

COX-2 inhibitors and adverse cardiovascular effects

The risk for adverse cardiovascular events due to treatment with COX-2 inhibitors has been discussed intensely. In September 2004, rofecoxib was withdrawn from the market worldwide. This decision was based on data from a study of rofecoxib in the prevention of adenomatous colonic polyps (APPROVe) in which 2586 patients were randomly assigned to rofecoxib or placebo. Cardiovascular safety was monitored by an independent committee. Thrombotic events included myocardial infarction, unstable angina, sudden death from cardiac causes, ischemic stroke, transient ischemic attack, peripheral arterial thrombosis, peripheral venous thrombosis and pulmonary embolism. A total of 46 patients in the rofecoxib group (1287 patients) experienced a thrombotic event during 3059 patient-years of follow-up (1.5 events per 100 patient-years). In comparison, 26 patients in the placebo group had a confirmed thrombotic event during 3327 patient-years (0.78 events per 100 patient-years). The corresponding relative risk was 1.92 (95% CI, 1.19-3.11, p=0.008). The increased

risk became first apparent after 18 months of treatment. During the first 18 months, the event rates were similar in both groups. The difference primarily reflected a greater number of myocardial infarctions and ischemic cerebrovascular events in the rofecoxib group. Overall mortality was similar in both groups (86).

The adenomatous polyp prevention trial (APC) studied the effect of celecoxib on prevention of colorectal adenomas. 2035 patients were randomised to two doses of celecoxib (200 mg or 400 mg daily) or placebo. An analysis of cardiovascular events (deaths form cardiovascular causes, myocardial infarction, stroke, heart failure) showed that risk after 3 years treatment was 1% in the placebo group, 2.3% (HR 2.3, 95% CI 0.9.5.5) in patients treated with celecoxib 200 mg x 2 and 3.4% (HR 3.4, 95% CI 1.4-7.8) in patients treated with 400 mg x 2. The incidence of thromboembolic events was also increased among patients receiving celecoxib: four in the group given 400 mg celecoxib twice daily, three in the group treated with 200 mg daily, compared with one in the placebo group. This difference was not statistically significant (87).

Data on adverse events from clinical trials, for example the CLASS study, comparing celecoxib to other NSAIDs in 8059 patients with rheumatoid arthritis, suggest that use of celecoxib might not be associated with an increase in risk of serious cardiovascular events (88). A second long term trial in patients with previous colon polyps (PreSAP) randomly assigned 1561 patients to celecoxib 400 mg x 2 or placebo. It did not show a statistically increased risk of cardiovascular events in the celecoxib group (89).

The risk for side effects of celecoxib in the CYCLUS study must be seen in relation to a potential benefit of the drug as anticancer agent and especially in relation to the risks for side effects of other treatment such as chemotherapy.

Toxicity, including cardiovascular events, is registered systematically in cancer trials. In the CYCLUS study, toxicity was registered by Common Terminology Criteria for Adverse Events v3.0 (CTCAE, (90)).

2. Aims of this thesis

The overall aim of this thesis was to study different aspects of the association between non-small cell lung cancer and inflammation – from a clinical point of view and with different research methods. The major project was the planning, organisation, coordination, performance and analysis of a prospective randomised double blind multicenter phase III trial (CYCLUS* study, paper III).

<u>The specific research questions of the studies were:</u>

Paper I, retrospective study
- Do C-reactive protein level and smoking have impact on prognosis in patients with advanced non-small cell lung cancer treated with palliative first-line chemotherapy?

Paper II, prospective methodological laboratory study
- Is it possible to evaluate cycoloxygenase-2 expression in cytologically diagnosed lung cancer?

Paper III, randomised double-blind phase III trial (CYCLUS study)
- Does addition of the COX-2 inhibitor celecoxib to palliative first-line chemotherapy prolong survival in patients with advanced non-small cell lung cancer?

- What are the effects of the COX-2 inhibitor celecoxib on quality of life?

3. Summary of papers

Paper I

Prognostic significance of C-reactive protein and smoking in patients with advanced non-small cell lung cancer treated with first-line palliative chemotherapy

Objectives

There is growing evidence for a causal relationship between inflammation and cancer and vice versa. C-reactive protein (CRP) is widely used as sensitive, but non-specific, marker of systemic inflammation. Smoking has been described as a prognostic factor in lung cancer. However, the majority of the studies included patients with various stages, and type of treatment was not taken into consideration. The objective of the study was to analyze if CRP and smoking status provide prognostic information in patients with advanced NSCLC receiving palliative first-line chemotherapy.

Patients and methods

This was a retrospective, single-institutional study, comprising all patients with NSCLC stage IIIB/IV and WHO performance status 0-2 who started palliative first-line chemotherapy between January 1, 2002, and January 31, 2007 at the Department of Pulmonary Medicine, University Hospital, Linköping, Sweden. Patient records were reviewed. Cox's proportional hazards model was used to identify prognostic factors.

Results

289 consecutive patients were available for analysis. 68% hade stage IV disease and 67% had performance status (PS) 0 or 1. Median survival was 7.4 months. At onset of chemotherapy, 206 patients (71%) had elevated CRP values ($\geq$10 mg/l). 144 patients (50 %) were current smokers. On univariate analysis, patients with elevated CRP levels had inferior survival (HR=1.67, 95 % CI, 1.28 – 2.19, P < 0.001). Smoking at onset of treatment was associated with shorter survival (HR 1.56, 95 % CI, 1.22 - 1.98, P<0.001). Ever smokers had shorter survival than never smokers (HR 1.80, 95 % CI, 1.25 – 2.59, P = 0.001). On multivariate analysis, with stage, PS, albumin and gender as covariates, both smoking at start of chemotherapy and CRP elevation were independent negative prognostic factors for survival.

Conclusions

CRP and smoking status are independent prognostic factors for survival in patients with advanced NSCLC receiving palliative first-line chemotherapy and provide additional information to established prognostic factors such as stage of disease and performance status.

Paper II

Cyclooxygenase-2 expression in lung cancer cells evaluated by immunocytochemistry

Objectives

Cyclooxygenase-2 (COX-2) expression may be a prognostic factor in lung cancer and may also be relevant for the effect of COX-2 inhibitors on cancer cells. In previous studies, COX-2 expression has almost exclusively been evaluated by immunohistochemical methods performed on histology sections of tissue biopsies. However, in clinical practice, lung cancer is often diagnosed with cytological techniques only. Methodology and results from analysis of COX-2-expression in cytological material from lung cancer patients by immunocytochemistry have, to our knowledge, not been described previously. The purpose of this study was to evaluate an immunocytochemical method for analysis of COX-2-expression in lung cancer patients.

Patients and methods

Fifty-three non-consecutive patients with lung cancer were prospectively examined. Material was obtained by transbronchial needle aspiration (TBNA) at routine diagnostic bronchoscopy or transthoracic needle biopsy performed at the Department of Pulmonary Medicine, University Hospital, Linköping, Sweden. A method for analysis of COX-2 expression with immunocytochemistry was developed at the Department of Pathology and Cytology, University Hospital, Linköping, Sweden. One experienced cytopathologist evaluated all slides as well as routinely stained parallel slides. Based on the microscopic examination, the percentage of stained tumour cells (<1%, 1-10%, 11-50%, >50%) and the intensity of staining (none, weak, strong) were estimated. Slides with less than 1% COX-2 expressing tumour cells were defined as negative.

Results

Preparation and staining with the method established at our laboratory were easy to perform and resulted in good quality slides. The percentage COX-2-stained cells and the intensity of staining varied widely between and within the different cases. The proportion of positively stained tumour cells was as follows: <1% in 20 pts., 1-10% in 7 pts., 11-50% in 17 pts., more

than 50% in 9 pts. In 17 cases, groups of cells with different intensity of COX-2-staining were found in the same slide.

Conclusions

Immunocytochemical analysis of COX-2-expression is technically easy to perform with routine diagnostic procedures. There is a great variation in the proportion of COX-2-positive cells between patients as well as in the intensity of staining between individual cells in many single cases.

Paper III

Effect of Celecoxib on Survival in Patients With Advanced Non-Small Cell Lung Cancer: a double blind randomised clinical phase III trial (CYCLUS study) by the Swedish Lung Cancer Study Group

Background

Increased expression of cyclooxygenase-2 (COX-2) is common in non-small cell lung cancer (NSCLC) and has been associated with poor prognosis. Experimental studies and clinical phase II trials have indicated that the addition of the COX-2 inhibitor celecoxib to palliative chemotherapy might increase survival time in patients with advanced NSCLC.

Patients and methods

We performed a double-blind, placebo-controlled multicenter phase III trial at 13 centres in Sweden. 319 patients with advanced NSCLC stage IIIB-IV and performance status 0-2 were randomised to receive celecoxib 400mg b.i.d. or placebo in addition to palliative chemotherapy. The primary objective was to compare overall survival. Other endpoints were quality of life, progression-free survival time, toxicity and biomarkers (vascular endothelial growth factor, proteomics).

Results

316 patients were included in the analysis, 158 in each treatment group. Median survival time was 8.5 months. There was no survival difference between the treatment arms. Small but not statistically significant differences in global quality of life and pain were seen favouring the celecoxib group. An increased frequency of leukopenia was observed in the celecoxib group. No increased incidence of cardiovascular events was observed in the celecoxib group.

Conclusions

This study failed to demonstrate a survival benefit of the addition of celecoxib to palliative chemotherapy.

4. Discussion

In this thesis, various aspects of inflammation in non-small cell lung cancer were investigated. The clinical point of view and the applicability of the methods in clinical practice were of great importance for the design of our projects. Three different research methods were applied: a retrospective register study, a methodological laboratory study and a randomised, double-blind, placebo-controlled, multicentre trial.

The three projects and the research questions posed in chapter 2 "Aims of this thesis" are discussed. As the CYCLUS study (paper III) was the main project of this thesis, I would like to devote a major part of this discussion to it.

C-reactive protein and smoking as prognostic factors in patients with advanced non-small cell lung cancer treated with palliative first-line chemotherapy (paper I)

The number of prognostic factors studied in lung cancer is extensive. In a review of the literature published between 1990 and 2001, Brundage et al. found 169 prognostic factors described in 887 articles, regarding survival of patients with NSCLC. There are variations in study populations, variations in the type of statistical analysis, variations in the type of treatment, and variations in the prognostic factors included in the analyses (12). A problem from the clinical point of view is that the majority of these factors are not readily available in routine clinical practice and that they generate extra costs.

C-reactive protein (CRP) is clinically widely used as a sensitive, but non-specific, marker of systemic inflammation (47). Elevated levels of circulating CRP, defined as CRP >10 mg/l, have been found in 75-80% of patients with inoperable NSCLC (49-50). Surprisingly, only few studies have analysed CRP as a prognostic factor in advanced NSCLC. CRP as a prognostic factor in patients with advanced NSCLC has only been studied in heterogeneous groups of patients without taking treatment into consideration (50, 57). Smoking has been described as a prognostic factor in lung cancer. However, as in the case of CRP, most of the studies included patients with various stages, and type of treatment was not taken into consideration (16-19, 91).

The aim of this retrospective study was to investigate the prognostic impact of CRP and smoking in patients with advanced non-small cell lung cancer treated with palliative first-line chemotherapy. Two-hundred and eighty-nine consecutive patients were included.

We found that CRP-levels $\geq$ 10 mg/l and smoking at start of treatment were negative prognostic factors for survival in univariate analysis, besides established prognostic factors such as stage and performance status. Smoking status had impact on prognosis, both when we compared current smokers with patients who did not smoke, and when we compared ever smokers with never-smokers. Both CRP and smoking remained independently associated with survival in multivariate analysis, with tumour stage, PS, albumin and gender as covariates.

The major strength of this study is a uniform patient population with respect to diagnosis, stage and treatment. Long follow-up time reduced the number of censored events. A weakness is that the analysis was performed retrospectively. Furthermore, patients were treated with various types of chemotherapy. However, the majority of patients (71%) received carboplatin + gemcitabine.

Do C-reactive protein and smoking have impact on prognosis in patients with advanced non-small cell lung cancer treated with palliative first-line chemotherapy?

In our study, CRP and smoking status at start of treatment were prognostic factors in patients with advanced NSCLC receiving palliative chemotherapy. To our knowledge, this is the first study analysing the prognostic value of both CRP and smoking in patients with NSCLC stage IIIB/IV receiving palliative first-line chemotherapy. Information on smoking status can be easily obtained from the patient and CRP level is part of routine blood analyses in many hospitals. Thus, in contrast to many other prognostic factors, information on these two factors is readily available for the clinician and does not generate extra costs or discomfort for the patients.

Never smokers may have a different biological subtype of lung cancer with less aggressive clinical features. Lung cancer not related to smoking will, however, also be found among smokers, thus obscuring the possibility to define prognostic characteristics of such tumours.

Our findings also indicate that CRP level and smoking history are relevant in the reporting of studies of chemotherapy in advanced NSCLC. This should be of particular interest in clinical research of inflammation in lung cancer.

Cyclooxygenase-2 expression in lung cancer cells evaluated by immunocytochemistry (paper II)

Increased expression of COX-2- has been described in lung cancer and has been associated with worse prognosis (68-72, 92-96). In previous studies, COX-2 expression was evaluated on histology sections of tissue biopsies. However, in clinical practice, lung cancer is often diagnosed with cytological techniques. In Sweden, 39% of all lung cancer diagnoses from 2002 to 2008 were based on cytological examination only (5). Analysis of COX-2 in cytological material in lung cancer patients has not been described earlier.

The aim of this project was to evaluate a method for analysis of COX-2 in patients with cytologically diagnosed lung cancer. Fifty-three patients with lung cancer were included from 2006 to 2008. Two main conclusions can be drawn from this study: The first one is that it is fully possible to perform COX-2 staining on lung cancer cells in a purely cytological material. The other conclusion is that there is great variation in the proportion and distribution pattern of COX-2 expressing tumour cells. The percentages of tumour cells expressing COX-2 were as follows: <1% in 20 pts., 1-10% in 7 pts., 11-50% in 17 pts., >50% in 9 pts. The intensity of staining varied between individual cells in many cases. In 17 cases (32%), groups of cells with different intensity of COX-2 staining were found on the same slide.

The variation of COX-2 expression in our study might have several explanations. Firstly, there might be methodological explanations. The technique of aspirating cells while moving the needle may collect material from several parts of the tumour and might have caused the variations in the distribution as well as in the intensity of staining. This would, anyway, imply that COX-2 expression varies within the same tumour. We have considered the possibility that the variation could be due to other methodological reasons, but the staining pattern seen speaks against this possibility. Secondly, there might be internal biological reasons. It should be remembered that what is seen in our and similar studies is just a snapshot of the COX-2 occurrence in lung cancer cells. Production of COX-2 might differ between different phases of the cell cycle. If so, the simultaneous presence of cells in different cell cycle phases could be a possible explanation for finding COX-2 negative cells close to positive cells. If this was the case, the extent of COX-2 expression within the same cell would differ if the aspiration was done at a different point of time. Thirdly, factors such as tumour cell differentiation (68, 97), pro-inflammatory cytokines (60), smoking status und use of non-steroidal anti-inflammatory drugs may influence COX-2 expression.

We believe that the variation of COX-2 expression in our study is a biological property and not primarily caused by methodological factors. This hypothesis is supported by the fact that a variation also has been described in studies on histological material (68, 97-98). In other studies, the variation is not explicitly commented upon (69, 71, 92, 95, 99). However, we interpret the use of different definitions of COX-2-positivity and different scores combining the percentage of positively stained cells and intensity as a way to manage variation and as an attempt to systematise and quantify it (68-69, 71, 92, 97, 99-100).

Is it possible to analyze cycoloxygenase-2 in cytological material in patients with lung cancer?

Technically, the analysis of COX-2 expression is feasible and results in good quality slides. This is a major advantage since lung cancer is often diagnosed with cytological techniques only. The method we described was technically easy to perform with routine diagnostic procedures and resulted in good quality slides.

Due to the variation in COX-2 expression, the interpretation of the results is, however, difficult. There is no consensus on how to quantify COX-2 expression. Several studies on the prognostic value of COX-2 in NSCLC have been published. In a meta-analysis published 2006, Mascaux et al. failed to demonstrate a statistically significant impact of COX-2 expression as a prognostic factor for survival in patients with NSCLC in univariate survival analysis. They emphasize the heterogeneity between the evaluable studies - various different types of patients, various disease characteristics, and diversity in the techniques used to analyze COX-2 expression. In the majority of studies, immunohistochemistry (IHC) was used to detect COX-2 expression. However, even the studies which used IHC are not easily comparable since various scoring systems as well as different antibodies and different protocols were used (69, 71, 92, 94-95, 101-102).

Clinical trials examining the effect of COX-2 inhibitors in lung cancer patients are ongoing. In 2008, Edelman et al. published a randomized phase 2 trial which indicated that COX-2 expression might be predictive for the effect of celecoxib in patients with advanced NSCLC (71).

Evaluation of the prognostic and/or predictive role of COX-2 expression, used in clinical research and, later on, in clinical routine, requires a well defined and well standardized technique. Optimally, such a method should be easy to perform, cheap, reproducible between laboratories, and associated with as little extra discomfort as possible for the patient.

Evaluation of COX-2 expression with the immunocytological technique as described here might be such a method. Interpretation of the results is, however, challenging.

The technique of analyzing COX-2 expression in patients with cytologically diagnosed lung cancer was not available when the CYCLUS study was planned. The method is of crucial importance for the prospective evaluation of COX-2 expression in a clinical trial since many lung cancer cases are diagnosed with cytological techniques only.

The effect of celecoxib in patients with advanced NSCLC receiving palliative first-line chemotherapy (CYCLUS study, paper III)

COX-2 is reported to interfere with angiogenesis, apoptosis and tumour invasiveness (79). Preclinical studies have shown that COX-2 inhibitors inhibit the growth of human lung cancer cells as single agents as well as in combination with chemotherapy (58, 84). Clinical phase II studies suggested that a combination of the selective COX-2 inhibitor celecoxib with chemotherapy might have better effect in NSCLC than chemotherapy alone (83, 85).

We performed an academic, multicenter, double-blind, randomised, phase III trial to investigate if the addition of celecoxib to first-line palliative chemotherapy would prolong survival in patients with advanced NSCLC. Secondary endpoints were quality of life, progression-free survival, toxicity and biomarkers. The study was designed to detect an increase of median survival from 7.5 months to 9.5 months. With a type I error of 5 % and a power of 80% (two-sided test), 760 patients were required, 380 in each treatment group. The study protocol was developed by a study committee on behalf of the Swedish Lung Cancer Study group and the trial was coordinated from the University Hospital in Linköping. The allocation sequence was generated at the data centre, Oncology Centre, Sahlgrenska University Hospital, Gothenburg, Sweden. The Hospital Pharmacy at the University Hospital Lund, Sweden, was responsible for distribution of the study drug. Thirteen centres in Sweden participated in the clinical part of the study. Blood samples for analysis of biological parameters were taken at four university hospitals. Planning of the trial started in 2003 and the first patient was included in May 2006. In accordance with the original time schedule, inclusion of patients was finished three years after start of the study, although we had not included the stipulated number of patients at that point.

Between May 2006 and May 2009, 319 patients with advanced NSCLC stage IIIB-IV and performance status 0-2 were randomised to receive celecoxib 400mg b.i.d. or placebo in addition to palliative chemotherapy. 316 patients were included in the analysis, 158 in each treatment group. There was no significant difference in overall survival between the two treatment groups (celecoxib: 8.9, 95 % CI 7.3-10.9 months vs. placebo: 7.9 months, 95% CI 7.2-10.0, p=0.92). The HR for OS was 1.00 (95% CI 0.79-1.26, p=0.97). No differences were found in progression-free survival or overall response rate.

Quality of life analyses showed a pattern of changes in global QoL and pain favouring celecoxib, but the differences were not statistically significant. A higher frequency of

leukopenia grade 3-4 was seen in the celecoxib group compared to placebo (59 vs. 39 respectively, p=0.02). The frequency of cardiac infarctions (three) and cerebrovascular ischemia (five) was low. Thrombosis/thrombus/embolism, all grades, was reported in 19 cases (celecoxib) and 16 cases (placebo).

The number of serious adverse events was similar in both groups (54 vs. 56 for celecoxib and placebo, respectively).

What has happened in the area since 2006, the start of the CYCLUS study?

There are currently two published phase III studies on the effect of COX-2 inhibitors in patients with advanced NSCLC receiving palliative chemotherapy. In an open-labelled phase III trial with a 2 x 2 factorial design, Gridelli et al. studied the effect of rofecoxib and prolonged constant infusion of gemcitabine in patients with NSCLC stage IIIB or IV, PS 0-1 and age < 70 years (GECO study). The two rofecoxib groups were closed early due to withdrawal of the study drug. The statistical analysis was limited to 240 patients who had at least 3 months of treatment. Rofecoxib did not prolong overall survival or progression-free survival but did improve response rate. Rofecoxib significantly improved several quality of life items, including global quality of life and pain-related items (103). Interpretation of the results is difficult for several reasons: 1. There are two main endpoints: the effect of rofecoxib and the effect of prolonged infusion with gemcitabine on survival. 2. The two treatment groups with rofecoxib were closed early which resulted in a lower number of patients and imbalance between the groups. 3. The study was not placebo-controlled.

A randomized placebo-controlled Dutch phase III study (NVALT-4) of docetaxel/carboplatin with celecoxib 400 mg b.i.d. in patients with advanced NSCLC was presented at the American Society of Clinical Oncology (ASCO) meeting 2009. Five hundred and sixty-one patients were randomized, 281 to celecoxib and 280 to placebo. Overall median survival was 8.3 months and median PFS was 5.5 months. No differences between the arms were seen. The response rate in evaluable patients was better in the celecoxib arm (p=0.05) (104).

A very interesting study was published in 2008. Edelman et al. performed a randomized phase II trial to test the hypothesis that inhibitors of two eicosanoid pathways, celecoxib and zileuton, added to chemotherapy, would improve outcome in advanced NSCLC. Patients with a PS of 0-2 and no prior chemotherapy were eligible. All patients received chemotherapy with carboplatin + gemcitabine and were randomly assigned to celecoxib, zileuton or celecoxib +

zileuton. One-hundred and thirty-four patients were included in analysis. There was no survival difference between the arms. COX-2 expression was a negative prognostic factor for overall survival in patients not receiving celecoxib. Patients with increased COX-2 expression receiving celecoxib had better survival than COX-2 expressing patients not receiving celecoxib (71).

Does addition of celecoxib to palliative first-line chemotherapy prolong survival life in patients with advanced non-small cell lung cancer? What are the effects of celecoxib on quality of life?

Celecoxib did not prolong survival in our study. The question if celecoxib has beneficial effects on survival in patients with advanced NSCLC in general cannot be answered by our trial for several reasons. A limitation of our study is the number of patients included. The study was designed to detect an increase of survival by two months with a two-sided test. 760 patients were required, but only 319 patients had been included when the trial was closed in accordance with the original study plan. However, the survival curves with the current number of 316 patients did not suggest any difference and it is unlikely that a number of 760 would have shown a significant difference.

Our results confirm the results of the Dutch study which did not show any survival benefit of adding celecoxib in patients with advanced NSCLC to palliative chemotherapy. In contrast to our study, response rate was improved in patients who were treated with a COX-2 inhibitor in both Gridelli's and Groen's trials (103-104).

We found a higher incidence of grade 3-4 leukopenia in the patients treated with celecoxib, compared to placebo (59 vs. 39 patients, respectively, p=0.02). Altorki et al. observed a higher frequency (62%) of neutropenia in 29 patients with NSCLC who were preoperatively treated with a combination of celecoxib 400 mg x 2 and paclitaxel/carboplatin (83). However, no similar observation was made in any other of the randomised trials (103-104), and it is not possible to draw any definitive conclusions about the clinical importance of this finding.

A comparison of the three trials must be carried out with caution. Rofecoxib and celecoxib differ in their ability to selectively inhibit COX-2, and different types of chemotherapy were combined with a COX-2 inhibitor in the three trials. Gridelli et al. combined rofecoxib with gemcitabine + cisplatin and Groen et al. combined celecoxib with docetaxel + carboplatin. We did not require a specific type of chemotherapy as there are two different regimens (gemcitabine + carboplatin and vinorelbine + carboplatin) mainly used in Sweden. The fact

that we permitted different types of chemotherapy can be criticised as the study drug might interact differently with different chemotherapeutics. Altorki et al. found that preoperative treatment with paclitaxel + carboplatin led to increase of intratumoural COX-2 levels in patients with NSCLC (105). The effect of other cytotoxic drugs on COX-2 expression is not clear. Our decision to permit different types of chemotherapy was due to the fact that there are two regimens in common use in Sweden. There is currently no evidence that any of the two regimens would be superior in combination with celecoxib. When analysing the overall survival in the CYCLUS study, no survival differences were seen when we compared the two regimens.

We could not find any statistically significant differences in QoL. However, a pattern of changes in some of the same items as in Gridelli´s study – global QoL and pain – favouring celecoxib were found. No quality of life data were published in the Dutch study. With a greater number of patients, a more reliable answer to the question if celecoxib affects quality of life could have been given.

In the phase II trial published by Edelman et al. 2008, COX-2 expression correlated with prognosis and benefit of celecoxib. COX-2 expression was a negative prognostic factor for overall survival in patients not receiving celecoxib. Patients with increased COX-2 expression receiving celecoxib had better survival than COX-2 expressing patients not receiving celecoxib. In contrast, there might be an adverse effect for patients who received celecoxib and did not express COX-2. This might be an explanation for the negative results in trials studying celecoxib not analysed with respect to COX-2 expression. The numbers of patients in the subgroups were, however, small and there was no placebo arm in this study (71).

Analysis of COX-2 expression was not planned when we started inclusion of patients in the CYCLUS study in 2006. The fact that we have treated an unselected population might be an explanation for the negative result of our study. An exploratory analysis has been initiated in patients where histological specimens are available.

Recruitment of patients in the CYCLUS study

The calculated time to include 760 patients was three years. Every year, about 2600 patients in Sweden are diagnosed with NSCLC. The majority, 65-70% or about 1800 patients, have stage IIIB/IV disease. About 20% of these patients have performance status 3-4 and will not be considered for chemotherapy. Among the remaining 1400 patients, a limited number will

receive curative chemoradiotherapy. The great majority is treated with palliative chemotherapy.

The study protocol was discussed and accepted by the Swedish Lung Cancer Study group. Fourteen out of 28 hospitals which were asked about participation agreed to include patients in the study. The major reasons for declining participation were lack of staff or concerns about cardiovascular toxicity of celecoxib. The average number of patients per hospital and year was estimated to be 15-20 by the responsible local investigators. With 14 participating centres, the conclusion was that 250 patients per year could be included in the study.

The actual number of included patients was 319 after three years, or 106 patients per year.

We decided to close the trial as originally planned. The study was organised to complete recruitment within three years, in order to permit subsequent studies in the same patient group. With the current or a slightly lower inclusion rate, a further 4-5 years would have been required to complete the trial. Fear for cardiovascular side effects of celecoxib may have made investigators more reluctant to include patients. Our data did, however, not show any significant increase of cardiovascular toxicity. With the current number of patients, the power of the study decreased to 47%. QoL analyses suggested small differences in favour of celecoxib, and with a larger number of patients, a more reliable answer to the question if celecoxib affects QoL could have been given.

In contrast, the Norwegian Lung Cancer Study Group included about 350 patients per year in two randomised phase III trials in patients with advanced NSCLC stage IIIB/IV treated with palliative first-line chemotherapy (28-29). The number of new cases of lung cancer in Norway is about 2500 every year (106). Replication of the Norwegian experience would permit inclusion of 500 patients/year in Sweden, around five times as many as in the CYCLUS trial.

It could be noticed that the total number of patients per hospital in the CYCLUS study varied between 0 and 86, figures that were not obviously in proportion to the numbers of patients with stage IIIB-IV disease diagnosed at the respective hospitals.

Generally, the proportion of patients with NSCLC included in clinical lung cancer trials in Sweden is about 5% (5). There are probably both local and national explanations for the poor inclusion rate in our study, as well as in other lung cancer trials. Insufficient doctor's time because of heavy workload and underpowered staff may be contributory. Lack of research nurses at the participating units may be an obstacle. Failure of researchers to publish previously finished clinical trials may decrease motivation.

An important question is how to achieve funding of academic clinical trials. Lack of resources was a major argument for several hospitals to decline the CYCLUS trial. In a British publication about the impact of the European 'Clinical Trials' Directive (2001/20/EC) (EUCTD) which was implemented in 2004, directors and senior staff in eight Clinical Trial Units where interviewed. All eight units reported that the EUCTD had made non-commercial trials more expensive. The cost increase was considered a major problem in areas of research without the support of a major funder (107).

The performance of an academic multicenter, randomised, double blind, phase III trial – some reflections

In May 2004, The European Union Clinical Trials Directive (2001/20/EC) was implemented in Sweden (108). The primary aims of this directive were to protect the rights of the patients enrolled in clinical trials and to harmonize clinical research across the EU.

Researchers from several EU countries have reported an excessive increase of bureaucracy, increased costs and a decrease of the number of clinical trials after the implementation of the directive. The situation seems particularly threatening for academic and non-commercial research supported by grants, often without one major funder (107, 109-111).

Our own experience from the CYCLUS study are in accordance with these reports. The idea to perform a clinical trial to test the hypothesis that a COX-2 inhibitor prolongs survival in patients with advanced NSCLC was discussed for the first time in October 2003. Writing of the protocol and planning of the study lasted from spring 2004 to autumn 2005. Approvals to perform the trial were achieved in December 2005 (Medical Products Agency) and January 2006 (Ethics Committee). The first patient was included on May 31, 2006. Thus, the process from idea to inclusion of the first patient took 2.5 years.

The trial was designed close to clinical routine regarding diagnostic procedures and follow-up. Furthermore, case report forms were simple and easy to complete. The performance of the CYCLUS study was, however, a logistic and economical challenge. The costs were mainly caused by the double-blind design, services provided by the data centre and salaries for some of the centrally involved staff. No compensation was offered to the participating centres. The study drug (celecoxib and placebo) was provided by Pfizer. The trial was mainly supported by grants from FORSS (The Research Council of Southeastern Sweden) and the County Council in Östergötland but would not have been possible to perform without independent research grants from two pharmaceutical companies, Pfizer and Pierre Fabre Norden AB.

It is likely that the increased administrative burden caused by the EUCTD has influenced the number of centres being able to participate in the CYCLUS study and thus the number of included patients.

The intention of the EUCTD to harmonize the performance of clinical trials and thus make research more standardised is positive. Unfortunately, there is a lack of infrastructure and financial resources in academic clinical research, which makes it difficult to implement the directive. I believe that there are possibilities to decrease the administrative workload, which slows down the process of clinical trials. Academic research is of great importance for the patients. We clinicians meet the patients and have the opportunity to see was is relevant and needed in the clinical context. Another important argument for academic research is its non-commercial character. Evaluation of cheap and easily applied treatment is of central interest for both patients, the medical community and public health care providers.

5. Concluding remarks and future perspectives

Various aspects of inflammation in non-small cell lung cancer have been studied in this thesis. Research questions were posed and studied from the perspective of the clinician and – as far as possible – close to clinical routine.

The CYCLUS study, a multicenter, double-blind, randomised, phase III failed to demonstrate a benefit of treatment with celecoxib on survival in patients with advanced NSCLC when added to first-line palliative chemotherapy. Survival was equal in both groups. The required number of patients could not be included, but the survival curves with the current number of 316 patients did not suggest any difference and it is unlikely that a number of 760 would have shown a significant difference. However, as for quality of life, a greater number of patients had probably given clearer answers. We found a pattern of changes in favour of celecoxib, but the differences were not statistically significant.

From the first study we can conclude that C-reactive protein and smoking status have impact on survival in patients with advanced NSCLC receiving palliative first-line chemotherapy. CRP and smoking status are easily available in clinical context. The second study showed that analysis of COX-2 expression in cytological material is technically easy to perform with routine diagnostic procedures and results in good quality slides.

Recent data on research in lung cancer research indicates that there is a need to individualize treatment in lung cancer. Individual factors as gender, histology, genetic variations and other conditions at diagnosis, such as weight loss or biological markers, seem to have impact on treatment results. This is confirmed in our studies. Factors related to inflammation, such as CRP and smoking, seem to be of importance for prognosis in advanced non-small cell lung cancer. More recent data suggest that COX-2, an important enzyme involved in inflammation, might have predictive value for the effect of celecoxib. Analysis of COX-2 expression was not part of the CYCLUS trial but may be relevant for its interpretation. An analysis of COX-2 expression in existing histopathological material has been initiated in which the results will be correlated to survival data from the CYCLUS study.

There is a link between inflammation and cancer, but the complex role of inflammation in cancer is far from completely understood. Further insights in molecular mechanisms are needed. Regarding COX-2 expression, there is an association between VEGF, a central factor for angiogenesis, and expression of COX-2. We are currently analysing VEGF content in plasma samples from a subgroup of patients in accordance with the study protocol.

Future studies on COX-2 inhibitors in advanced NSCLC should take COX-2 expression, CRP, smoking status and probably even gender into consideration. Lung cancer trials with preplanned subgroup analyses require the inclusion of a larger number of patients. Multinational trials will become even more important. Currently, the performance of a clinical phase III trial is a heavy, time-consuming and expensive process. The European Clinical Research Infrastructures Network (ECRIN), a not-for-profit infrastructure supporting multinational clinical research projects in Europe, and The Swedish Clinical Research Infrastructures Network (SweCRIN) work to facilitate the performance of both commercial and academic clinical trials (112).

Academic research is needed to ensure the independence of clinical research and to optimize the use of the scarce financial resources in public health services.

I would like to finish with a citation from the excellent book *Diagnosis and Treatment of Lung Cancer (113)* about the importance of clinical research: "Although the process at times be unwieldy, cumbersome, and painstakingly slow, support is needed because there is no other way to make progress in the clinical care of patients".

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7. Acknowledgements