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Prognostic Factors in Non-Small Cell Lung Cancer (NSCLC)

GEORG HOLGERSSON



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Abstract

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Background: Non-small cell lung cancer (NSCLC) is the cancer disease with the highest mortality globally. About 75% of NSCLC patients are diagnosed in an advanced stage where surgical treatment is not possible. For patients with locally advanced disease without distant metastases, the treatment of choice is curatively intended radiotherapy. However, this treatment has considerable side effects and many patients relapse. To individualize the treatment strategy for these patients, it is essential to have as much prognostic information as possible. The aim of this thesis was to investigate the prognostic significance of histology and pre-treatment hematopoietic blood parameters.

Material and Methods: Data were collected retrospectively for NSCLC patients treated between 1990 and 2000 with curatively intended radiotherapy. The data were obtained by manually searching patient records from all radiation oncology departments in Sweden. The prognostic significance of histology, and pre-treatment levels of hemoglobin (Hgb), white blood cells (WBC) and platelets (Plt) were analyzed in relation to overall survival using univariate and multivariate statistical methods. These prognostic factors were further analyzed in a chemoradiation patient cohort and in a cohort of patients with recurrent NSCLC treated with palliative docetaxel, or the insulin-like growth factor 1 receptor (IGF-1R) modulator AXL1717.

Results: In the cohort of NSCLC patients treated between 1990 and 2000, squamous cell carcinoma (SCC) histology and pre-treatment anemia (Hgb <110 g/L), leukocytosis (WBC > 9.0 x10⁹/L), and thrombocytosis (Plt >350 x10⁹/L) were independent prognostic factors for shorter overall survival. However, in the chemoradiation cohort only thrombocytosis retained independent prognostic significance in a multivariate analysis. In the cohort of patients with recurrent disease treated with palliative systemic therapy, only leukocytosis was significantly associated with worse survival.

Conclusions: Routine pre-treatment hematopoietic blood parameters—together with other prognostic factors such as disease stage and performance status—can provide decision-making support when individualizing treatment of NSCLC. The prognostic role of histology is unclear and further research is warranted to determine its significance.

Keywords: NSCLC, prognostic factors, survival, histology, anemia, leukocytosis, thrombocytosis

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*To my mother, who would have
loved to read this*

List of Papers

This thesis is based on the following papers, which are referred to in the text by their Roman numerals.

- I. Holgersson G, Bergström S, Bergqvist M, Nyman J, Hoyer E, Helsing M, Friesland S, Holgersson M, Birath E, Ekman S, Blystad T, Ewers SB, Mörth C, Löden B, Henriksson R. (2011) Swedish lung cancer radiation study group: predictive value of histology for radiotherapy response in patients with non-small cell lung cancer. *European Journal of Cancer*. Nov;47(16):2415-21.
- II. Holgersson G*, Sandelin M*, Hoyer E, Bergström S, Henriksson R, Ekman S, Nyman J, Helsing M, Friesland S, Holgersson M, Lundström KL, Janson C, Birath E, Mörth C, Blystad T, Ewers SB, Löden B, Bergqvist M. (2012) Swedish lung cancer radiation study group: the prognostic value of anaemia, thrombocytosis and leukocytosis at time of diagnosis in patients with non-small cell lung cancer. *Medical Oncology*. Dec;29(5):3176-82.
- III. Holgersson G, Bergström S, Hallqvist A, Liv P, Nilsson J, Willén L, Nyman J, Ekman S, Henriksson R, Bergqvist M. The Prognostic Value of Pre-treatment Thrombocytosis in Two Cohorts of Patients with Non-small Cell Lung Cancer Treated with Curatively Intended Chemoradiotherapy. *Accepted for publication in Neoplasma*.
- IV. Holgersson G, Bergqvist M, Nilsson J, Thureson M, Harmenberg J, Bergström S. (2017) The Prognostic Value of Pre-treatment Leukocytosis in Patients with Previously Treated, Stage IIIB/IV Non-small cell Lung Cancer Treated with the IGF-1R pathway modulator AXL1717 or Docetaxel; A Retrospective Analysis of a Phase II Trial. *Asian Pacific Journal of Cancer Prevention*. Jun 25;18(6):1555-1560.

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Abbreviations

95% CI	95% confidence interval
AC	Adenocarcinoma
ALK	Anaplastic lymphoma kinase
ASCO	American Society of Clinical Oncology
AUC	Area under curve
BMI	Body mass index
CK	Cytokeratin
CNS	Central nervous system
COPD	Chronic obstructive pulmonary disease
CT	Computed tomography
CTC	Circulating tumor cell
ctDNA	Circulating tumor DNA
CTLA-4	Cytotoxic T-lymphocyte antigen 4
DLCO	Diffusing capacity of the lungs for carbon monoxide
EBUS-TBNA	Endobronchial ultrasound-guided transbronchial needle aspiration
ECG	Electrocardiogram
ECOG	Eastern Cooperative Oncology Group
EGFR	Epidermal growth factor receptor
EMA	European Medicines Agency
EML4	Echinoderm microtubule-associated protein-like 4
EUS-FNA	Endoscopic ultrasound-guided fine needle aspiration
FDA	Food and Drug Administration
FEV1	Forced expiratory volume in 1 second
FGF	Fibroblast growth factor
FISH	Fluorescent in situ hybridization
G-CSF	Granulocyte colony-stimulating factor
GM-CSF	Granulocyte macrophage colony-stimulating factor
Gy	Gray (unit)
Hgb	Hemoglobin
HR	Hazard ratio
IARC	International Agency for Research on Cancer
IASLC	International Association for the Study of Lung Cancer
IGF-1R	Insulin-like growth factor 1 receptor
IL-6	Interleukin 6
KPS	Karnofsky Performance Status

LCC	Large cell carcinoma
<i>MET</i>	MET proto-oncogene
NGS	Next-generation sequencing
NLR	Neutrophil-to-lymphocyte ratio
NOS	Not otherwise specified
NSCLC	Non-small cell lung cancer
ORR	Overall response rate
OS	Overall survival
PAH	Polycyclic aromatic hydrocarbons
PCR	Polymerase chain reaction
PD-1	Programmed cell death 1
PD-L1	Programmed cell death ligand 1
PDGF	Platelet-derived growth factor
PET/CT	Positron emission tomography/Computed tomography
PFS	Progression-free survival
Plt	Platelets
PS	Performance status
PTH	Parathyroid hormone
<i>ROS1</i>	ROS proto-oncogene 1
RTOG	Radiation Therapy Oncology Group
SBRT	Stereotactic body radiotherapy
SCC	Squamous cell carcinoma
SCLC	Small cell lung cancer
SEER	Surveillance, Epidemiology, and End Results
SIADH	Syndrome of inappropriate antidiuretic hormone secretion
TKI	Tyrosine kinase inhibitor
TNM	Tumor-node-metastasis
TRL	Tumor-related leukocytosis
TTF-1	Thyroid transcription factor 1
VEGF	Vascular endothelial growth factor
WBC	White blood cells
WHO	World Health Organization

Introduction

Epidemiology

Around 1.8 million people are diagnosed worldwide with lung cancer each year[1]. This accounts for about 13% of total cancer diagnoses making it the most common cancer disease. Lung cancer is also the type of cancer that has the highest mortality, killing approximately 1.6 million people annually[1]. The highest incidence rates among men are in the United States and Eastern Europe, whereas the highest among women are in North America and Northern Europe[2].

Lung cancer incidence rates reflect tobacco smoking trends and these vary with time and region[3, 4]. In countries such as China and others in Asia and Africa, the tobacco epidemic has been established relatively recently and the smoking prevalence is still rising. Lung cancer rates are therefore increasing and will continue to do so in the coming decades[3, 5]. On the other hand, in some Western European countries, the tobacco epidemic peaked in the middle of the 20th century for men and a few decades later for women. In these countries lung cancer incidence has begun to decrease in men and has plateaued in women[6, 7].

In Sweden, about 4000 people were diagnosed with lung cancer in 2015 according to the statistical database of the Swedish National Board of Health and Welfare (Socialstyrelsen)[8]. The same year, over 3600 people died of the disease making it the cancer disease that kills most people of both genders in Sweden. Both the incidence and mortality of lung cancer in Sweden has historically been considerably higher in men; however, the incidence and mortality is now slightly higher in women[8].

Lung cancer incidence increases with age and about one-third of lung cancer deaths occur after the age of 75. Lung cancer before the age of 45 is rare and accounts only for about 3% of lung cancer deaths[9]. Squamous cell carcinoma (SCC) has been the most prevalent histological lung cancer subtype until the 1990s when it was surpassed by adenocarcinoma (AC) histology. In women, however, AC has always been the most prevalent histological subtype[8, 10]. In recent years, the proportional incidence of AC has increased in both men and women replacing SCC as the most common histological subtype in men[10, 11]. This histological shift was first observed in Europe and North America in the late 1990s; however, this trend has also been evident in developing nations such as China and India[10, 12, 13].

Risk Factors

Tobacco Use

Tobacco smoking is the most well-established risk factor for developing lung cancer and the association between tobacco smoking and lung cancer has been demonstrated in numerous epidemiological studies since the early 1950s[14, 15]. In 1957, a report from the British Medical Research Council and an American study group appointed by the National Cancer Institute, the National Heart Institute, the American Cancer Society, and the American Heart Association all concluded that there was no doubt of a causal relationship between cigarette smoking and lung cancer[16, 17].

Duration of smoking is considered to be the strongest risk factor for lung cancer development in smokers and early cessation of smoking lowers the risk substantially[18]. Smoking cessation before the age of 30 reduces almost all of the tobacco-attributable risk compared to continuing smokers and there is a considerable protection even for those who stop in middle-age[19]. Tobacco smoking also impacts the histology of lung cancer—AC is the most prevalent histological subtype among never smokers, whereas SCC and small cell carcinomas (SCLC) are more common with increasing exposure to tobacco smoke[20].

Although the epidemiological evidence is stronger for first-hand smoking, second-hand exposure to smoking also increases the risk of lung cancer[21]. The main risks include workplace and spousal exposure, with an increased risk of lung cancer of about 20-30% for a non-smoker who is married to a smoker or exposed to tobacco smoke at the workplace[22].

The few studies that have been made concerning risk of lung cancer among users of smokeless tobacco, such as chewing tobacco and moist snuff, show inconsistent results; some suggest an increased risk and other studies not[23].

Electronic cigarettes are increasing in popularity as a tobacco-free alternative to regular smoking. Although this type of cigarette does not produce the harmful chemicals associated with tobacco combustion, the high temperature reached in the vapor can still generate dozens of toxic substances including polycyclic aromatic hydrocarbons (PAH)[24]. The long-term effects of electronic cigarette use remain to be determined[25].

Radon

Exposure to ionizing radiation has been extensively studied in Japanese atomic bomb survivors and has been reported to increase the risk of developing malignancies, including lung cancer[26]. The radioactive, naturally occurring noble gas radon-222 (radon) is responsible for about half of all non-

medical exposure to ionizing radiation[27]. It originates from the decay series of uranium-238 which is a radioactive mineral found in the earth crust. Epidemiologic studies of miners show a linear relationship between radon exposure and lung cancer risk[28].

Residential exposure can occur from airborne radon released from the ground which enters a home through structural defects in the walls and floor[29]. Radon can also be found in low concentrations in certain building materials such as concrete. Furthermore, radon has high water solubility and can enter sources of ground water through diffusion from rocks of the earth crust, which can be a potential health risk for people using ground water as drinking water[30].

Radon exposure is considered to be the second most important risk factor for developing lung cancer overall and the most important risk factor in never smokers[31]. In addition, there is a synergistic effect of radon exposure and tobacco smoking—the absolute risk of developing lung cancer by age 75 is about 25 times greater for a smoker than a non-smoker who has been exposed to the same accumulated radon[29].

Occupational Exposures

Asbestos is a collection of naturally occurring crystalline silicate fibers. Due to their stability and durability, these fibers have been widely used in building materials and thermal insulators. Asbestos is strongly linked to the development of malignant pleural mesothelioma, a rare form of cancer that arises from the thin layer of tissue that lines the lungs and chest wall[32].

Data on the association between asbestos exposure and lung cancer were published already in 1955 by Doll *et al*[33] and other studies have confirmed this relationship[34]. It is estimated that 5-7% of all lung cancers can be attributed to occupational exposure to asbestos[35]. Furthermore, the synergism between co-exposure of smoking and asbestos for lung cancer risk seems to be between additive and multiplicative[34]. The use of asbestos has been prohibited in Sweden since 1982 and is nowadays banned in more than 50 countries[36, 37]. However, significant amounts of asbestos are still being used in India, China, Russia and some developing countries[37].

Polycyclic aromatic hydrocarbons (PAH) are formed during incomplete combustion of organic material. An increased risk of lung cancer has been reported in some occupations that involve exposure to PAH, such as aluminum production industries and iron and steel foundries[38]. Motor vehicle exhausts also contain PAH mixtures, and an increased risk of lung cancer risk has been observed among workers with high occupational exposure to diesel engine exhaust, for example, those engaged in underground mining and tunnel construction[39].

Occupational exposure to inhaled inorganic arsenic by copper smelters and workers engaged in manufacturing arsenical pesticides has also been associated with a higher risk of lung cancer[40]. Inorganic arsenic has been classified as a group 1 carcinogen by the International Agency for Research on Cancer (IARC) since 1980[41]. Arsenic in drinking water is also a risk factor for the development of lung cancer[42].

Air Pollution

Cooking meals or heating homes by open fires with coal or biomass in poorly ventilated houses is commonplace in the developing world. The resulting fumes contain particles and gaseous chemicals that are carcinogenic[43]. Studies from Asia show this type of air pollution is a risk factor for developing lung cancer, especially in non-smoking women[44-47].

Concerning outdoor air pollution, one large study of 17 European patient cohorts suggests an association between residential exposure to air pollution and risk for lung cancer[48]. The IARC has classified outdoor air pollution as a lung carcinogen in humans[49].

Family History

The increased risk of lung cancer in the offspring of lung cancer patients has been described in several registry-based studies, including segregation analyses[50]. It is reasonable to believe that this increased relative risk is partly due to genes that affect smoking behavior; however, most familial cases of lung cancer are not primarily due to shared smoking habits[51].

The evidence of familial aggregation of lung cancer suggests that certain genes are associated with lung cancer susceptibility. A linkage analysis has found a major susceptibility locus in chromosome 6q23-25 in lung cancer pedigrees[52]. An increased lung cancer risk is also seen in patients with the Li-Fraumeni syndrome, a rare autosomal dominant hereditary disorder characterized by germline mutations of the tumor-suppressor gene *p53*[53].

Histology

Lung cancer is classified based on histology according to the 2015 WHO classification of lung tumors[54]; this replaces the previous WHO classification from 2004[55]. Lung carcinomas are traditionally, and for therapeutic purposes, divided into two main entities based on histology: small cell lung cancer (SCLC) and non-small cell lung cancer (NSCLC). NSCLC accounts

for approximately 80% of all primary lung cancers[56]. The WHO classification of lung cancer applies to both surgically resected tumors and specimens from diagnostic small biopsy/cytology.

The main histological NSCLC categories used for surgical specimens include adenocarcinomas (AC), squamous cell carcinomas (SCC), and large cell carcinomas (LCC). SCLC, which will not be further discussed in this thesis, consists of highly aggressive neuroendocrine lung tumors with morphological and clinical features that clearly distinguish them from NSCLC[57].

The subtyping of NSCLC did not, until only a decade ago, have any impact on the choice of treatment. Thus, little effort was made to distinguish AC and SCC in small tissue samples. However, this changed following the results of a phase III study in 2008 that compared cisplatin plus gemcitabine with cisplatin plus pemetrexed as first-line treatment of advanced NSCLC. Patients with a non-squamous histology given cisplatin/pemetrexed had a superior overall survival compared to the patients that had received cisplatin/gemcitabine, whereas the opposite was true for patients with squamous cell histology[58]. Treatment with the vascular endothelial growth factor (VEGF) monoclonal antibody bevacizumab is recommended for advanced non-squamous NSCLC, but is contraindicated in SCC due to the risk of life threatening or fatal hemoptysis[59]. Furthermore, histology is of major importance for molecular pathologists since mutations in the epidermal growth factor receptor (*EGFR*) and rearrangements of the anaplastic lymphoma kinase (*ALK*) or the ROS proto-oncogene 1 (*ROS1*) genes are almost exclusively found in non-SCC histology. Tumors harboring *EGFR* mutations or *ALK/ROS1* rearrangements can be effectively treated with targeted tyrosine kinase inhibitors, TKIs[60-62] and therefore molecular testing for these is now recommended for those NSCLC tumors classified as non-SCC[63, 64].

The importance of correct histological classification, as part of personalized medicine in NSCLC, will likely increase as the histology determines whether certain types of molecular testing are relevant to the treatment strategy choice.

Adenocarcinoma

AC histology is the most prevalent subtype of lung cancer. ACs are especially common in never smokers, females, and Asian patients[65]. The tumors tend to arise peripherally in the lung and may be associated with scarring and pleural retraction. They are histologically characterized by the formation of glands and/or production of mucin[66]. The malignant glands are composed of columnar or mucinous epithelial cells with large nuclei and prominent nucleoli[67].

ACs often show histological heterogeneity. Surgically resected ACs can be further divided into numerous subgroups including acinar, papillary, micropapillary, and solid, depending on the predominant growth pattern[68]. This subdivision has little clinical importance, although it might help distinguish multiple primary tumors from intrapulmonary metastases[69].

In well- and moderately-differentiated ACs, the histologic features are often easily demonstrated by routine microscopy[67]. In poorly differentiated tumors, however, immunohistochemical staining is usually needed for diagnosis. The most important methods to verify AC histology include positive immunohistochemical staining for mucin, thyroid transcription factor 1 (TTF-1), and/or napsin-A[68].

Squamous Cell Carcinoma

SCC is nowadays the second most prevalent NSCLC histology. Since SCCs are strongly associated with smoking, the declining incidence is primarily thought to be a consequence of changes in smoking behavior, in particular the decrease of non-filtered cigarette smoking[70, 71]. SCCs typically arise centrally in the lung in the large bronchi. They tend to have a locally aggressive growth pattern and distant metastases occur less frequently than in ACs.

Histological characteristics of well-differentiated SCCs include keratinization, intercellular bridges, and pearl formations. The tumor cells are usually large with an abundant cytoplasm, hyperchromatic nuclei and small nucleoli[72]. In poorly differentiated non-keratinizing tumors, diagnosis can be challenging and immunohistochemical staining is often required. In addition to a negative TTF-1 staining, the most important immunohistochemical SCC markers include p63, p40 and cytokeratin 5/6 (CK5/6)[68, 72].

Large Cell Carcinoma

Lung cancer with no specific features of SCLC, AC or SCC is classified as LCC. It is a diagnosis of exclusion which requires a thorough morphological assessment of the whole tumor to rule out focal areas of differentiation. Thus, for small biopsies and cytology samples, an LCC diagnosis is not allowed. Instead, NSCLC-NOS (not otherwise specified) is used to describe lung cancer with no specific features of SCLC, AC, or SCC[68].

LCC tumors tend to be composed of large, polygonal cells arranged in sheets or nests with vesicular nuclei, a moderate amount of cytoplasm, and prominent nucleoli[73]. If a morphological diagnosis of LCC has been made, immunohistochemical staining should be conducted for evidence of squamous or glandular differentiation. If any such evidence is found, the pathol-

ogy report should comment whether the tumor differentiation favors SCC or AC[74]. The increasing use of immunohistochemistry has reduced the proportion of undifferentiated LCC in NSCLC as more tumors can be classified as either AC or SCC. However, about 70% of lung cancers are diagnosed with a small biopsy or cytology, where pathological assessment is more challenging than in surgical specimens. In this group, NSCLC-NOS still accounts for about 20% of diagnoses[66].

From a clinical viewpoint, LCC and NSCLC-NOS are usually categorized together with AC as non-squamous and treated accordingly[75].

Diagnostic Workup

Symptoms and Initial Evaluation

More than 90% of patients with lung cancer are symptomatic at the time of diagnosis. The most common debut symptom is a persistent cough which is present in up to 75% of patients[76]. Symptoms can be related to the primary tumor itself, (e.g., cough and hemoptysis), to loco-regional spread (e.g., chest pain and superior vena cava obstruction), and/or to distant metastases in other organs such as the skeleton or the brain (e.g., bone pain, dizziness). Brain metastases are a common finding in lung cancer; > 8% of NSCLC patients have brain metastases already at disease presentation[77]. Symptoms can also be non-specific (e.g., anorexia, weight loss, and fatigue) or related to paraneoplastic syndromes, such as the syndrome of inappropriate antidiuretic hormone secretion (SIADH), which causes hyponatremia, or ectopic production of PTH (parathyroid hormone), which causes hypercalcemia[76, 78].

When a patient presents with symptoms that raise suspicions about lung cancer, the initial evaluation should always include a chest x-ray. If any abnormalities are found, the patient should be referred to a specialist clinic for further investigations[78].

The association between time intervals from symptoms to early investigations and cancer outcomes is under investigation by the International Cancer Benchmarking Partnership[79]. A project dedicated to increasing awareness of lung cancer symptoms in primary care and reducing the time interval for referral to a lung clinic was conducted between 2010 and 2012 in Dalarna County, Sweden. Patients who called their primary care nurse with symptoms that fulfilled certain criteria—cough with a duration of > 6 weeks, chronic cough that had changed character, or hemoptysis—were referred for a chest x-ray the same day and for an appointment with their primary care doctor within five days. The project was considered successful and has now been implemented at all primary care centers in Dalarna County[80].

Tissue Diagnosis

A patient with an abnormal chest x-ray where a lung cancer is suspected undergoes a diagnostic workup that includes three investigations performed simultaneously: tissue diagnosis, disease staging, and functional evaluation. An adequate tissue sample is required to verify a diagnosis of lung cancer and to determine histological subtype. Tissue samples can be obtained in several ways and the choice of procedure depends on the location and size of the tumor[81]. Conventional fiberoptic bronchoscopy with biopsy and/or cytology from washings and brushings is best suited for central tumors, whereas computed tomography (CT)-guided transthoracic biopsy generally works better for peripheral lesions. Other feasible methods for tissue diagnosis include: biopsy or fine-needle aspiration from distant metastases or lymph nodes; thoracocentesis with pleural fluid cytology; and pleural biopsy[78]. In Sweden, lung cancer diagnoses are confirmed by histology or cytology in > 90% of cases[82].

An important part of the tissue analysis is molecular profiling of the tumor, which means testing for *EGFR* mutations or *ALK* or *ROS1* rearrangements[83] as these affect the choice of treatment. Traditionally single-gene testing using real-time polymerase chain reaction (PCR) and Sanger sequencing has been viewed as the gold standard for detecting *EGFR* mutations, whereas fluorescence in situ hybridization (FISH) is usually used to detect *ALK* or *ROS1* rearrangements[84-86]. In recent years, mutation detection has moved from single-gene testing towards sequencing of entire genes in order to detect genetic alterations occurring at both hotspot and non-hotspot regions. This high-throughput sequencing technique is known as next-generation sequencing (NGS) and holds great promise for identifying oncogenic driver mutations in NSCLC[87].

In addition to conventional tissue sampling, a new diagnostic method called liquid biopsy is currently in the process of moving from research into clinical practice. This technique refers to blood sampling from cancer patients to detect materials such as circulating tumor cells (CTCs) and circulating tumor DNA (ctDNA) originating from the primary tumor or metastases[88]. Advantages with this technique include its non-invasive sampling and the possibility to gather material from multiple metastatic sites. It provides an alternative for patients who cannot undergo biopsy or where standard biopsies do not provide enough tissue[89]. It can also be used to monitor cancer disease because ctDNA correlates with changes in tumor burden[90]. Studies with NSCLC indicate that ctDNA is suitable for monitoring treatment response and for mutation detection, such as *EGFR* driver mutations or acquired therapy-resistance mutations[89, 91].

Disease Staging

For disease staging, most patients undergo a CT exam of the chest and upper abdomen. This can be done to identify distant metastases, but is not sufficient for evaluating disease spread to mediastinal lymph nodes. For a more accurate staging, especially in patients who are eligible for curative treatment, a positron emission tomography/computed tomography (PET/CT) is performed[92].

PET/CT has a high sensitivity and if there are no enlarged (≥ 15 mm) or PET-positive mediastinal lymph nodes, the risk of mediastinal lymph node metastases is considered to be low[93]. However, a positive PET/CT finding can be due to reasons other than metastases, such as infection or inflammation. Therefore, PET-positive lymph nodes should be verified with invasive techniques if it will affect the treatment decision. According to the European Society of Thoracic Surgeons guidelines, pre-operative invasive mediastinal staging should be performed where there is a central tumor, a PET-negative primary tumor, PET-positive mediastinal lymph nodes, or mediastinal lymph nodes ≥ 15 mm regardless of fluorodeoxyglucose-uptake[94]. Historically, mediastinoscopy has been the gold standard for invasive mediastinal staging of potentially operable patients due to a very high sensitivity and specificity[95]. However, the minimally invasive techniques of endobronchial ultrasound-guided transbronchial needle aspiration (EBUS-TBNA) and endoscopic ultrasound-guided fine needle aspiration (EUS-FNA) have become increasingly used and thereby have reduced the need for mediastinoscopy[96, 97].

Stage classification is performed to describe the anatomic extent of a cancer disease in a common and consistent way. Correct stage classification in NSCLC is of major importance as it helps determine which treatment to choose and affects the prognosis. Staging can be classified as clinical, which is determined by using all available information prior to treatment, or pathologic, which is determined after a surgical resection. Stage classification is based on the tumor-node-metastasis (TNM) staging system. The first edition of the TNM staging system was presented in 1974[98]. Since then, it has undergone several revisions until the current 7th edition from 2009, which is based on data from over 100,000 patients diagnosed between 1990-2000[99, 100].

The T in TNM describes the size and location of the primary tumor and the degree of invasion of nearby tissue. The T stages range from T1 to T4 where T1 and T2 tumors are further subdivided into T1a/T1b and T2a/T2b depending on their size. When the primary tumor is not found, T0 is used to denote the T stage. Cancer *in situ* is designated Tis[101].

The N in TNM describes the involvement of the regional lymph node stations. Depending on which lymph nodes are involved, the N is staged from 0 to 3 where N0 means that there are no involved lymph nodes. A lymph node

map is used to classify the involved lymph nodes from N1 to N3[102]. Direct growth of a primary tumor into a lymph node is classified as nodal involvement and extrathoracic nodal involvement is classified as having distant metastases[101].

The M stage in TNM is binary: either M0 (no distant metastases) or M1 (distant metastases present). M1 is further subdivided, where M1a means that there are separate tumor nodules in the contralateral lung, pleural or pericardial nodes, or any malignant pleural/pericardial fluid, whereas M1b represents any other form of distant metastasis[101]. By combining the T, N and M stages of a tumor, the combined TNM classifies the patient's disease stage. The TNM stage groups range from I-IV with stage I-III being subdivided into a and b groups (see Table 1-2).

Table 1. *TNM definitions*

T/N/M	Description
TX	Primary tumor cannot be assessed
T0	No evidence of primary tumor
Tis	Carcinoma <i>in situ</i>
T1	Tumor ≤ 3 cm in greatest dimension surrounded by lung or visceral pleura, no bronchoscopic evidence of invasion more proximal than the lobar bronchus
T1a	Tumor ≤ 2 cm in the greatest dimension
T1b	Tumor > 2 cm but ≤ 3 cm in the greatest dimension
T2	Tumor > 3 cm but ≤ 7 cm or any of the following: <ul style="list-style-type: none"> • Invades visceral pleura • Involves the main bronchus ≥ 2 cm distal to the carina • Associated with atelectasis/obstructive pneumonitis extending to the hilar region but not involving the entire lung
T2a	Tumor > 3 cm but ≤ 5 cm in the greatest dimension
T2b	Tumor > 5 cm but ≤ 7 cm in the greatest dimension
T3	Tumor > 7 cm or any of the following: <ul style="list-style-type: none"> • Direct invasion of the chest wall, diaphragm, phrenic nerve, mediastinal pleura or parietal pericardium • Involves the main bronchus < 2 cm distal to the carina without involvement of the carina • Associated with atelectasis/obstructive pneumonitis of the entire lung or separate tumor nodules in the same lobe
T4	Tumor of any size that invades any of the following: mediastinum, heart, great vessels, recurrent laryngeal nerve, esophagus, vertebra, carina, or separate tumor nodule(s) in ipsilateral lobe
NX	Regional lymph nodes cannot be assessed
N0	No regional node metastasis
N1	Metastasis in ipsilateral peribronchial and/or ipsilateral hilar lymph nodes and intrapulmonary nodes, including involvement by direct extension
N2	Metastasis in ipsilateral mediastinal and/or subcarinal lymph nodes
N3	Metastasis in contralateral hilar, contralateral mediastinal, ipsilateral or contralateral scalene or supraclavicular lymph nodes
M0	No distant metastases
M1	Distant metastases
M1a	Separate tumor nodules in a contralateral lobe; tumor with pleural nodules or malignant pleural or pericardial effusion
M1b	Distant metastasis in extrathoracic organ(s)

Table 2. Overview of TNM staging

T/M Stage	Subgroup	N0	N1	N2	N3
T1	T1a	IA	IIA	IIIA	IIIB
	T1b	IA	IIA	IIIA	IIIB
T2	T2a	IB	IIA	IIIA	IIIB
	T2b	IIA	IIB	IIIA	IIIB
T3	-	IIB	IIIA	IIIA	IIIB
T4	-	IIIA	IIIA	IIIB	IIIB
M1 (any T)	M1a, M1b	IV	IV	IV	IV

An 8th edition of the NSCLC TNM staging system has been developed and internally validated using the International Association for the Study of Lung Cancer (IASLC) database and will be clinically implemented worldwide in January 2018[103]. Changes from the 7th edition include a further-particularization of the T categories; tumors between 5 and 7 cm are now T3 whereas tumors > 7 cm are classified as T4. Central tumors ≤ 5 cm involving a main bronchus (but not the carina) or causing atelectasis are classified as T2 regardless of the distance to the carina or the size of the atelectasis. Tumors involving the diaphragm are classified as T4. The N categories have not been changed. The M category now distinguishes tumors with a solitary distant metastasis (M1b) from tumors with multiple distant metastases (M1c)[104].

Functional Evaluation

In addition to tissue diagnosis and staging, functional evaluation of the patient is an important part of the diagnostic work-up. It includes a full medical history with tobacco consumption, co-morbidities, and current symptoms taken into account as well as a physical examination[105]. Routine blood samples are analyzed to assess bone marrow function (Hgb, WBC, Plt), electrolyte balance (sodium, potassium), and kidney function (creatinine).

A performance status score is commonly used as a grading tool to quantify patient well-being and daily life activities[106, 107]. The Karnofsky Performance Status (KPS) scoring was introduced in the 1940s and is still frequently used by clinicians[107]. It scores the patient's functional status from 0 (dead) to 100 (normal status) that corresponds to percentage values. Another frequently used scoring system is the Eastern Cooperative Oncology Group (ECOG) score that runs from 0 (asymptomatic) to 5 (dead)[106]. It is sometimes also referred to as the World Health Organization (WHO) or Zubrod score. For comparison of the KPS and the ECOG/WHO scoring systems, see Table 3.

Table 3. Comparison of performance status by KPS and ECOG/WHO scores

Karnofsky Performance Status (KPS)	ECOG Performance Status	Description (KPS)	Description (ECOG/WHO)
100	0	Normal, no evidence of disease	Normal activity
90		Normal activity with only minor symptoms	
80	1	Normal activity with effort, some symptoms	Restricted in physically strenuous activity but ambulatory, cares for self
70		Able to care for self but unable to carry on normal activity	
60	2	Requires occasional assistance, capable of most self-care	Ambulatory >50% of the time, capable of all self-care but unable to work
50		Requires assistance, frequent medical care	
40	3	Disabled, requires special care/assistance	Ambulatory ≤50% of the time, capable of only limited self-care
30		Severely disabled, hospitalization indicated	
20	4	Hospitalization necessary, requires active supportive care	Bedridden, not capable of self-care
10		Moribund	
0	5	Dead	Dead

For patients who are considered eligible for surgery, a pre-operative pulmonary evaluation including a dynamic spirometry and diffusing capacity of the lungs for carbon monoxide (DLCO) test is routinely performed[105]. To be eligible for curatively intended surgery (lobectomy or pneumonectomy), the predicted post-operative forced expiratory volume in 1 second (FEV1) and DLCO have to be at least > 30-40% of the expected values. A pre-operative FEV1 and DLCO > 80% of the expected values is usually sufficient for a pneumonectomy and FEV1, and DLCO > 60% usually enough for a lobec-

tomy without increased risk. Post-operative values of FEV1 and DLCO can be estimated using combined data from spirometry and a ventilation/perfusion scintigraphy[108]. A cardiac stress test is performed; an exercise capacity of 80-100W is usually required for pneumonectomy and 55W for lobectomy[105]. Other risk factors, such as previous or current heart disease, current smoking, and obesity are also taken into account before a decision regarding surgery is made.

A substantial proportion of patients do not pass the pre-operative functional evaluation and for these medically inoperable patients, curatively intended radiotherapy may be an alternative[109]. The greatest limitation of radiotherapy for lung tumors, apart from acute esophagitis, is radiation-induced lung toxicity[110]; hence, a pre-treatment pulmonary evaluation is mandatory. However, the evidence-based recommendations regarding lower limits of respiratory function parameters are not as clear as those for surgically treated patients, partly due to heterogeneity in the scoring systems used to grade radiotherapy-induced lung toxicity[108]. In general, radiotherapy is rarely considered for patients with FEV1 < 1.0 L/s[105]. Patients with pre-existing pulmonary conditions such as COPD (chronic obstructive pulmonary disease) have an increased risk of radiation toxicity, which should also be taken into consideration[111].

Treatment

Surgery

The first successful resection of a lung cancer by pneumonectomy was reported in 1933 by Graham and Singer[112]. In the 1950s, lobectomy was introduced as an alternative when a complete resection of the tumor could be accomplished[113]. Still today, complete resection of the tumor by pneumonectomy or lobectomy is the treatment of choice for stage I-II NSCLC—and selected cases of stage IIIA—when there are no medical contraindications, since this offers the best chance of cure[114].

The type of surgical resection depends on the location of the tumor, the extent of the disease, and the cardiopulmonary condition of the patient[115]. A sublobar resection (segmentectomy or wedge resection) is only recommended for patients who cannot tolerate a lobectomy, as it often carries a higher risk of loco-regional recurrence[116]. Mediastinal lymph node assessment is recommended during surgery for accurate surgical staging; however, the extent of the assessment remains controversial[117].

If the resection is not radical, re-resection is recommended. If this is not feasible, postoperative radiotherapy improves overall survival and should be considered[118]. Postoperative radiotherapy may also be considered in radically resected disease with incidental N2 metastases found postoperatively;

however, the benefit remains controversial[119, 120]. Adjuvant chemotherapy with four cycles of a platinum-doublet yields an absolute survival benefit of 4% at 5 years and has become standard of care for patients in stage II and above, and in high-risk stage IB (primary tumor > 4 cm)[121-123]. For stage IA, adjuvant chemotherapy is associated with worse survival than surgery alone and is not used in clinical practice[124].

Stage IIIB disease is, with very few exceptions[125], considered technically inoperable due to either invasion of mediastinal structures or vertebrae (T4), or involvement of contralateral mediastinal lymph nodes or supraclavicular nodes (N3). In stage IV, surgery may be considered in carefully selected cases of oligometastatic disease, e.g., a single adrenal or brain metastasis, if complete resection of both the primary tumor and the metastasis is possible[126].

Radiotherapy

Radiotherapy plays an important role in NSCLC treatment. Curatively intended radiotherapy can be offered to patients with early stage disease (I-II) who are considered medically inoperable due to co-morbidities, high age, or poor cardiopulmonary function[109]. These patients can be offered conventionally fractionated radiotherapy (typically ≥ 60 Gy in 1.8-2.0 Gy fractions) as an alternative to surgery; however, the local control and survival is substantially poorer than in operated patients[127].

A more recent, increasingly used technique, is high-precision stereotactic body radiotherapy (SBRT), where a few, high-dose fractions are delivered to small target volumes. In the first randomized phase II trial comparing SBRT (66 Gy in 22 Gy fractions) with conventionally fractionated radiotherapy (70 Gy in 2.0 Gy fractions) in stage I NSCLC, there were no differences in PFS (progression-free survival) and OS (overall survival) between the treatments but less toxicity and better quality of life in the SBRT group[128]. In a systematic review of SBRT vs. other types of radiotherapy in early-stage medically inoperable NSCLC, SBRT was associated with similar or improved results in terms of survival and local control and similar or fewer adverse effects[127]. The ESMO guidelines recommend SBRT as the standard of care for stage I NSCLC, and radical radiotherapy using conventionally fractionated radiotherapy for tumors larger than 5 cm or centrally located[129].

In unresectable stage III NSCLC, the combination of conventionally fractionated radiotherapy (60-66 Gy in 1.8-2.0 Gy fractions) and platinum-based chemotherapy is considered the standard treatment; it confers loco-regional control rates of about 40%[130]. The role of chemotherapy in this setting is to act as a radiosensitizer and to eliminate micrometastatic spread of the disease[131]. Numerous studies regarding dose-escalation and alternative

fractionation schedules, including hyperfractionation, have been published; however, the optimal dose-fractionation schedule remains to be determined[132-135]. Radiochemotherapy, compared to radiotherapy alone, has been associated with a statistically significant survival benefit corresponding to an absolute benefit of 4% at two years[136]. In a comparison of concomitant and sequential radiochemotherapy, the concomitant approach has a slightly better overall survival primarily because of a better loco-regional control rate[137-139]. However, it is also associated with higher toxicity, including pneumonitis and esophagitis. Therefore, some patients—due to age, performance status, and co-morbidities—receive sequential treatment instead[137, 140].

Chemotherapy

About 40% of patients with lung cancer present with an advanced disease stage where there are no treatments with curative potential[141]. For many of these patients, chemotherapy is considered the standard of care. However, the use of chemotherapy in NSCLC was disputed well into the 1990s due to its limited efficacy and significant toxicity[142]. The first randomized trial showing a survival benefit of platinum-based chemotherapy over best supportive care was published in 1988[143]. This finding was later confirmed in a meta-analysis of 52 randomized clinical trials[144]. Subsequent studies have confirmed the superiority of combining cisplatin with other drugs versus cisplatin alone[145, 146].

Today, four cycles of a platinum-based doublet chemotherapy—including cisplatin or carboplatin together with a third-generation cytotoxic agent (taxane, vinorelbine, gemcitabine, pemetrexed)—is the first-line treatment of advanced NSCLC in all patients with a good PS (≤ 2) and without an *EGFR* mutation or *ALK/ROS1* gene rearrangement[147]. Large clinical trials have compared the different cytotoxic combination regimens; however, no single regimen has proven to be significantly superior to the other[148-151]. A meta-analysis comparing cisplatin-based combinations versus carboplatin-based combinations showed a slightly longer survival for the cisplatin-containing regimens, albeit at the cost of a higher incidence of non-hematologic toxicity, which is important to consider in a palliative situation[152]. Given the similar efficacy of the different chemotherapy regimens, the choice of which to use has to be individualized, based on factors such as the toxicity profile and patient performance status. Histology is also important in regimen selection as pemetrexed has a survival benefit that is limited to patients with non-squamous NSCLC[153].

Historically, a watch-and-wait strategy has been applied after completion of the pre-defined four cycles of chemotherapy; second-line treatment is

only started at disease progression. However, many patients will progress a few months after completion of first-line therapy and almost half of them will be unable to receive another treatment line due to poor performance status[154]. Therefore, patients with good performance status and disease control after the standard four cycles of first-line chemotherapy may be offered maintenance treatment—until disease progression or unacceptable toxicity—with a single agent non-platinum drug[155]. The theoretic rationale for maintenance therapy is that the early use of non-cross-resistant drugs leads to elimination of more cancer cells before the resistant clones arise[156]. Due to its relatively mild side effects, pemetrexed is the cytotoxic agent with the most promising results in this clinical context. Maintenance pemetrexed was first shown to be associated with significantly improved PFS and OS in a phase III study by Ciuleanu *et al.* The 663 patients with advanced stage NSCLC without progressive disease had four cycles of platinum-based, first-line chemotherapy that did not include pemetrexed. They were then randomized to receive maintenance pemetrexed or placebo[157]. This procedure is referred to as switch maintenance as treatment is switched to an agent with a mode of action different from those in the first-line regimen. In the subsequent PARAMOUNT trial, patients with non-squamous NSCLC who did not progress after four cycles of first-line cisplatin-pemetrexed were randomized to receive maintenance pemetrexed or placebo until disease progression. Also in this trial, maintenance pemetrexed was associated with a significantly prolonged PFS and OS compared to placebo[158]. This strategy is referred to as continuation maintenance because treatment is continued with one of the compounds of the first-line regimen. Although the benefits of switch and continuous maintenance with respect to improved survival have been demonstrated in several randomized controlled trials, there is also an increased toxicity[159, 160]. Therefore, patients have to be selected carefully and maintenance therapy should only be considered in patients with WHO performance status 0-1[160].

In the second-line setting, patients with performance status 0-2 without an *EGFR* mutation or *ALK/ROS1* gene rearrangement, or patients who did not respond to a first-line tyrosine kinase inhibitor may be treated with chemotherapy[147]. Docetaxel was the first drug approved for second-line treatment of NSCLC and may be used in both squamous and non-squamous histology[161, 162]. The second drug approved was pemetrexed, which was compared to docetaxel in a randomized phase III trial of NSCLC patients previously treated with chemotherapy; it showed equivalent efficacy outcomes but fewer adverse effects[163]. Since pemetrexed is more effective in patients with non-squamous histology in the first line, the use of pemetrexed in the second-line setting is also reserved for non-squamous histology[147]. Unlike in the first-line setting, trials comparing mono-chemotherapy with doublet chemotherapy have not found any significant overall survival differ-

ences[164]. Therefore, single-drug chemotherapy remains the standard of care in the second-line setting.

In the third-line setting and beyond, there is not enough evidence to recommend for or against chemotherapy, but it may be offered in selected cases of patients with good performance status[147].

Targeted Therapies

For many years, chemotherapy was the only effective treatment for lung cancer patients with an advanced disease stage. In the 1990s, increasing attention was given to cancer-cell signaling pathways and, in particular, the EGFR pathway[165, 166]. The EGFR is located in the cell membrane and has an intracellular and an extracellular domain. When activated by an extracellular protein ligand (epidermal growth factor, EGF), it is involved in a number of processes important for cancer cell progression including cell proliferation and DNA synthesis[167]. In cancer cells, mutations occur in the *EGFR* that make it constantly activated; this makes it an attractive therapeutic target. In lung cancer adenocarcinomas, *EGFR* mutations are present in 10-15% of cases, particularly in females, never smokers, and patients of Asian descent[168, 169]. The discovery of *EGFR* mutations in NSCLC made possible treatments specifically targeting EGFR. This changed the treatment paradigm from a one-size-fits-all approach to treatment based on the molecular properties of the tumor[170].

The EGFR tyrosine kinase inhibitor (TKI), gefitinib, was the first treatment specifically approved for *EGFR*-mutated NSCLC adenocarcinoma patients following the results of the IPASS trial. There it showed superiority to carboplatin/paclitaxel as a first-line treatment in terms of PFS and ORR (overall response rate)[60]. Shortly following, the EGFR-TKI erlotinib demonstrated the same efficacy in *EGFR*-mutated NSCLC adenocarcinomas, in a comparison with chemotherapy as the first-line treatment[171, 172]. A second generation EGFR-TKI, afatinib, which acts as an irreversible covalent inhibitor of EGFR, has recently been approved for first-line treatment of *EGFR*-mutated NSCLC adenocarcinomas[173, 174]. Treatment with EGFR-TKIs in *EGFR*-mutated NSCLC is associated with high response rates. However, a majority of patients progress after about one year of treatment due to acquired resistance to first and second generation EGFR-TKIs[175]. One known mechanism behind this is the T790M resistance mutation in the *EGFR*, which is found in about 60% of the patients at the time of progression[175, 176]. To overcome this, a third generation EGFR-TKI, osimertinib, selective for both EGFR-TKI sensitizing and T790M resistance mutations in NSCLC, has been developed and it has shown greater efficacy than platinum therapy plus pemetrexed in T790M-positive NSCLC[175].

Other important therapeutic targets in NSCLC include ALK and ROS1. In about 2-5% of NSCLC patients, the ALK transmembrane protein becomes oncogenic due to rearrangement between the *ALK* and the *EML4*-genes. This rearrangement leads to the production of an EML4-ALK fusion tyrosine kinase, which is involved in cell proliferation, differentiation and anti-apoptosis[177]. The *ROS1* oncogene encodes a receptor tyrosine kinase related to ALK. This gene is also activated by chromosomal rearrangement with a selection of partner genes resulting in a ROS1 fusion protein that promotes proliferation and inhibits apoptosis[178]. *ROS1* rearrangements are present in about 1-2% of NSCLC and these rearrangements seem to be mutually exclusive of *ALK* rearrangements[179, 180]. Patients with *ALK* or *ROS1* rearrangements tend to be younger and have a history of light or no smoking[180].

The clinical importance of *ALK* and *ROS1* rearrangements has led to the development of crizotinib, a selective inhibitor of the ALK and MET tyrosine kinases with additional ROS1 kinase inhibitory activity[181]. In ALK-positive patients, crizotinib is superior to standard chemotherapy in terms of PFS and ORR both in first-line and second-line settings[61, 182]. In addition, crizotinib has shown remarkable response rates (72%) in *ROS1*-rearranged NSCLC[62]. A second generation ALK-inhibitor, ceritinib, has been approved for use in the second-line treatment of ALK-positive NSCLC, on the basis of encouraging response rates (58%) from a phase I single arm trial[183]. Recent advances in ALK inhibitor treatment were given much attention at the annual American Society of Clinical Oncology (ASCO) meeting in Chicago in June 2017. Preliminary data were presented from the ALEX randomized phase III trial comparing the new ALK-inhibitor alectinib with crizotinib in patients with previously untreated advanced ALK-positive NSCLC. Alectinib was superior in terms of PFS, less toxic, and associated with an 84% risk reduction of progression in the CNS[184].

Angiogenesis refers to the formation of new blood vessels from pre-existing vasculature and is essential to supply oxygen and nutrition for tumor growth[185]. This process is regulated by complex mechanisms involving angiogenesis-related factors. One of the most potent stimulators of angiogenesis is the signal molecule, vascular endothelial growth factor (VEGF)[186]. The VEGF monoclonal antibody, bevacizumab, inhibits angiogenesis and improves survival for non-squamous NSCLC when added to first-line platinum-based chemotherapy or erlotinib[59, 187]. However, this comes at the cost of an increased risk of side effects, in particular pulmonary hemorrhages, which in some cases have been fatal[59].

Another way of blocking angiogenesis is by targeting the tyrosine kinase activity of pro-angiogenetic receptors. Nintedanib is a triple angiokinase inhibitor that targets the VEGF receptors 1-3, platelet-derived growth factor (PDGF) receptors α and β , and fibroblast growth factors (FGF) 1-3[188]. In the large randomized phase III study LUME-lung 1, docetaxel/nintedanib

was compared with docetaxel/placebo as second-line treatment of NSCLC[189]. Patients who received nintedanib had a significantly longer PFS with a manageable toxicity profile, but there was no difference in OS in the overall population of patients. However, when only taking adenocarcinoma patients into account, nintedanib was associated with a significant increase in both PFS and OS.

Immunotherapy

The interaction between cancer and the immune system has been known for many decades[190]. This interaction is complex and the immune system can both suppress and promote tumor growth[191]. Of the different immune cells associated with antitumor activity, T-cells/T-lymphocytes have received the most attention[191, 192]. Tumor cells can, in their turn, suppress and evade the immune system through various mechanisms[193]. One of the first recorded examples of oncological immunotherapy took place in the late 19th century when New York surgeon William Coley reported regression of sarcomas after injection of bacterial toxins[194]. However, for the treatment of most solid tumors, there has been very little clinical progress in immunotherapy until only recently. A breakthrough came with the discovery of tumor-infiltrating T-cells that up-regulate negative co-stimulatory receptors including cytotoxic T-lymphocyte antigen 4 (CTLA-4), programmed cell death 1 (PD-1) receptor, and several others[195]. Up-regulation of these immune checkpoint proteins leads to T-cell inactivation and inefficient T-cell mediated killing of the tumor cells. These immune checkpoints became targets of a new type of drug called immune checkpoint inhibitors. The first clinical success with these inhibitors was seen in malignant melanoma with the CTLA-4 inhibitor ipilimumab followed by the PD-1 inhibitors nivolumab and pembrolizumab[196-198].

Immune checkpoint inhibitors have also displayed impressive results in NSCLC. In two large phase III studies of nivolumab versus docetaxel in the second-line treatment of patients with squamous[199] and non-squamous[200] NSCLC, the median OS was about 3 months longer for the patients receiving nivolumab. Furthermore, the toxicity was significantly milder in the nivolumab group. In non-squamous NSCLC, the superiority of nivolumab was only seen in patients with a tumor expression of the programmed cell death ligand 1 (PD-L1) of $\geq 1\%$, whereas in the SCC patients, the effect of nivolumab was independent of PD-L1 expression. Similar results in terms of survival benefit and reduced toxicity have been seen for pembrolizumab compared to docetaxel in the second-line treatment of PD-L1-positive NSCLC[201]. Pembrolizumab has also shown better PFS and OS and a lower rate of adverse effects than standard platinum-doublet chem-

otherapy in the first-line setting of EGFR- and ALK-negative NSCLC patients with a PD-L1 expression of $\geq 50\%$ [202]. However, in another randomized phase III trial comparing nivolumab with platinum-based chemotherapy in the first-line setting of advanced NSCLC with a PD-L1 expression of $\geq 5\%$, there were no benefits in terms of PFS or OS for the nivolumab group[203]. As a consequence, pembrolizumab is currently (August 2017) approved for both first- and second-line treatment of advanced NSCLC in Sweden, whereas nivolumab has only been approved for treatment of NSCLC in the second-line setting.

In addition to PD-1 inhibitors, immune checkpoint drugs targeting PD-L1 are receiving increasing attention. One such drug, the monoclonal PD-L1 antibody atezolizumab, significantly improved survival compared to docetaxel in previously treated NSCLC patients; the improvement significantly correlated with PD-L1 expression[204]. These results were confirmed in the OAK randomized phase III trial in which previously treated NSCLC patients receiving atezolizumab had a significantly better overall survival and favorable toxicity profile than patients treated with docetaxel[205]. Atezolizumab has been approved in the USA by the FDA (Food and Drug Administration) for treatment of patients with advanced NSCLC previously treated with chemotherapy. In Europe, atezolizumab is currently awaiting approval by the EMA (European Medicines Agency)[206].

The historical view of lung cancer as a non-immunogenic disease has now changed and extensive research on immune-based treatments is being pursued. Challenges for the future include understanding the mechanisms of resistance to immunotherapy drugs, development of predictive biomarkers, and combining immunotherapy with other treatment modalities for an optimal outcome[191].

Palliative Treatment

Despite all the advances in lung cancer treatment, the disease still carries a poor prognosis and the majority of the patients will not be cured[207]. In many cases, treatment will be given with a palliative intention to prolong survival and/or improve quality of life. Advanced lung cancer is associated with a great burden of symptoms that tend to worsen in the final months of life[208]. In a study of inoperable NSCLC patients with good performance status, the patients reported on average 14.3 symptoms; the most common were fatigue, lack of energy, dyspnea, cough, worrying, and chest pain[209].

The benefits of early introduction of palliative care were demonstrated in a randomized study by Temel *et al.*, where patients with newly diagnosed metastatic NSCLC were randomized to receive either early palliative care integrated with standard oncologic treatment or only standard oncologic

treatment[210]. The addition of early palliative care was associated with higher quality of life and a statistically significant 2.7 months longer median OS. Based on these results, the American Society of Clinical Oncology recommends integrating palliative care into standard oncologic care for patients with metastatic cancer[211].

Radiotherapy plays an important role in the palliative treatment of lung cancer. Palliative radiotherapy of the primary tumor should be considered in cases of local symptoms such as chest pain, hemoptysis, dyspnea, hoarseness, or superior vena cava syndrome[212]. As an alternative to external radiotherapy, endobronchial laser resection can be used to relieve obstruction, particularly with tumors growing into the central airways[213]. Placing an airway stent is another option in this situation[214]. Endobronchial brachytherapy is another palliative option to treat central obstructive lesions and can be used in combination with laser therapy/stenting or on its own[215].

Brain metastases are a relatively common finding in lung cancer patients, occurring in about 25% of cases during the course of the disease[216]. Neurologic symptoms caused by brain metastases can be palliated with corticosteroids to reduce edema, followed by radiotherapy[217, 218]. Stereotactic radiosurgery should be considered when there are one to few metastases; otherwise, whole brain radiotherapy is the main option[218]. Radiotherapy is also an effective way to treat painful bone metastases. Usually, a single fraction of 8 Gy is enough to provide adequate pain control[219].

Prognosis

Survival

The prognosis for patients diagnosed with NSCLC is poor. The National Cancer Institute's Surveillance, Epidemiology, and End Results (SEER) database in the USA has reported a 5-year survival rate of 18% across all stages, based on data from 2006-2012[207]. Similar survival results are seen in data from the Swedish National Lung Cancer Registry which includes all patients diagnosed with NSCLC from 2002 to 2015[82]. However, > 75% of patients are diagnosed in stage III-IV[82] and there are major survival differences for the different clinical stages. In stages IA and IB NSCLC, 5-year survival is about 50% and 40%, respectively[220, 221]. In selected cases of resected T1a tumors, 5-year survival rates of 90% have been reported[222]. The survival rates rapidly decrease with increasing tumor stage; for stage II the 5-year survival rate is about 25-30%, and for stage IIIA and IIIB the survival rate is about 15-20% and 5-10%, respectively[220, 221]. Patients in stage IIIA who are eligible for surgery have a 5-year survival of about 25%, however the surgical approach is only possible for a minority of these pa-

tients due to either technical or medical inoperability[223]. For patients with clinical stage IV NSCLC, the 5-year survival rate is only about 1-2% and the median overall survival in this group is about 6 months[220, 221]. Stage IV is, however, very heterogeneous, ranging from a single or a few distant metastases to generalized dissemination in multiple organs and the prognosis varies accordingly. In a meta-analysis of 757 NSCLC patients with control of the primary tumor and one to five distant metastases that were all treated with locally ablative therapy, the median OS was 26 months and 5-year OS was 29%[224]. Thus, although the prognosis in stage IV NSCLC is poor, some carefully selected patients with oligometastatic disease may benefit from aggressive treatment[225].

Prognostic Versus Predictive Factors

Accurate estimation of survival is one of the core skills of medicine. It has special importance in oncology for the planning of treatment strategies and for avoiding harm, discomfort and unnecessary therapies in vulnerable patients[226, 227]. Unfortunately, accurate survival estimations are often hard to achieve and doctors tend to be overoptimistic[228, 229].

Prognostic factors identify patients with different risks of a specific outcome (such as disease progression or survival) regardless of the treatment given[230]. Prognostic factors can be patient-related (e.g., performance status, age, gender), or tumor-related (e.g., clinical stage, histological type). The purpose of prognostic markers is to identify subpopulations of patients with significantly different anticipated outcomes, and who therefore might benefit from different treatment strategies. A prognostic factor, however, does not give information regarding the benefits of a specific therapy. Patients with many beneficial prognostic factors may require less aggressive treatment to achieve a cure, while patients with many negative prognostic factors may require additional treatment to achieve the same survival as patients without these factors. The opposite could also be true, for example if the patient has many bad prognostic factors—this might warrant shifting from an aggressive treatment strategy with curative intent towards a less aggressive, palliative treatment.

Predictive factors are patient- or tumor-related characteristics that predict benefit (in terms of treatment response or survival) from a specific treatment[230]. Thus, there will be differences in treatment response or survival in patients who receive a specific treatment depending on the predictive factor in question. In some cases, a factor can be both prognostic of shorter survival and predictive for a specific treatment (see Table 4-6).

Table 4. *Prognostic factor example*

	Median survival (months)		
	No Treatment	Treatment A**	Treatment B**
Factor X*			
Present	4	8	8
Not present	8	16	16

*Factor X is prognostic of shorter survival

** Treatment A and B are considered equally effective

Table 5. *Predictive factor example*

	Median survival (months)		
	No Treatment	Treatment A**	Treatment B**
Factor Y*			
Present	8	16	24
Not present	8	16	16

*Factor Y is predictive for therapy response from treatment B

** Treatment A and B are considered equally effective

Table 6. *Prognostic and predictive factor example*

	Median survival (months)		
	No Treatment	Treatment A**	Treatment B**
Factor Z*			
Present	4	8	12
Not present	8	16	16

*Factor Z is prognostic of shorter survival and predictive for therapy response from treatment B

** Treatment A and B are considered equally effective

Prognostic Factors

TNM Stage

The strongest prognostic factor in NSCLC is the clinical stage according to the TNM classification[231]. The International Association for the Study of Lung Cancer (IASLC) international database of > 80,000 lung cancer cases is used to assess the impact of three, independently strong, prognostic factors (tumor size, nodal involvement, and distant metastases); the results are extensively validated and regularly updated by the IASLC[232]. However, there is considerable heterogeneity within each stage group, with some patients rapidly progressing in their disease and others surviving a long time with no recurrence. Thus, there is a need to find patient- and tumor-related characteristics to stratify patients within each stage group and individualize treatment accordingly[233].

Performance Status

Performance status is a tool to quantify the general well-being and daily activities of patients. In addition to being simpler to use than the KPS, the ECOG score has shown better prognostic ability in lung cancer patients and is therefore more commonly used for performance status assessment in NSCLC[234]. One concern regarding the use of performance status is its inherent subjectivity and the weak-to-moderate correlation in grading between the patients themselves and the physician[235]. Nevertheless, performance status is one of the most extensively studied prognostic factors and its prognostic significance has been demonstrated in several major malignancies[236-238]. The correlation between worse performance status and shorter survival in NSCLC has been consistently demonstrated in the literature[239].

Gender

Gender has been widely studied as a prognostic factor in NSCLC and with female gender being associated with longer survival[239]. This could be due to biologic differences between the genders and/or confounding factors associated with female NSCLC patients, such as young age, smoking habits or histology[240, 241].

A meta-analysis of 39 articles looked at gender and survival differences for NSCLC in > 80,000 patients. Survival for the women was significantly longer than for the men, irrespective of disease stage, histology, and smoking status[241]. The etiology for the better prognosis in women is not fully understood but underlying biologic differences such as gender hormones (in particular estrogen), and variations in mutation rates of regulating gene pathways seem to play an important role[240, 242, 243].

Age

Age has been frequently studied as a prognostic factor in NSCLC; however, the results are inconsistent[244, 245]. Despite the high incidence of elderly patients (> 65 years) diagnosed with cancer, these patients are often underrepresented in clinical trials[246, 247]. Although a randomized trial comparing single agent vinorelbine with best supportive care in patients \geq 70 years showed a significant survival benefit and an improved quality of life for the chemotherapy group[248], the use of cisplatin-based doublets in elderly patients is controversial[249].

In a comprehensive review of the literature, 39 studies examined the prognostic impact of age on survival; four reported a significant finding. Of

these four, older age was associated with better outcome in three of them and worse outcome in one[239]. Thus, the prognostic value of chronological age is weak and current recommendations state that treatment strategies should rather be guided by functional status and comorbidities[250, 251].

Histology

The prognostic and predictive value of histology in NSCLC received increased interest after the discovery that non-squamous patients treated with pemetrexed had longer overall survival than SCC patients[58, 252]. However, although histology is considered to be a predictive factor for treatment with pemetrexed, the prognostic role is still unclear, despite extensive research.

On the basis of data from an IASLC staging project including > 12,000 patients, SCC histology was associated with better overall survival, but only statistically significant for patients in stage IIIA[253]. In a comprehensive review of the literature, 31 studies examined the prognostic impact of histology on survival. Of these, five studies (16%) reported a significant association—adenocarcinoma histology was associated with better outcome in four of the them and SCC histology was in one[239].

Weight Loss

Involuntary weight loss is an ominous sign in oncology and its negative impact on prognosis has been reported in numerous malignancies, including NSCLC[239, 254]. In addition, loss of skeletal muscle mass (sarcopenia) seems to be a strong negative prognostic factor, independent of weight loss[255].

In the literature, weight loss is often categorized by the percentage of body weight lost over a specific time[239]. The current international definition of cancer cachexia is weight loss >5% over the previous 6 months (in patients with a BMI < 20, >2%), or signs of sarcopenia[256]. Patients who manage to stabilize their weight during treatment have a better prognosis than those patients who continue to lose weight[257].

Anemia

Anemia is usually defined as a decrease in the oxygen-carrying capacity of blood due to a decrease in the amount of red blood cells or hemoglobin (Hgb) in the blood[258]. Anemia in adults was defined by a WHO study

group in 1968 as Hgb < 130 g/L for men and < 120 g/L for non-pregnant women[259]. Although these cut-offs are still used today, they are to a certain extent arbitrary as mild anemia (defined as Hgb 100-129 g/L in men and 100-119 g/L in women) can be found in about 10% of people > 65 years[260].

Anemia is common in cancer patients and $\geq 50\%$ of cancer patients will be anemic at some point during the course of their disease; about 20% will require blood transfusion[261]. Anemia in cancer patients is often multifactorial and can be caused by disruption of hematopoiesis due to metastatic bone marrow infiltration, tumor-related bleeding, or cytokines produced by the tumor itself[261]. The role of anemia as a prognostic factor in NSCLC is controversial—some studies support a negative prognostic effect of pre-treatment anemia[262-265] whereas others show no such correlation[266]. One possible reason for a negative prognostic impact of anemia is that it contributes to tumor hypoxia, which has been associated with increased resistance to radiotherapy and chemotherapy in several types of cancer[267, 268].

Leukocytosis

Leukocytosis refers to an abnormally high number of white blood cells (WBC) in the peripheral blood and it is frequently found in patients with NSCLC[269]. It can be caused by one or a combination of factors including infection, bone marrow metastasis, and corticosteroid treatment[270]. However, some patients with NSCLC present with leukocytosis without any apparent reason; these patients may be considered to have tumor-related leukocytosis (TRL)[271]. This paraneoplastic syndrome is mainly caused by production and secretion of hematopoietic cytokines by the tumor, including granulocyte colony-stimulating factor (G-CSF), granulocyte macrophage colony-stimulating factor (GM-CSF), and interleukin 6 (IL-6)[270, 271].

Leukocytosis has been associated with poor prognosis in several trials of early and advanced stages of NSCLC[264, 271-274]. The reason is not fully elucidated and leukocytosis is currently regarded as an epiphenomenon of a high, intrinsic biologic aggressiveness of the underlying cancer[270].

Thrombocytosis

Thrombocytosis refers to an abnormally high level of platelets (Plt) in the blood circulation and its association with malignant disease has long been recognized[275]. Similarly to leukocytosis, thrombocytosis can be a paraneoplastic phenomenon caused by increased cytokine production by the

tumor[276]. It has been a negative prognostic factor for survival in lung cancer in several studies[272, 276-283]. However, there are also studies showing no such association, including a pooled analysis of the North Central Cancer Treatment Group trials with data from > 1000 patients with advanced stage NSCLC[264].

The mechanism behind an association between thrombocytosis and poor prognosis is currently not clear. Several theories have been proposed including that thrombocytosis might facilitate cell invasion and metastasis formation by affecting the blood vessel endothelium[284-286].

Aims of the Thesis

General Aim

The general aim of this thesis was to identify accurate and readily available prognostic factors in patients with NSCLC treated with curatively intended radiotherapy and in patients with recurrent disease.

Specific Aims

- To retrospectively assess the prognostic value of histology in patients with NSCLC treated with curatively intended radiotherapy between 1990 and 2000 in Sweden (**Paper I**).
- To retrospectively assess the prognostic value of the pre-treatment hematopoietic blood parameters Hgb, WBC, and Plt in the same patient dataset (**Paper II**).
- To verify the prognostic value of histology and pre-treatment Hgb, WBC, and Plt in a pooled dataset from two phase II studies of patients with stage III NSCLC treated with curatively intended chemoradiotherapy between 2002 and 2007 in Sweden (**Paper III**).
- To verify the prognostic value of histology and pre-treatment Hgb, WBC, and Plt in a dataset from a multicenter phase II study of patients with previously treated stage IIIB/IV NSCLC receiving palliative systemic treatment with docetaxel, or the insulin-like growth factor 1 receptor (IGF-1R) modulator AXL1717 (AXL), between 2011 and 2013 (**Paper IV**).

Patients and Methods

Papers I and II

Patients with NSCLC subjected to curatively intended radiotherapy (defined as total radiation dose ≥ 50 Gy) between 1990 and 2000 at any of the 15 departments of radiation oncology in Sweden were retrospectively identified with the help of the medically responsible doctor at the respective site. The patients' radiation charts and medical records were reviewed and data regarding the following variables were collected: gender, age, histology, TNM stage, pre-treatment blood parameters of Hgb, WBC and Plt, all treatment in addition to radiotherapy, occurrence of relapse, and cause of death. Patient- and treatment-related characteristics were analysed in relation to overall survival.

The aim was to include all eligible patients at all 15 treatment sites, but one site did not make the data for all patients available. Data were also missing for the investigated variables for some patients. These patients, however, were not excluded from the study unless the missing data were required for survival estimation. In total, 1,146 patients were identified and available for further analysis. Follow-up data was collected until the end of 2008.

The reference limits for thrombocytosis (Plt $>350 \times 10^9/L$) and leukocytosis (WBC $>9.0 \times 10^9/L$) were based on the normal intervals used at the Uppsala University Hospital. Hgb <110 g/L was used to define anemia in both men and women.

Paper III

Data were collected retrospectively from two Swedish phase II trials of patients with stage IIIA/IIIB NSCLC treated with curatively-intended radiochemotherapy.

The RAKET trial[287], conducted from 2002 to 2005, was a three-arm randomized trial of 151 patients with NSCLC stage IIIA/IIIB treated with two cycles of induction chemotherapy (carboplatin AUC 6 and paclitaxel 200 mg/m^2) followed by either: A) hyperfractionated accelerated radiotherapy 1.7 Gy twice a day to 64.6 Gy concurrent with a third cycle of chemotherapy; B) radiotherapy with 2 Gy daily to 60 Gy concurrent with daily

paclitaxel 12 mg/m²; or C) radiotherapy with 2 Gy daily to 60 Gy concurrent with weekly paclitaxel 60 mg/m².

The Satellite trial[288], conducted from 2006 to 2007 was a one-arm phase II trial of 71 patients with NSCLC stage IIIA/IIIB treated with two cycles of induction chemotherapy (cisplatin 75 mg/m² and docetaxel 75 mg/m²) followed by radiotherapy, 2 Gy daily, to 68 Gy concurrent with weekly cetuximab (initial dose of 400 mg/m² followed by 250 mg/m²). Data regarding gender, age, histology, WHO performance status, weight loss, TNM stage, and the blood parameters of Hgb, WBC, and Plt at enrollment were collected for all patients and analyzed in relation to overall survival. Follow-up data were available until 2011 for the patients in the RAKET trial and until 2010 for the patients in the Satellite trial.

Paper IV

Data were collected from a randomized, multicenter phase II study (AXL003) conducted from 2011 to 2013 in five Eastern European countries with 99 previously treated stage IIIB or IV NSCLC patients with AC or SCC histology[289]. In the primary study treatment period, the patients were treated in 21-day cycles for up to four cycles with either the IGF-1R modulator AXL1717 (AXL) twice a day as an oral suspension, or docetaxel 75 mg/m² on day 1 of each treatment cycle. Baseline data, including gender, age, histology, WHO performance status, and the pre-treatment blood parameters of hemoglobin (Hgb), white blood cells (WBC), and platelets (Plt) were collected for all patients and analyzed in relation to overall survival. Follow-up data were available until December 2014.

Statistics

The patient characteristics and treatments were presented using standard descriptive statistics. Overall survival was analysed using Kaplan-Meier product-limit estimates. Survival curves for different subgroups were compared using the log-rank test. The follow-up time was calculated from the date of diagnosis (Papers I & II), date of enrollment (Paper III), or date of randomization (Paper IV) until death or last follow-up date. Patients lost to follow-up were censored at the last date known alive. Age was categorized into four age groups in Papers I-III and into two groups in Paper IV. Overall survival was analysed using Cox proportional hazards regression models; both univariate and multivariate Cox analyses were performed. Results were presented as hazard ratios with 95% confidence intervals (95% CI). P-values <0.05 were regarded as statistically significant.

In **Paper I**, only patients with AC or SCC histology were included in the statistical analyses; patients with other histology or lacking data on histology were excluded. Two multivariate models were used—one included histology and the patient-related factors gender and age at diagnosis and the other also included stage and additional treatments (surgery and chemotherapy).

In **Paper II**, analyses were performed on all patients in the dataset. Two multivariate models were used—one included hematological markers (Hgb, WBC and Plt), gender, age at diagnosis, stage, and additional treatments (surgery and chemotherapy), and the other also included combinations of pathological laboratory markers.

In **Paper III**, no univariate Cox-analyses were performed and only one multivariate model including gender, age, histology, performance status, weight loss, stage, Hgb, WBC, and Plt was used. In addition, correlation between WBC and Plt levels was analyzed using Spearman's correlation coefficient. A comparison of the Plt levels in patients with PS 0 and PS 1 was performed using Mann-Whitney's test and illustrated with a Tukey's boxplot.

In **Paper IV**, no univariate Cox-analyses were performed and only one multivariate model including gender, age, histology, performance status, Hgb, WBC, Plt and treatment arm was used.

Results

Paper I

Of the 1146 patients in the dataset, 919 patients were diagnosed with either AC or SCC histology and thus included in this study. A majority of the patients were men (68%) and SCC was the predominant histology (65%). For the patients with AC, the gender distribution was roughly equal, whereas the male proportion was higher in patients with SCC (76%). All TNM stages were represented; however, most patients were diagnosed with TNM stage IIIA-IIIB (61%). The age range was 25-86 years and the median age was slightly lower in the AC group (63 years) than in the SCC group (66.5 years). The mean radiation dose given was roughly the same in the AC and SCC groups (59 and 58 Gy respectively). The prevalence of patients receiving chemotherapy (induction and/or concomitant) was higher in the AC group (37%) than the SCC group (26%). The prevalence of surgical treatment prior to radiotherapy was also higher in the AC group (33%) than in the SCC group (23%).

The median overall survival of all 919 patients was 14.8 months (95% CI 14.1 to 16.2 months) and the 5-year survival rate was estimated to be 9.5%. Patients with AC had a significantly longer median overall survival (17.0, 95% CI 15.0–19.4 months) compared to patients with SCC (14.0, 95% CI 12.8–14.9 months); the difference was statistically significant ($p=0.0063$, log-rank test). The overall survival for patients with AC and SCC histologies is shown in Figure 1. The survival benefit in AC patients was confirmed in a univariate Cox analysis and in two multivariate models. The effect of histopathology was adjusted for the patient-related factors gender and age in the first model, and also for the treatment-related factors surgery, stage, and chemotherapy in the second model.

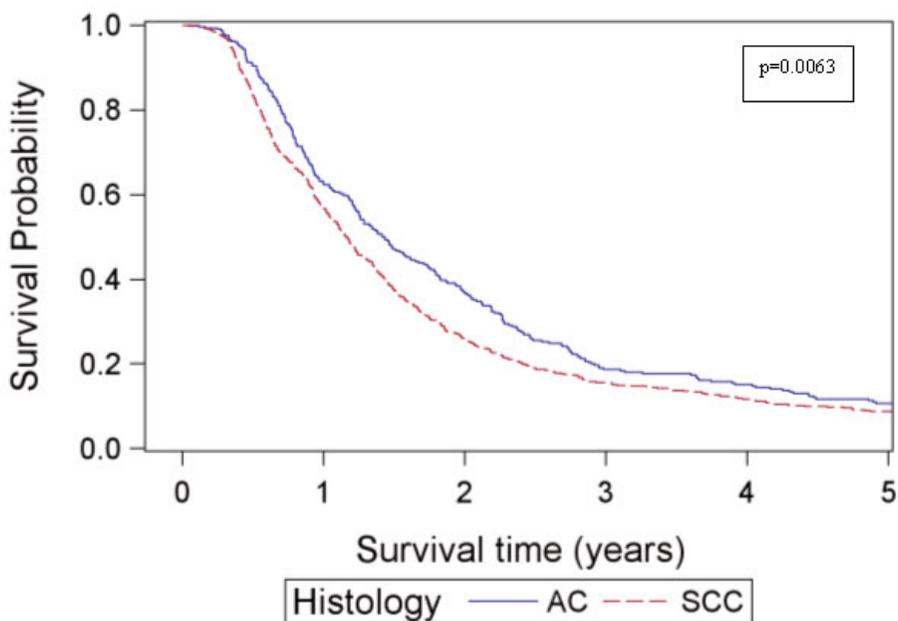


Figure 1. Overall survival for patients with AC and SCC histologies. Figure reproduced from Paper I.

Paper II

Of 1146 patients, data were available for 835 patients for at least one of the pre-treatment blood parameters Hgb, WBC, and Plt; 818 patients had data for all three. Histologically, 56% of the patients had SCC, 28% had AC, and 15% had other histology. With respect to patient- and treatment-related characteristics, there were no major differences from those in Paper I.

For the patients with data for all three blood parameters (n=818) the median survival was 13.9 (95% CI: 12.7-14.7) months. The median overall survival was significantly shorter for patients with anemia at diagnosis—11.2 (95% CI: 9.6 -13.1) months compared to 14.5 (95% CI: 13.4-15.4) months for patients without anemia (p=0.0032). Patients with leukocytosis had a median survival of 11.6 (95% CI: 10.8-13.6) months whereas patients with normal WBC at baseline had a median survival of 15.4 (95% CI: 14.0-17.0) months, which was significantly longer (p<0.0001). For patients with thrombocytosis, the median survival was 11.2 (95% CI: 10.0-12.7) months, whereas patients with normal Plt levels had a median survival of 14.9 (95% CI: 13.9-16.4) months, which was significantly longer (p<0.0001).

The statistically significant, negative prognostic impact on survival for anemia, leukocytosis, and thrombocytosis was demonstrated in a multivariate Cox-regression analysis that also included gender, age, stage, surgery and chemotherapy. When examining combinations of pathological pre-treatment blood parameters (Figure 2), the median survival was 16.0 (95% CI: 14.2-17.9) months in the group without pathological results in the studied parameters. In contrast, the median survival was 8.0 (95% CI: 6.9-10.0) months in the group with pathological results in all three blood parameters ($p<0.0001$).

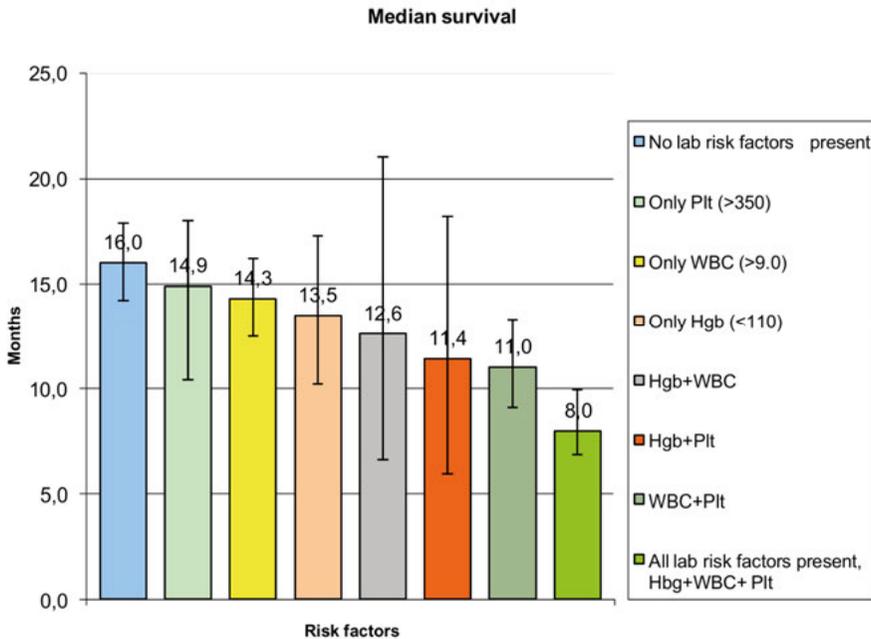


Figure 2. Median survival (in months) for patients with single or combinations of pathological pre-treatment blood parameters of Hgb, WBC and Plt. Figure reproduced from Paper II.

Paper III

In the pooled dataset of the RAKET and Satellite studies, 222 patients were available for analysis. Of these, half (51%) were men and the median age was 62 years. Histologically, 34% had SCC, 48% had AC, and 17% had other types of histology. Concerning WHO performance status, 123 patients (55%) were PS 0, whereas the other 99 patients (45%) were PS 1. The patients were either in TNM stage IIIA (35%) or stage IIIB (65%).

The median overall survival was 17.7 (95% CI: 15.1-21.5) months. The median survival was similar in the RAKET study, 17.8 (95% CI: 14.9-22.0)

months and the Satellite study, 17.0 (95% CI: 14.7-25.0) months. Patients with AC and SCC histology had similar median overall survival, 20.1 (95% CI: 15.8-25.0) and 18.3 (95% CI: 13.9-26.3) months, respectively, whereas patients with other histology had a shorter median survival; 13.0 (95% CI: 7.3-19.6) months. The difference was not statistically significant ($p = 0.099$).

Patients with anemia (Hgb < 110 g/L) had a median survival of 12.5 (95% CI: 7.9-53.7) months, and patients without had 18.2 months (95% CI: 15.9-22.9); however, the difference was not statistically significant ($p = 0.38$, log-rank test). Patients with leukocytosis (WBC > $9 \times 10^9/L$) had a median survival of 14.9 (95% CI: 10.5-19.8) months whereas patients with a normal WBC at baseline had 22.5 (95% CI: 17.8-29.0) months. This difference was statistically significant ($p = 0.016$, log rank test). Likewise, patients with thrombocytosis (Plt > $350 \times 10^9/L$) had a shorter median survival of 14.5 (95% CI: 12.0-18.2) months compared to 23.7 months (95% CI: 20.1-33.6) for the patients with normal Plt at baseline (Figure 3). The difference was statistically significant ($p = 0.0025$, log rank test).

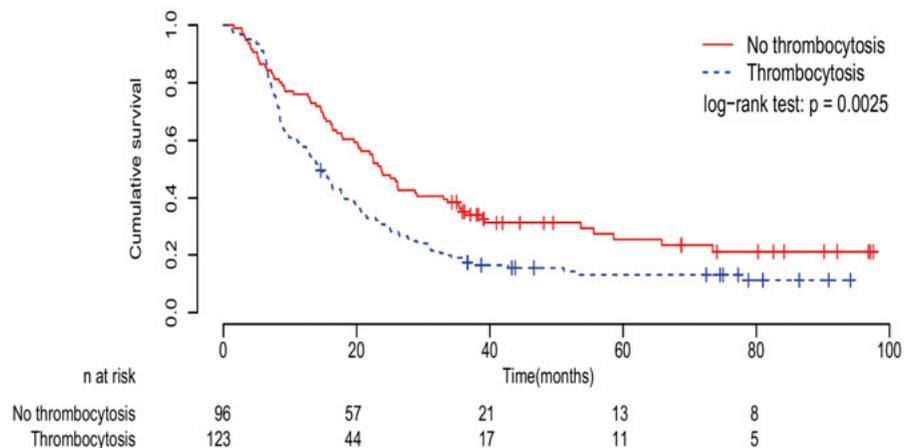


Figure 3. Overall survival in patients with and without thrombocytosis. Figure reproduced from Paper III.

In a multivariate Cox analysis that included gender, age, histology, PS, weight loss, TNM stage, anemia, leukocytosis, and thrombocytosis, the prognostic significance was still evident for PS (HR: 1.61, 95% CI: 1.15-2.26, $p = 0.0051$) and thrombocytosis (HR: 1.66, 95% CI: 1.12-2.48, $p = 0.012$), whereas leukocytosis was no longer significantly associated with worse overall survival (HR: 1.09, 95% CI: 0.75-1.58, $p = 0.64$). WBC and Plt correlated with a Spearman's correlation coefficient of 0.44 ($p < 0.0001$) and higher Plt levels were seen in patients with PS 1 than in patients with PS 0 ($p = 0.037$, Mann-Whitney's test).

Paper IV

The dataset of the AXL003 study contained 99 patients. Of these, 58 were treated with AXL and 41 were treated with docetaxel. Most patients were men (72%) and the median age was 57 years (range: 42 – 81 years). The proportion of AC (49%) and SCC (51%) histology was almost equal. Most patients (72%) were considered to be PS 1, 24% were PS 0 and only 4% were PS 2.

The median overall survival for all patients was 8.9 (95% CI: 6.1-13.6) months and there was no statistically significant survival difference between the patients receiving docetaxel and those receiving AXL. Patients with SCC histology had a median overall survival of 8.1 (95% CI: 6.6-13.3) months compared to 12.6 (95% CI: 4.9-18.3) months for patients with AC histology. The difference did, however, not reach statistical significance ($p = 0.12$, log rank test).

Patients with anemia had a median survival of 6.1 months (95% CI: 3.9-18.3) and 9.4 months (95% CI: 6.5-15.0) for patients without, a non-significant difference with an HR of 1.63 (95% CI: 0.91-2.93, $p = 0.097$, log rank test). Patients with thrombocytosis had a median survival of 4.2 months (95% CI: 3.5-23.6) and those without had 12.6 months (95% CI: 6.6-16.1). This was a non-statistically significant difference with an HR of 1.54 (95% CI: 0.93-2.65, $p = 0.094$, log rank test). Patients with leukocytosis had a median survival of 4.2 months (95% CI: 3.2-11.3) and those without had 12.3 months (95% CI: 7.5-17.1), see Figure 4. This difference was statistically significant with an HR of 2.10 (95% CI: 1.29-3.43, $p = 0.002$, log rank test).

In a multivariate Cox analysis including gender, age, histology, WHO performance status, treatment arm, anemia, leukocytosis, and thrombocytosis, only leukocytosis had a prognostic significance (HR: 1.83, 95% CI: 1.06-3.13, $p = 0.029$).

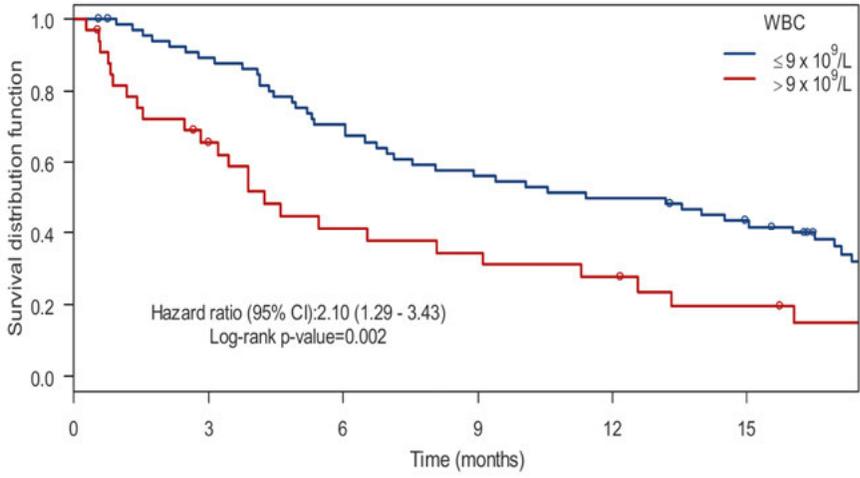


Figure 4. Overall survival in patients with and without leukocytosis. Figure reproduced from Paper IV.

Discussion

In Papers I-IV, we investigated prognostic factors for NSCLC with special emphasis on histology (Paper I) and three pre-treatment blood parameters (Papers II-IV). We investigated prognostic factors in patients treated with curatively intended radiotherapy, with or without chemotherapy (Papers I-III), and patients receiving systemic, palliative treatment after disease recurrence (Paper IV). Two of the studies focused on patients in common clinical practice outside of clinical trials (Papers I-II), and two studies included patients from specific clinical phase II trials (Papers III-IV). The studies included data from the 1990s (Papers I-II), 2000s (Paper III) and 2010s (Paper IV).

Paper I included 919 patients with AC or SCC histology. Two-thirds of them were men and two-thirds had SCC histology. This reflects the epidemiological situation in Sweden in the 1990s when SCC was the most prevalent histology and NSCLC was significantly more common in men than in women[8]. Since then, a shift in NSCLC epidemiology has made AC the most common histology and nowadays the incidence of NSCLC is almost equal in men and women in Sweden[8, 10]. Stage III was the most prevalent (61%) TNM stage, which is as expected since curatively intended radiotherapy is the standard treatment for these patients. The percentage of patients receiving treatment in addition to radiotherapy (surgery or chemotherapy) was higher in the AC than the SCC group. The more aggressive treatment approach in the AC group is likely because this group is usually younger and thus more suitable for chemotherapy and surgery.

The median overall survival of 14.8 months and the 5-year survival rate of 9.5% for the whole patient cohort is in line with the results of previous reports of NSCLC treated with curatively intended radiotherapy in the 1990s[290, 291]. The median overall survival was significantly longer in the AC group than the SCC group (17.0 months and 14.0 months, respectively). The survival benefit in AC patients was confirmed in two multivariate Cox analyses in which patient- and treatment-related factors were taken into consideration. On the basis of these results, AC histology is independently associated with better overall survival in patients with NSCLC treated with curatively intended radiotherapy.

Histology is controversial as a prognostic factor in NSCLC. In a large, retrospective study by the IASLC, SCC histology was a prognostic factor for better overall survival, but the result was only significant for stage IIIA[253].

This differed from the results in Paper I, where the prognostic impact of histology was most pronounced for stage II, and AC, rather than SCC histology, was associated with better survival. In a Japanese study of 83 patients with stage I NSCLC receiving curatively intended radiotherapy (60-80 Gy in 2 Gy fractions), there was no statistically significant difference in 5-year overall survival for AC and SCC[292]. However, the 5-year primary control rate of SCC was significantly poorer than that of AC, whereas the opposite was seen for the 5-year metastasis-free survival; this suggests that the failure pattern is histology dependent. This histologically related failure pattern is also seen in a large, retrospective study, of over 1400 patients treated with curatively intended irradiation alone and 350 patients treated with radiochemotherapy[293]. In this study, the authors found that death with no clinical progression was more likely with SCC than non-squamous histology regardless of treatment. Furthermore, SCC was more likely to progress at the primary site than AC, whereas AC was more likely to metastasize to the brain and other distant sites.

Hirsch *et al.* made a comprehensive review of over 400 publications (phase II and III studies, retrospective studies, and meta-analyses) on the prognostic and predictive value of histologic subtype for clinical outcome in NSCLC. They concluded that the data were inconsistent. Some studies suggested more favorable outcomes for patients with AC, others for SCC, and some no difference at all[252]. The reason for this could be that histology is indeed not significantly prognostic for NSCLC outcome. However, it could also be due to study design issues, such as insufficient sample sizes, heterogeneous study populations, and inadequate statistical analyses. It could also be due to the difficulties in classification of histology. Within one histological subtype, such as AC, multiple subtypes with different prognosis may exist. Another issue concerns misclassification of tumors due to small samples and inter-observer and inter-institutional variations[294, 295]. In addition, the definition of histologic subtypes in NSCLC has changed over time, making comparisons between studies difficult[54, 55].

In **Paper II**, the same dataset was used as in Paper I, but the exclusion criteria yielded a slightly different patient cohort. Patients with anemia, leukocytosis, and/or thrombocytosis at diagnosis had a worse overall survival than patients without these laboratory abnormalities. Furthermore, combinations of these pathological pre-treatment blood parameters resulted in a worse survival than single abnormalities.

Anemia has been associated with a poor response to radiotherapy in several cancers including cervix cancer[296], head and neck cancer[297] and NSCLC[298]. Hypoxia may directly contribute to treatment resistance by reducing the level of oxygen-derived free radicals in the tumor, which are needed for the cytotoxic actions of radiotherapy and chemotherapy[268]. Tumor hypoxia may also indirectly affect resistance through modulation of

gene expression and cell-cycle position, leading to malignant progression[267, 268]. Thus it is reasonable to attribute the prognostic value of pre-treatment anemia to tumor hypoxia.

Leukocytosis in cancer patients is commonly caused by infection, corticosteroid treatment, or bone marrow metastases. However, leukocytosis can also occur in absence of these precipitating factors as a paraneoplastic phenomenon caused by production of hematopoietic cytokines by the tumor. This cytokine production has been associated with a higher intrinsic biologic aggressiveness of the tumor which might explain the poorer prognosis in these patients[270].

Similarly, thrombocytosis can be caused by increased cytokine production by the tumor, also indicating a more aggressive disease[276]. Results from previous studies suggest that thrombocytosis can facilitate tissue invasion and metastatic spread by affecting the blood vessel endothelium[284-286]. The combined prognostic effect of pre-treatment anemia, leukocytosis, and thrombocytosis has been reviewed previously in a study of patients with NSCLC treated surgically; these results are in accordance with the findings in Paper II[273].

The main strengths of Papers I and II are the size of the patient cohorts and the thorough manual review of individual medical records and radiation charts for each patient. Main limitations include the retrospective nature of the studies, the uncertainty regarding histological classification, and the heterogeneity of the patient cohort treatments, (curative radiotherapy alone, chemoradiotherapy, or postoperative radiotherapy). Another important weakness is the lack of data regarding performance status, which is an established prognostic factor that could have had a significant impact on the multivariate models. Also, the actual coverage of patients receiving curatively intended radiotherapy during the specified time period is a topic of discussion. In Sweden, 8,163 patients were diagnosed with stage III NSCLC from 1990 to 2000[8] whereas there were only 1,146 patients included in the dataset of Papers I and II. However, it cannot be expected that all patients in stage III during the time period were treated with curatively intended radiotherapy. Some were likely treated with palliative chemotherapy or received only best supportive care.

In **Paper III**, we attempted to obtain a more homogeneous patient cohort. To do so, prognostic factors found in Papers I and II were tested within the context of a clinical trial setting. A pooled dataset was created through a retrospective gathering of data from the RAKET and Satellite phase II trials, which had a similar study design[287, 288]. This dataset included an almost equal number of men and women; AC rather than SCC was the dominant histology; and the median age was a few years lower than in Papers I and II. The trials in Paper III were conducted between 2002 and 2007, and thus the patient characteristics are more representative of the recent findings that

show an increasing prevalence of ACs and female patients[10]. The median overall survival was higher in this study than in Papers I and II despite that only patients with locally advanced disease (stage IIIA/IIIB) were included. This is likely due to the inherent selection of patients with good PS (0-1), i.e., they were deemed fit enough to receive chemoradiotherapy in the clinical trial setting of Paper III unlike the more average patient populations in Papers I and II.

Patients with SCC and AC histology had similar median overall survival, whereas patients with other histology had significantly shorter survival. Thus, we were unable to confirm the results from Paper I, in which AC histology was associated with a more favorable prognosis. One reason could be that histology has a stronger prognostic value in early disease stages. There could also have been other subgroups of patients within the larger patient population in Paper I, who were not present in Paper III, where histology had a prognostic significance. The smaller sample size of Paper III also made it more difficult to obtain statistically significant results.

Anemia was associated with a shorter median survival although the difference was not statistically significant. The survival difference was similar to the findings from Paper II, and the lack of significance might be explained by the small number of patients in the anemia group (n = 21). Patients with leukocytosis had significantly shorter median survival than patients with a normal WBC at baseline. Likewise, patients with thrombocytosis had a significantly shorter overall survival than patients with normal Plt at baseline.

These results are in line with the findings in Paper II. However, in the multivariate analysis—which included potentially confounding factors such as PS—only thrombocytosis retained a statistically significant association with shorter survival. Thrombocytosis correlated with worse performance status and both of these were independently associated with shorter median survival with similar hazard ratios in the multivariate analysis. Unlike in Paper II, leukocytosis was not independently associated with shorter survival in the multivariate analysis. There was a significant correlation between leukocytosis and thrombocytosis and even though both may present as markers of systemic inflammation, thrombocytosis seems to have the greater prognostic value in stage III NSCLC.

The mechanisms behind the effects of thrombocytosis on NSCLC prognosis are unclear. In an *in vitro* study by Li *et al.*, platelets promoted migration of lung AC cells when cultured together with them. Furthermore, in a mouse model, AC cell lines co-infused with platelets led to more lung metastases than infusions with the AC cells alone[299]. This suggests that thrombocytosis might favor hematogenous spread of NSCLC; the elevated platelets in stage I-III may indicate disease spread that is not visible on radiological imaging. In several retrospective studies of patients in stage I-III NSCLC, elevated platelet counts are associated with poorer prognosis[277, 281-283]. In addition, an association has been demonstrated between throm-

bocytosis in newly diagnosed lung cancer and metastasis to bone, soft tissue, lymph nodes, and malignant pleural effusion[300].

The main strength of Paper III is the clear definition of and similarity in treatment of the patients—i.e., stage III NSCLC in PS 0-1 treated with curatively intended chemoradiotherapy in a phase II trial setting. Nevertheless, there were some sources of heterogeneity. First, the data came from two separate cohorts with patient populations treated slightly differently. Second, stage III NSCLC consists of a heterogeneous population of lesions (i.e., T4N0-3, T3N1-3, and T1a-2aN2-3) where the significance of different prognostic factors may vary.

The main purpose of **Paper IV** was to further examine the validity of the prognostic factors found in the previous papers and to investigate the generalizability of these prognostic factors in NSCLC. The dataset included patients that had been given systemic treatment in a palliative setting rather than curatively intended radiotherapy. As expected, the median overall survival in this cohort was shorter than in the previous papers and, like in the original study report, neither of the treatments (AXL vs. docetaxel) was significantly superior to the other in terms of survival[289]. The patient characteristics differed somewhat from those of the previous cohorts, with a clear male predominance and an almost equal number of AC and SCC histologies.

Like in Paper I, patients with SCC histology had a shorter median overall survival than those with AC histology, and like in Papers II and III, patients with anemia, leukocytosis or thrombocytosis also had a shorter median overall survival than patients without these abnormalities. However, only leukocytosis displayed a statistically significant association with shorter survival, in both univariate and multivariate analysis. A reasonable explanation for this is that, due to the relatively small number of patients (n=99), the study lacked power to estimate the impact on survival. Not even poor PS—an established prognostic factor in NSCLC[239]—was significantly associated with shorter survival in either the univariate or the multivariate analysis. In this context, the statistically significant hazard ratio attributed to leukocytosis, despite the small sample size, becomes even more noteworthy.

In addition to leukocytosis, other surrogate markers of systemic inflammation have received increased interest in recent years. One closely related blood parameter, a high pre-treatment neutrophil-to-lymphocyte ratio (NLR), has been associated with poorer outcome in various cancer types[301]. In NSCLC, this association has been shown in two meta-analyses[302, 303]. In both of them, the hazard ratio was higher for the patients treated non-surgically, suggesting a higher prognostic significance in a more advanced disease. In a recently published retrospective study of 175 patients treated with the PD-1 inhibitor nivolumab, pre-treatment NLR was independently associated with poorer OS and PFS[304]. It is unclear, however, whether the

effect was prognostic or predictive. More studies are needed to determine the usefulness of leukocytosis in this clinical setting.

Similarly to Paper III, the main strength of Paper IV is its homogenous and well-defined patient cohort, i.e., previously treated stage IIIB or IV that had received palliative treatment with either docetaxel or AXL1717 in a phase II trial setting. A main disadvantage is the small sample size which makes statistical analyses more uncertain. Other limitations include the retrospective nature of the study and the *post hoc* analyses of data that were not planned in the original study protocol.

On the basis of the results from this thesis, AC histology may be associated with a more favorable prognosis for patients with NSCLC treated with curatively intended radiotherapy. The reason for this is unknown although one explanation could be histology-dependent biological differences that lead to different failure patterns. However, the observed difference in median overall survival was small and the results could not be verified in a more homogenous chemoradiation cohort where PS was taken into account as a confounding factor. Thus, the potential prognostic role of histology in NSCLC treated with curatively intended radiotherapy is still unclear.

Our results also indicated that pre-treatment anemia, leukocytosis, and thrombocytosis were associated with shorter survival for patients with NSCLC treated with curatively intended radiotherapy. However, in a more homogenous chemoradiation cohort, where PS was taken into account as a confounding factor, only thrombocytosis was independently associated with shorter survival. In contrast, in a cohort of patients with recurrent NSCLC given systemic treatment, only leukocytosis was independently associated with shorter survival. One reason for this could be that thrombocytosis is an indicator of undetected metastatic disease. This would explain the poorer prognosis in stage III NSCLC treated with a curative intent and the smaller impact on the prognosis for the patients already known to be metastatic. On the other hand, leukocytosis might be a more general marker of tumor aggressiveness. More research is needed to validate these findings and to clarify the underlying mechanisms.

Given the poor prognosis and the significant side effects associated with treatment of advanced stage NSCLC, it is important to have as much available prognostic information as possible at the start of treatment. However, apart from TNM stage and PS, few prognostic factors are routinely used in clinical practice. Thus, there is a need for additional prognostic factors, which should preferably be accurate, non-expensive, and readily available for all patients.

A complete blood count including Hgb, WBC and Plt is one of the most common blood analyses carried out at a hospital. This low-cost procedure is simple and every NSCLC patient will have had this blood test before referral to the specialist physician. For the patient presenting with inoperable

NSCLC, concurrent chemoradiotherapy is the standard of care. Although the benefit of chemoradiotherapy over radiotherapy alone has been associated with a fairly modest survival benefit[136], there is currently no way to tell in advance who will benefit from the addition of chemotherapy. At the same time, there is sometimes a desire to omit concurrent chemotherapy in order to reduce toxicity[140]. In this situation, pre-treatment thrombocytosis, as an indicator of increased risk of metastatic spread, could be a useful addition to other prognostic information in making decisions regarding the aggressiveness of treatment.

In the setting of NSCLC relapse, adequate prognostic information is vital both to decide on further palliative treatment and to provide the patient with realistic information about the future. Patients with PS ≥ 2 have a poor prognosis[305] and many aspects have to be carefully considered before giving another line of treatment. In this situation, WBC could be a valuable addition to other available prognostic information. The benefits of early introduction of palliative care in metastatic NSCLC has been demonstrated previously[210]. By combining prognostic factors, including leukocytosis, the physician can better decide when to introduce a palliative discussion with the patient.

Conclusions

- The prognostic role of histology in NSCLC is unclear. A prognostic significance in a subgroup of patients cannot be ruled out, but further studies are needed in order to verify this.
- Routine pre-treatment hematopoietic blood parameters (Hgb, WBC, Plt) can be used to support treatment decisions in NSCLC when considered together with other prognostic factors such as stage and performance status.
- Thrombocytosis as a prognostic factor may be of particular interest in patients with stage III NSCLC eligible for curatively intended chemoradiotherapy as thrombocytosis was shown to be associated with shorter survival in this setting.
- Leukocytosis as a prognostic factor may be of particular interest in patients with recurrent NSCLC eligible for systemic palliative treatment, as leukocytosis was shown to be associated with shorter survival in this setting.

Future Perspectives

- Determination of the optimal cutoff points for anemia, leukocytosis, and thrombocytosis that would provide the most prognostic information in NSCLC.
- Investigation of the prognostic value of thrombocytosis in prospective trials that include patients with stage III NSCLC.
- Investigation of the prognostic value of leukocytosis in stage IV NSCLC with emphasis on patients with PS 2 and patients treated with immunotherapy.
- Creation of a composite prognostic index for patients with NSCLC that can be used to guide treatment strategy.

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Sammanfattning på svenska (Summary in Swedish)

Lungcancer är den cancersjukdom som orsakar flest dödsfall såväl i Sverige som globalt. I Sverige avlider varje år närmare 4000 människor till följd av sjukdomen. Lungcancer delas utifrån mikroskopiskt utseende (histologi) in i två huvudtyper, småcellig lungcancer (SCLC) och icke-småcellig lungcancer (NSCLC) där NSCLC är vanligast och står för ca 80% av alla lungcancerfall.

Prognosen vid NSCLC är dålig med en 5-årsöverlevnad på ca 15-20% för alla sjukdomsstadier tillsammans. Detta kan till stor del förklaras av att över 75% av patienterna diagnostiseras i ett sjukdomsstadium där cancer inte går att operera bort. Om sjukdomen är inoperabel men inte har hunnit sprida sig till andra organ är strålbehandling, med eller utan tillägg av cytostatika, den enda möjligheten till botande behandling. Strålbehandling erbjuder en chans till bot men prognosen är ändå relativt dålig med en 5-årsöverlevnad på ca 15%. Dessutom är behandlingen förenad med en risk för allvarliga biverkningar, som i vissa fall kan vara livshotande. Hos patienter som får återfall i NSCLC efter tidigare behandling är prognosen mycket dålig. I vissa fall finns det möjlighet att ge ytterligare medicinsk behandling i tumörbromsande syfte medan man i andra fall får inrikta sig på enbart symptomlindrande behandling.

För att kunna anpassa behandlingsstrategin för den enskilda patienten finns ett stort behov av att finna s.k. prognostiska faktorer som ger information angående patientens förväntade överlevnad. Förutom TNM-stadium, som anger tumörsjukdomens utbredning i kroppen, är patientens funktionsnivå (performance status) den mest etablerade prognostiska faktorn vid NSCLC. Dock finns det betydliga variationer i prognos även hos patienter inom samma stadium och funktionsnivå varför behovet av ytterligare prognostiska faktorer är stort. På senare år har intresset för den prognostiska betydelsen hos vanliga rutinblodprover vid NSCLC ökat. Studier på dessa faktorer samt på andra kliniska prognostiska faktorer vid NSCLC som ålder, kön, histologi, m.m. har påvisat vissa samband med överlevnad men resultaten har inte varit helt samstämmiga. Få studier har gjorts specifikt på prognostiska faktorer hos patienter som strålbehandlats för NSCLC eller hos patienter som fått återfall i sjukdomen.

I den här avhandlingen har den prognostiska betydelsen av tumörhistologi, samt blodprovsvärden för hemoglobin, vita blodkroppar

(leukocyter) och blodplättar (trombocyter) vid NSCLC analyserats. Detta har gjorts genom en manuell insamling av data från patientjournaler hos de patienter som har genomgått strålbehandling mot NSCLC i botande syfte under åren 1990-2000 i Sverige. Genom statistiska analyser av dessa data har samband mellan tumörhistologi och patienternas överlevnad undersökts i delarbete ett. I delarbete två har liknande analyser utförts för att undersöka om det finns något samband mellan blodprovsvärden för hemoglobin, leukocyter och trombocyter vid behandlingsstart och överlevnaden hos patienterna. I delarbete tre har resultaten från delarbete ett och två testats retrospektivt på ett patientmaterial som genomgått kombinerad strål- och cytostatikabehandling mot NSCLC i botande syfte inom ramen för två fas 2-studier som genomfördes i Sverige under åren 2002-2007. I delarbete fyra har resultaten från delarbete ett och två testats retrospektivt på ett patientmaterial som genomgått palliativ medicinsk behandling mot återfall av NSCLC inom ramen för en multicentrisk fas 2-studie som genomfördes i Östeuropa under åren 2011-2013.

Resultaten visar att hos patienter som strålbehandlats i botande syfte mot NSCLC är skivepitelhistologi (SCC), lågt hemoglobinvärde (anemi), högt antal vita blodkroppar (leukocytos) och högt antal blodplättar (trombocytos) associerat med kortare överlevnad. Vid fördjupad analys visar det sig att trombocytos har störst betydelse för prognosen hos patienter som genomgår strålbehandling i botande syfte medan leukocytos har störst betydelse för prognosen hos patienter med återfall i sjukdomen efter tidigare behandling. Resultaten kan ge vägledning i situationer där många prognostiska faktorer behöver vägas samman för att kunna ta beslut om behandling. Genom att utnyttja avvikande blodprovsvärden i kombination med andra prognostiska faktorer kan välavvägda beslut fattas avseende om behandlingsstrategin ska vara aggressiv med sjukdomsbot som målsättning alternativt mer palliativ inriktad.

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