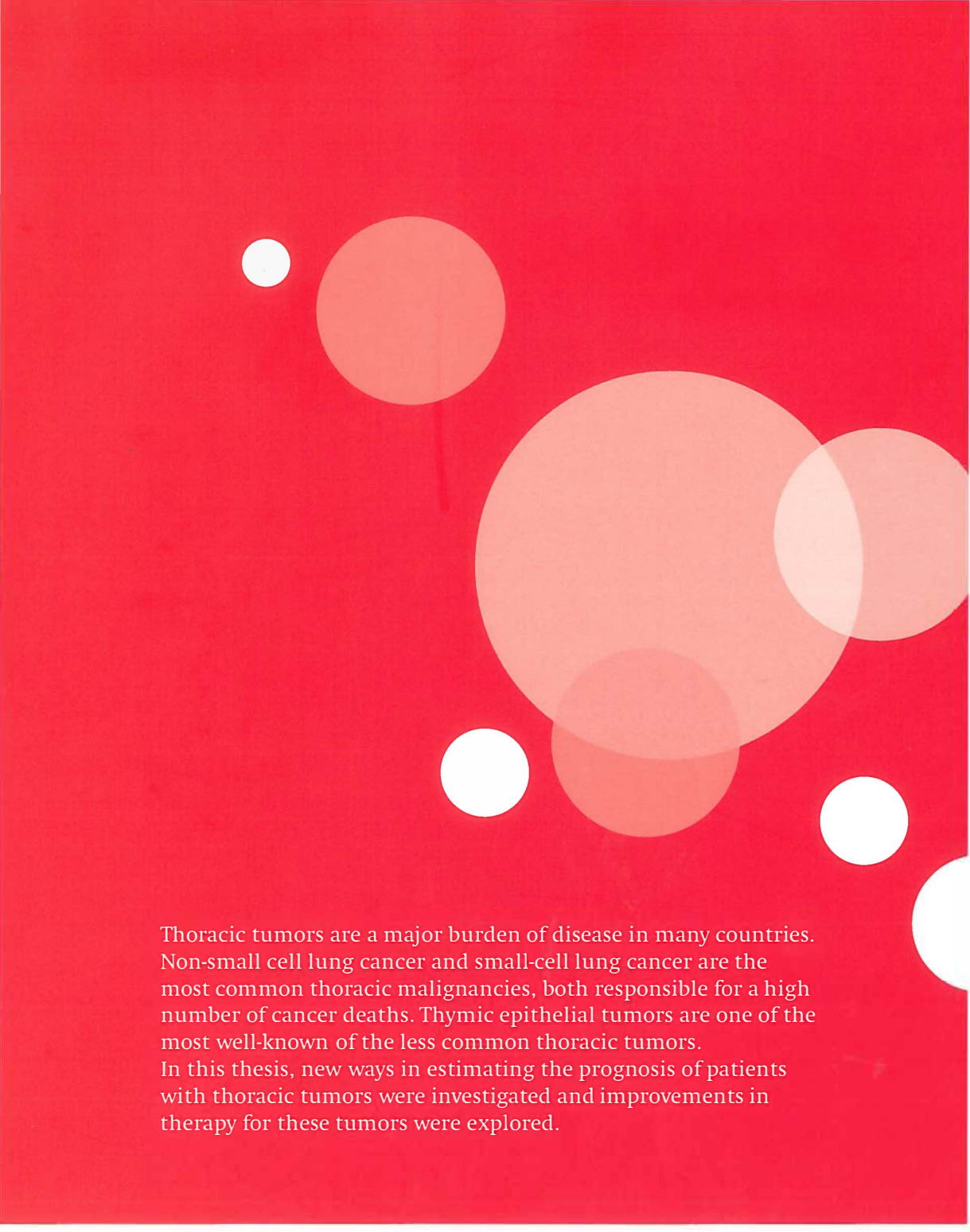
Thoracic tumors: prognostic and therapeutic improvements

Wouter Karst de Jong



Thoracic tumors are a major burden of disease in many countries. Non-small cell lung cancer and small-cell lung cancer are the most common thoracic malignancies, both responsible for a high number of cancer deaths. Thymic epithelial tumors are one of the most well-known of the less common thoracic tumors.
In this thesis, new ways in estimating the prognosis of patients with thoracic tumors were investigated and improvements in therapy for these tumors were explored.

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Stellingen

behorend bij het proefschrift

Thoracic tumors: prognostic and therapeutic improvements

door Wouter Karst de Jong

1. De dalende populariteit van het kleincellig longcarcinoom onder onderzoekers is een kwalijke zaak en dient gekeerd te worden.

2. Prognostische modellen gebaseerd op laboratorium parameters in combinatie met de klinische conditie van een patiënt voorspellen de levensverwachting van patiënten met een kleincellig longcarcinoom even goed als modellen gebaseerd op een combinatie van beeldvorming en klinische conditie. *(dit proefschrift)*

3. Patiënten met een resectabel niet-kleincellig longcarcinoom kunnen met behulp van de mediane waarde van SUVmax in twee goed te onderscheiden prognostische groepen ingedeeld worden. *(dit proefschrift)*

4. Promoter methylatie is voornamelijk een eigenschap van endobronchiale tumorcellen, en niet van andere endobronchiale epitheelcellen blootgesteld aan tabaksrook. *(dit proefschrift)*

5. De meerderheid van patiënten met een epitheliale thymustumor heeft kans op tumor-gerelateerde sterfte; een thymoom mag dus niet beschouwd worden als goedaardig. *(dit proefschrift)*

6. Een incomplete resectie, in combinatie met aanvullende therapie, is te prefereren boven helemaal geen resectie voor epitheliale thymustumoren. *(dit proefschrift)*

7. In Nederland dient een landelijk thymomenpanel opgericht te worden om de diagnostiek en therapie voor deze zeldzame tumoren te optimaliseren.

8. De combinatie cyclofosfamide, doxorubicine en etoposide is obsoleet geworden als eerstelijns behandeling voor patiënten met extensive disease kleincellig longcarcinoom. *(dit proefschrift)*

9. Derdelijns chemotherapie is een goede behandelingsoptie voor patiënten met kleincellig longcarcinoom in een goede conditie. *(dit proefschrift)*

10. Het adagium "stage dictates treatment" wordt mogelijk in de toekomst vervangen door "stage and molecular profile dictate treatment".

11. De functies "CC" en "BCC" in het emailverkeer worden te vaak gebruikt.

12. Fluimen kennen vele toepassingen binnen de longgeneeskunde; fluimen in het openbaar is echter eerder onsmakelijk dan klinisch waardevol.

13. Praatjes vullen wèl gaatjes in de agenda.

Groningen, 6 februari 2008

CIP-GEGEVENS KONINKLIJKE BIBLIOTHEEK, DEN HAAG

De Jong, W.K.
Thoracic tumors: prognostic and therapeutic improvements

Proefschrift Groningen. –Met lit. opg. – Met samenvatting in het Nederlands

ISBN 978-90-367-3243-7

Lay-out and cover: Helga de Graaf, Studio Eye Candy te Groningen,
(www.proefschrift.info).
Printed by: Drukkerij van der Eems, Heerenveen.

RIJKSUNIVERSITEIT GRONINGEN

Thoracic tumors: prognostic and therapeutic improvements

Proefschrift

ter verkrijging van het doctoraat in de
Medische Wetenschappen
aan de Rijksuniversiteit Groningen
op gezag van de
Rector Magnificus, dr. F. Zwarts,
in het openbaar te verdedigen op
woensdag 6 februari 2008
om 14.45 uur

door

Wouter Karst de Jong
geboren op 10 februari 1977
te Noordlaren

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Paranimfen: Mr. V. van Dorst
Dr. J. Cohen

Publication of this thesis was generously supported by:
Integraal Kankercentrum Noord-Nederland, Rijksuniversiteit Groningen, Oncomethylome Sciences SA, Eli Lilly Nederland BV, Astellas Pharma Europe, Pfizer BV, Novartis Pharma BV, Cephalon BV, Roche Nederland BV, Teva Pharmachemie, Sanofi-Aventis Nederland BV, Janssen-Cilag BV, Actelion Pharmaceuticals Nederland BV, Amgen BV, Meda Pharma BV, Boehringer Ingelheim BV, Groningen University Institute for Drug Exploration, AstraZeneca BV, Bayer Schering Pharma, Zambon Nederland BV, LEO Pharma, Merck Sharp & Dohme BV.

Their support is gratefully acknowledged.

A pessimist sees the difficulty in every opportunity;
an optimist sees the opportunity in every difficulty.

Sir Winston Churchill

The broad-backed hippopotamus
Rests on his belly in the mud;
Although he seems so firm to us
He is merely flesh and blood.

"The hippopotamus" by T.S. Elliot

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Treatment of thoracic tumors: small-cell lung cancer

Chapter 1

Introduction

Thoracic tumors are common, and arise from intrathoracic organs, such as lung, oesophagus, lymphatic organs, pleura, neurogenic tissue, or thymus but can also be metastases from other malignancies. This thesis will focus on two of these thoracic tumors, namely lung cancer and thymic epithelial tumors.

Lung cancer is the most common thoracic tumor, and has a world-wide age-standardized incidence of 40-80 per 100,000 each year in males and 10-20 per 100,000 in females[1]. The incidence of lung cancer is still increasing, especially in females and in the developing world. The recent temporal changes in incidence of lung cancer in the Netherlands are not known. The overall 5-year survival of all lung cancer patients is approximately only 14%[2]. Altogether, lung cancer is the leading cause of cancer-related death, and is responsible for almost 9000 deaths annually in the Netherlands[3] and over 160,000 deaths in the United States each year[4]. Lung cancer is therefore a major burden for both patients and health care.

Lung cancer is generally divided into small-cell lung cancer (SCLC) and non-small cell lung cancer (NSCLC). Approximately eighty-five percent of all lung cancers are NSCLC, and 15% or less are SCLC. NSCLC and SCLC combined constitute the overall majority of pulmonary tumors. A very heterogeneous group of tumors together forms the remaining 1-2% of intrapulmonary tumors. These rare other tumors may develop from orthotopic pulmonary tissue in the lung, from ectopic tissue in the lung, or from lymphatic tissue.

One of the relatively more known intrathoracic tumors other than lung cancer are thymic epithelial tumors. These tumors arise from the thymus gland in the anterior mediastinum. Most thymomas and thymic carcinomas are diagnosed and staged by pulmonologists.

This thesis will discuss improvements in the estimation of prognosis and progress in therapeutic options of both very common thoracic tumors (NSCLC and SCLC) and of rarer thoracic tumors. To better introduce the subject of this thesis, first an overview of the current knowledge on NSCLC, SCLC, and thymic epithelial tumors will be described. At the end of this chapter, the aims and outline of this thesis will be presented.

Non-small cell lung cancer

The sudden increase in the incidence of lung cancer cases in the 1930s and 1940s was from early on recognized as the probable result of the increasing popularity of tobacco smoking, with over 80% of all males smoking at that time[5]. However, it lasted until 1954 before the relationship between smoking and lung cancer was formally established in the famous "British Doctor Study" by Austin Bradford Hill and Richard Doll[6]. In effect, over 90% of all lung cancers are caused by tobacco smoke[7]. In the Netherlands, it is estimated that 92% of all lung cancers in males are caused by smoking[8]. This also means that approximately 10% of all lung cancers occur in never-smokers (mostly adenocarcinoma), which is therefore as common as myeloma in men or cervical cancer in females[7]. The chance of life-long smokers

to develop lung cancer is about twenty times as high as compared to life-long never smokers[9]. To continue, the risk of lung cancer is higher in (former) smokers with a higher number of packyears and/or the presence of chronic obstructive pulmonary disease (COPD, or smoking-related decline in lung function)[10,11]. Apart from tobacco smoking, few other risk factors for lung cancer have been identified, of which exposure to radon and asbestos are the most important[7]. The role of environmental tobacco smoke (passive smoking) in the development of lung cancer becomes more and more defined[12-14]. Passive smoking increases the (low) risk of lung cancer in never smokers by 20-30%. Although lung cancer tends to occur more frequently in some families, and some polymorphisms have been associated with an increased risk of lung cancer[15], a postulated true genetic predisposition for lung cancer has not been detected to date. Despite the overwhelming evidence that smoking causes lung cancer (and not to forget other conditions such as COPD and cardiovascular disease), about 35% of all Dutch adults still smoke (www.cbs.nl). Smoking cessation therefore remains a big challenge for governments, patients, and physicians.

NSCLC is generally subtyped into adenocarcinoma (45-50%), squamous cell carcinoma (30-40%) and (undifferentiated) large cell carcinoma(15-20%)[16]. These percentages have not been constant during the years in many countries. The relative portion of squamous cell carcinoma has decreased, while in the meantime the relative number of adenocarcinomas is rising, especially among women[16-18]. This shift in histopathological pattern is likely caused by the introduction of the low-tar filter cigarette[19]. This type of cigarette requires deeper inhalation, and therefore results in a more peripheral deposit of the carcinogenic substances. Adenocarcinomas tend to have a more peripheral localization than squamous cell carcinomas and small-cell lung cancer[20]. Thus it is postulated that the introduction of the filter cigarette is responsible for the relative increase in the number of adenocarcinomas. Recent information about changes in histological subtypes in the Netherlands is lacking.

Lung cancer develops over the course of many years through the subsequent accumulation of genetic damage in susceptible pulmonary cells. Over 50 potential carcinogens have been identified in tobacco smoke[21], of which the polycyclic aromatic hydrocarbons are the most well-known. On the molecular level, multiple critical alterations have been described, including loss of tumor suppressor genes, activation of (proto)oncogenes, deregulation of apoptosis, sustained angiogenesis, and tissue invasion[22]. These alterations consist of genetic processes such as mutation, loss of heterozygosity, and microsatellite instability, and epigenetic phenomena like aberrant DNA-methylation. Chromosome 3p is the most frequently involved chromosome in lung cancer pathogenesis, containing among others the RASSF1A, RAR-ß, and FHIT tumor suppressor genes. Other well known involved genes are the tumor-suppressor genes p53 and p16, the (proto)oncogenes k-RAS, c-MYC, and EGFR, and the Bcl-2, BAX, and survivin genes involved in apoptosis[22]. It is estimated that at least 20 genetic and epigenetic abnormalities are required before clinically evident lung cancer develops[23].

One of the most well-known epigenetic processes is DNA-methylation[24]. DNA-methylation refers to the binding of a methyl group to a cytosine nucleotide in the DNA sequence. Methylation is facilitated by the DNA methyl transferase enzyme. Methylation preferentially occurs if this cytosine base is followed by a guanine nucleotide. In some areas of the genome, especially in the promoter regions, these CpG dinucleotides are common, the so-called CpG islands. Hypermethylation, i.e. the binding of numerous methyl groups in a promoter region, leads to the transcriptional inactivation of the involved gene. In this way, tumor-suppressor genes can be silenced. Aberrant methylation can be detected using methylation-specific PCR. In lung cancer, aberrant methylation of at least 40 genes has been observed, of which RASSF1A, RAR-β, APC, and P16 are most well-known[25,26]. Inhibition of the DNA methyl transferase enzyme by for instance 5-aza-2'-deoxycytidine reverses hypermethylation and gene silencing[27]. Altogether, the presence of aberrant methylation might offer new opportunities in diagnosis, early detection, prevention, and therapy of lung cancer[26], but first a better understanding of the occurrence and spread of methylation events is required. In addition, the value of methylation tests in order to identify people at high-risk for lung cancer is currently not well-defined.

Parallel to the increasing number of (epi)genetic abnormalities, a histological spectrum of premalignant and pre-invasive lesions develops[28], with lung cancer being the end-point of both the molecular and histological spectra (Figure 1). This histological range is most extensively characterized for squamous cell carcinoma, from cellular hyperplasia and metaplasia to squamous dysplasia and carcinoma in situ. Most of these precursor lesions are invisible using white-light bronchoscopy or macroscopical examination. The use of autofluorescence bronchoscopy and microscopical techniques could reveal these lesions. All these structural abnormalities carry a risk of progression, but it is difficult to quantify the risk of progression of squamous dysplasia[28,29]. Similarly, atypical adenomatous hyperplasia, and probably also bronchoalveolar carcinoma, are established as precursors of adenocarcinoma. Again, little is known about the rate of progression from premalignant to malignancy of these lesions[28]. More data are coming from lung cancer screening studies.

The classical lung cancer patient is portrayed as a male of approximately 60 years old, with COPD and other co-morbidities. Obviously, this is not true for all patients. Although the average age at diagnosis is indeed around 60 years for both males and females[16], age at diagnosis ranges from 30 to 100 years old[3]. In addition, one or more co-morbidities, such as COPD or cardiovascular disease, are present in 45% of patients younger than 60 years, and in more than 75% of patients older than 70[30]. The vast majority of patients are symptomatic at the time of presentation[31]. Asymptomatic lung cancers are either incidental findings at imaging performed for other indications or are detected in lung cancer screening programs. Symptoms can be caused by the primary lesion or by local or distant spread of the tumor. None of the symptoms is pathognomonic for lung cancer.

The most common symptom of lung cancer is cough, with or without sputum

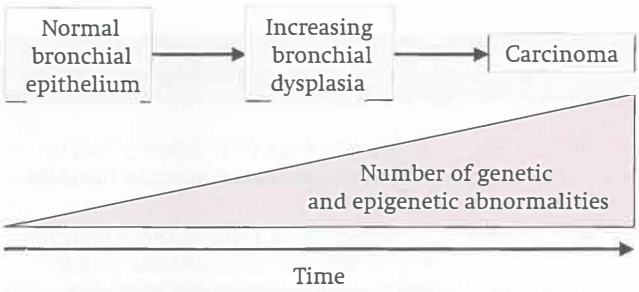

Figure 1. Model for the histologic, genetic, and epigenetic progression in the development of (squamous cell) lung cancer.

production, in 45-70% of patients[31,32]. Also dyspnea is very common. Hemoptysis in variable volumes occurs in 20-30% of patients with lung cancer. One quarter to one half of all patients experiences mild or more severe chest pain. Local spread of tumor may lead to pleural effusion, hoarseness, superior vena cava syndrome, and invasion of the brachial plexus (Pancoast tumor). Symptoms of metastases depend on the location, with bone pain or neurological symptoms being the most common. Paraneoplastic syndromes are not very common in NSCLC. General symptoms may include weight loss, anorexia, fatigue, and fever.

If lung cancer is suspected in a patient, it is important to obtain a pathological diagnosis and to evaluate the locoregional and distant spread of the tumor. The diagnostic and staging procedures are usually performed in concert, because the choice of the optimal diagnostic method is influenced by the stage of the tumor[33]. Several diagnostic procedures are used, each with their own specificity, sensitivity, and clinical applicability[33]. Almost every patient with suspected lung cancer will undergo a flexible fiberoptic bronchoscopy in order to examine the endobronchial situation and to obtain a tumor biopsy, endobronchial brush, or bronchioalveloar fluid for pathological examination. Other methods for obtaining a diagnosis are sputum cytology, fine needle aspirate of lymph nodes or metastatic lesions, or surgical procedures such as mediastinoscopy. Whether methylation tests on sputum or endobronchial brushes may improve the diagnostic process remains to be resolved. After one or more diagnostic procedures, a definitive diagnosis is established in the vast majority of the patients.

In this thesis, lung cancer is clinically staged according to the TNM classification from 1997, where T stands for Tumor, N for (lymph) Nodes, and M for Metastasis (Table 1)[34].

The TNM status of a patient is primarily established by non-invasive imaging techniques, eventually followed by invasive procedures to obtain pathological confirmation of suspected lesions[35,36]. Chest X-ray, Computed Tomography (CT) scanning, and, in the recent years, Positron Emission Tomography (PET) scanning, are more or less standard imaging procedures.

Currently, the TNM classification is being revised by the International Association for the Study of Lung Cancer (IASLC)[37]. The revision results in a better prognostic

Table 1. Staging of NSCLC (1997) used in this thesis (adapted from[34]).

Stage	Tumor	Node	Metastasis	General description
Local				
IA	T1	N0	M0	T1 tumor: ≤ 3 cm, surrounded by lung or pleura; no tumor more proximal than lobe bronchus
IB	T2	N0	M0	T2 tumor: > 3 cm, involving main bronchus ≥ 2 cm distal to carina, invading pleura; atelectasis or pneumonitis extending to hilum but not entire lung
IIA	T1	N1	M0	N1: involvement of ipsilateral peribronchial or hilar nodes and intrapulmonary nodes by direct extension
Locally advanced				
IIB	T2	N1	M0	
	T3	N0	M0	T3 tumor: invasion of chest wall, diaphragm, mediastinal pleura, pericardium, main bronchus < 2 cm distal to carina; atelectasis or pneumonitis of entire lung
IIIA	T1	N2	M0	N2: involvement of ipsilateral mediastinal or subcarinal nodes
	T2	N2	M0	
	T3	N1	M0	
	T3	N2	M0	
IIIB	Any T	N3	M0	N3: involvement of contralateral (lung) nodes or any supraclavicular node
Advanced				
IIIB	T4	Any N	M0	T4 tumor: invasion of mediastinum, heart, great vessels, trachea, esophagus, vertebral body, carina; separate tumor nodules in same lobe; malignant pleural effusion
IV	Any T	Any N	M1	M1: distant metastasis

classification based on clinical and pathological staging. The most important changes are in the T and M descriptors. Tumor size becomes more important (leading to a and b descriptors for T1 and T2), and nodules in the same lobe are now defined as T3, and nodules in the ipsilateral lung as T4. Also new is the distinction made between metastases in the contralateral lung or pleural or pericardial dissemination (M1a) versus distant metastases (M1b)[37]. The new classification is presented in Table 2.

Based on the TNM status, lung cancer is distributed into stages, ranging from IA to IV[34,37]. At diagnosis, many patients already have advanced disease (stage IV, presence of metastatic disease). Survival is strongly dependent on stage, with the five-year overall survival decreasing from 65-70% (with pathological stage defini-

Table 2. Staging (new IASLC proposal 2007) and prognosis of NSCLC (adapted from[2,34,37,38]).

Stage	Tumor	Node	Metastasis	General description	Survival*	
					MST	5yr
IA	T1a, T1b	N0	M0	T1 tumor: ≤ 3 cm, surrounded by lung or pleura; no tumor more proximal than lobe bronchus. T1a ≤ 2 cm, T1b > 2 cm but ≤ 3 cm	119	73%
IB	T2a	N0	M0	T2 tumor: > 3 cm, involving main bronchus ≥ 2 cm distal to carina, invading pleura; atelectasis or pneumonitis extending to hilum but not entire lung	81	58%
IIA	T1a, T1b	N1	M0	N1: involvement of ipsilateral peribronchial or hilar nodes and intrapulmonary nodes by direct extension	49	46%
	T2a	N1	M0	T2a > 3 cm but ≤ 5 cm		
	T2b	N0	M0	T2b > 5 cm but ≤ 7 cm		
IIB	T2b	N1	M0		31	36%
	T3	N0	M0	T3 tumor: tumor > 7 cm or invasion of chest wall, diaphragm, mediastinal pleura, pericardium, main bronchus < 2 cm distal to carina; atelectasis or pneumonitis of entire lung; separate tumor nodule(s) in same lobe		
IIIA	T1, T2	N2	M0	N2: involvement of ipsilateral mediastinal or subcarinal nodes	14	19%
	T3	N1, N2	M0			
	T4	N0, N1	M0	T4: tumor of any size with invasion of mediastinum, heart, great vessels, trachea, esophagus, vertebral body, carina; separate tumor nodule(s) in different ipsilateral lobe		
IIIB	T4	N2	M0		10	7%
IIIB	Any T	N3	M0	N3: involvement of contralateral (lung) nodes or any supraclavicular node		
IV	Any T	Any N	M1a,M1b	M1: distant metastasis. M1a: separate tumor nodule(s) in contralateral lobe, pleural nodules or pleural or pericardial dissemination, M1b: distant metastasis	6	2%

*Survival is based on pathological stage for local tumors, and on clinical stage for (locally) advanced tumors. MST median survival time (months).

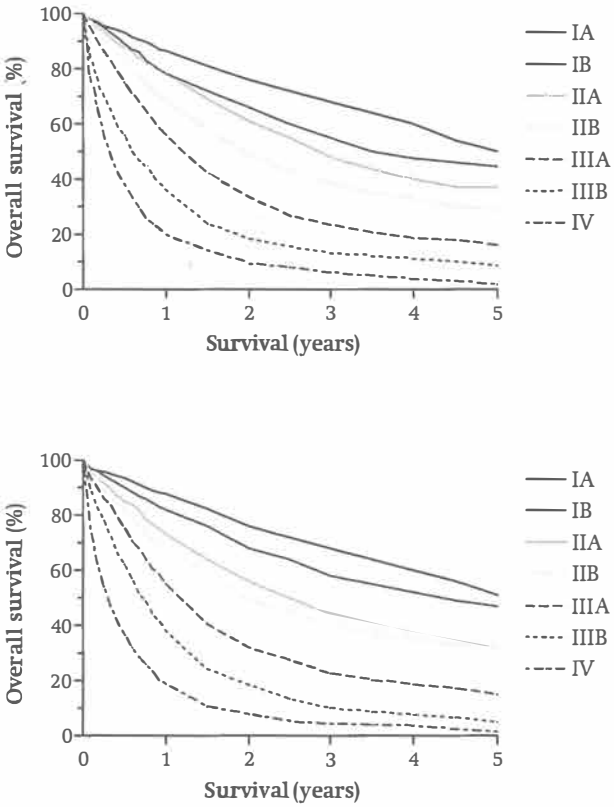

Figure 2.
Overall survival curves of over 30,000 patients with NSCLC according to 1997 TNM classification (upper panel) and 2007 IASLC proposal (lower panel) by best stage (pathological stage when available and clinical stage otherwise).
Adapted from[38].

tion) for stage IA (peripheral tumor less than 3 cm in size without nodal or systemic involvement) to 1-2% for stage IV (Table 2, Figure 2)[34,37,38]. The use of technically improved CT scanners and PET scanning could have led to an increased proportion of patients in higher disease stages at presentation. We have no information whether such stage shift has occurred on population level in the Netherlands.

Currently, several CT-based lung cancer screening programs are under investigation[39,40]. These programs and other early detection methods using methylation assays aim at detecting lung cancer in early, curable stages. In the Netherlands, the NELSON study has included over 16,000 participants aiming to show a reduction in lung cancer mortality[39]. However, it is not clear yet whether such screenings programs will result in a reduced mortality for the screened participants.

Estimation of a patient's success of treatment and chances of survival is of paramount importance for both patient and doctor. Numerous prognostic and predictive markers have been identified for NSCLC. The difference between a prognostic and a predictive marker is important. A prognostic marker or factor informs about

different outcomes of the disease for patients with and patients without the marker, irrespective of any therapy[41]. A predictive marker is a marker that predicts the differential efficacy (benefit) of a particular therapy based on marker status[41]. Without question, disease stage is the most prognostic factor for patients with NSCLC[2,42]. In fact, disease stage is so important that in order to evaluate other prognostic factors one should stratify NSCLC patients according to disease stage.

In general, prognostic and predictive factors can be categorized into host-related factors and tumor-related factors. The most important host-related prognostic factors are presence of weight loss and clinical performance scores, e.g. the Eastern Cooperative Oncology Group (ECOG) or the Karnofsky scores. The prognostic value of the other host-related factors gender and age are less clear. The tumor-related prognostic factors consist of laboratory parameters and histopathological and molecular tumor characteristics. Elevated serum calcium, elevated lactate dehydrogenase (LDH), and decreased hemoglobin are laboratory parameters associated with poor survival[42]. The number of known molecular markers able to predict prognosis of NSCLC is rapidly expanding[42,43]. These prognostic and/or predictive markers range from immunohistochemical assays for e.g. Ki-67, mutation analysis for e.g. K-ras, hypermethylation assays of e.g. RASSF1A or p16, growth factor analysis, e.g. vascular growth factor (VEGF) and epidermal growth factor (EGF), to complete molecular signatures based on micro-array testing[42-46]. Finally, the prognosis of patients with NSCLC can be predicted by evaluation of PET scans[47] or by quantification of the metabolic activity of a tumor on a PET scan using the standard uptake value (SUV)[48]. SUVs can be based on the maximal value measured within a hotspot, or on the mean value within a hotspot. The prognostic value of the different SUVs is not known and is studied in this thesis. Altogether, these prognostic and predictive factors help physicians to predict the prognosis of individual patients with lung cancer and help in choosing therapies.

A multidisciplinary approach is required for the treatment of NSCLC, because surgery, radiotherapy, chemotherapy, or a combination of these modalities all may play a role (Table 3).

Radical surgery offers the best chances of survival, and is therefore the primary treatment option for early stages, i.e. NSCLC without involvement of the mediastinal nodes (stages IA-IIB, and some patients with stage IIIA). After complete resection, the 5-year overall survival is 40-65%, depending on the stage[2]. If patients are inoperable due to for instance impaired pulmonary function, hypofractionated stereotactic radiotherapy on the primary tumor may be an alternative treatment option for early stage NSCLC[50,51]. Recently, several studies showed that adjuvant chemotherapy after a complete resection for stages IB-IIIA resulted in a 5-15% improvement in 5-year overall survival[49,52-54]. Although the largest trial reported up to now did not show a better survival after neo-adjuvant chemotherapy compared with direct surgery, it is nevertheless probable that a meta-analysis involving larger patient numbers will lead to the conclusion that neo-adjuvant and adjuvant chemotherapy have a similar

Table 3. Preferred treatment modalities for NSCLC according to disease stage (adapted from[2,49]).

Stage	Preferred first-line treatment
IA	Radical surgery
IB	Radical surgery with adjuvant chemotherapy
IIA	Radical surgery with adjuvant chemotherapy
IIB	Radical surgery with adjuvant chemotherapy
IIIA (N1)	Radical surgery with adjuvant chemotherapy
IIIA (N2)	Combination chemo-radiotherapy
IIIB	Combination chemo-radiotherapy
IV	Platinum-based combination chemotherapy, palliative radiotherapy

overall survival benefit[55].

A combination of radio- and chemotherapy results in a 15-20% 5-year overall survival in patients with stage IIIA with positive mediastinal lymph nodes and for patients with stage IIIB (locally advanced tumors)[2]. This combination therapy consists of two to three cycles of platinum-based chemotherapy followed by concurrent chemoradiotherapy[56,57]. Downstaging of patients with stage III NSCLC with induction chemotherapy followed by surgical resection has not resulted in superior survival compared to the standard chemoradiotherapy treatment[58,59].

For patients with stage IV NSCLC, advanced disease, the goal of treatment is palliation and prolongation of life. The mainstay of the treatment is chemotherapy with a combination of two cytotoxic drugs. Extending a two drug regimen with a third drug resulted in higher toxicity, without effects on survival[60]. In a comparison of four platinum-based regimens, no significant differences in overall survival were observed between the combinations[61]. All combinations resulted in a tumor response rate of 19%, with a median overall survival of 8 months[61]. Chemotherapy is associated with considerable, but acceptable, toxicity. The most well-known toxicities are myelosuppression, nausea and vomiting, alopecia, neurotoxicity, and nephrotoxicity. The frequencies in which these toxicities occur are dependent on the choice of the drug[2]. Second-line chemotherapy with docetaxel or pemetrexed for progressive and recurrent disease results in improved survival and quality of life[62,63]. The introduction in the recent years of targeted therapy starts to change the classical treatment with cytotoxic drugs[64]. These targeted therapies are currently extensively studied in numerous clinical trials testing these drugs as either single-agent therapy or in combination with chemotherapy, with other targeted therapies or with radiotherapy in all NSCLC stages[64-75].

Small-cell lung cancer

SCLC is the most aggressive and lethal subtype of all lung cancers. SCLC accounts for 10-15% of all lung cancers, and is diagnosed in more than 77,000 patients in Europe and the USA each year[76,77]. This means that the incidence of SCLC is higher than the incidence of ovarian cancer or oesophageal cancer[4,77]. Unfortunately, despite that SCLC is quite common, in the last decades a decreasing number of scientific publications concerning SCLC is observed. This is strikingly visualized by the number of abstracts submitted to the Annual Meeting of the American Society of Clinical Oncology. In 1980, 55% of all lung cancer abstracts considered SCLC, which decreased to less than 10% in 2007. The reason for SCLC being more and more dealt with as an orphan disease, despite the large number of patients, is not known, but the reason is definitely not that all patients are cured with the current treatment options. Therefore, improvements in treatment are highly needed in this disease with relapses as an important hallmark.

Also for SCLC, the relation between smoking and the development of SCLC is strong, with SCLC being more common among the heavy smokers. In the USA, the incidence of SCLC rapidly decreases, currently affecting an equal number of both sexes, which is probably caused by a change in smoking habits[77]. The situation in the Netherlands is unknown and will be studied in this thesis. In addition, a 1999 revision of the pathological classification system for small-cell lung cancer could also explain the less frequent occurrence of SCLC. In this classification, a new category of neuro-endocrine carcinoma was introduced[78]. Despite histological similarities, the biological behavior and genetic profile of SCLC and neuro-endocrine carcinoma are distinct[79]. Thus, it is likely that many cancers diagnosed as SCLC before 1999 at the present would be classified as neuro-endocrine carcinoma. Pathologically and clinically SCLC is different from NSCLC.

SCLC is pathologically characterized by cells with scant cytoplasm and many mitotic figures. Based on molecular similarities, it is suggested that SCLC resembles primitive, neuro-ectodermal tumors, such as neuroblastoma, which might provide new goals for targeted therapies[80]. The exact underlying biology of SCLC is not clear, but similar to NSCLC, SCLC is the endpoint of several genetic alterations. Autocrine-stimulated growth loops, activation of proto-oncogenes such as MYC, and inactivation of tumor-suppressor genes (FHIT, RB1, RASSF1A) all play a role[76].

The clinical presentation of SCLC is characterized by the rapid doubling time, paraneoplastic syndromes, and the inclination for early metastasis. The doubling time of SCLC is approximately 60 days, considerably less than the doubling time of NSCLC (120 days or more depending on histology)[81,82]. Patients generally present with a short history and a bulky disease, which is often centrally located[76]. Five to 15% of patients with SCLC present with or develop a paraneoplastic syndrome[76]. This is a consequence of either ectopic hormone production by tumor cells or of antibody-mediated damage to neural cells. The most common are the syndrome of inappropriate antidiuretic hormone (SIADH), Cushing syndrome, and

the Lambert-Eaton myasthenic syndrome. More than 60% of patients have clinically evident metastatic disease at the time of the diagnosis.

Because most patients present with disease that is too far spread for surgical resection, the TNM classification for staging is not often used in SCLC. Instead, patients are staged as limited disease (LD) or extensive disease (ED), originally proposed by Veterans' Affairs Lung Study Group[83]. LD is defined as disease which can be encompassed within a single radiotherapy port, and is generally confined to one hemithorax, with or without regional lymph node metastases. ED is defined as tumor beyond the bounds defined above, including metastatic disease and malignant pleural effusion. A small, third group (very limited disease) can be discerned, corresponding to T1-2N0 tumors, in which cases surgery remains an option[84,85]. Staging is primarily performed by imaging techniques, such as chest X-ray and CT scanning. Also PET scanning is valuable in staging, especially for the detection of unsuspected nodal and distant metastases[86]. The definitive diagnosis is pathologically established on endobronchial samples or on samples of metastatic disease.

The survival of patients with SCLC is strongly dependent on disease stage (Table 4). Apart from disease stage, several other prognostic factors have been identified. The most well-known factors associated with poor survival are a poor performance score, weight loss, elevated LDH and male sex[87-89]. Combinations of laboratory parameters and performance score – cheaper and more easily available than imaging techniques - proved to be of prognostic value[90]. However, it is unknown whether combinations of clinical parameters predict survival better than information from imaging techniques. Nevertheless, disease stage is not only an important prognostic factor, but is also important for treatment decisions, because LD and ED are treated in a different way.

Chemotherapy is the mainstay of treatment for all patients with SCLC (Table 4). SCLC is extremely sensitive to chemotherapy, and response rates (partial and complete) to chemotherapy are as high as 80-90% for LD and 60-80% for ED. Unfortunately, despite these excellent response rates, relapses occur in the vast majority of patients within one year after diagnosis. Thus, the major problem is not achieving tumor response to chemotherapy, but more to achieve a long-term, sustained response.

In LD, the addition of thoracic radiotherapy to chemotherapy improves survival and local control compared to chemotherapy alone[93]. In the recent years, it has become clear that cisplatin combined with etoposide is the optimal regimen for LD-SCLC. This combination was evidently superior to older regimens, consisting of cyclophosphamide, epirubicin, and vincristine[94,95]. Apart from a better survival, cisplatin and etoposide were also associated with less hematological toxicity, and are more easily combined with thoracic radiotherapy. Four to six cycles of chemotherapy are usually administered. Maintenance therapy, i.e. continuation chemotherapy for more than six cycles, or the extension of the combination with a third drug did not improve survival rates[96,97]. Radiotherapy is given in a total dose of 45-60 Gray

Table 4. Treatment and survival of SCLC according to stage (adapted from[2,76,91,92]).

Stage	MST (months)	5-yr survival	Treatment
LD	12-20	5-15%	Chemotherapy with concurrent thoracic radiotherapy followed by PCI
ED	7-12	1-2%	Chemotherapy followed by PCI (in case of response)

MST median survival time, PCI prophylactic cranial irradiation.

once or twice daily, concurrent with chemotherapy. Concurrent thoracic radiotherapy was associated with a better survival than sequential radiotherapy[98]. Besides the widely-used combination cisplatin and etoposide, several new drugs are subject of investigation for the treatment of LD-SCLC. In Asian and Western trials, irinotecan combined with cisplatin has resulted in better or equal results than cisplatin and etoposide[99-101], but in these trials all patients had extensive disease. Whether or not the combination of irinotecan and cisplatin with thoracic radiotherapy is similarly beneficial in patients with LD, and against what toxicity, is not known. Moreover, the optimal dosing and timing of irinotecan is an issue of debate.

Patients with ED are solely treated with chemotherapy. Treatment also consists of 4-6 cycles, but the optimal combination of drugs is less clear than for LD. In many European countries including the Netherlands, the combination of cyclophosphamide, doxorubicin, and etoposide (CDE) was the long-time standard treatment for ED-SCLC, with good results, but at the cost of a considerable toxicity[102]. In North-America, platinum-based regimens are considered standard treatment. The platinum agent can be combined with several second- or third-generation cytotoxic agents, such as etoposide[103], paclitaxel[104], pemetrexed[105] or irinotecan[99-101]. The combination of carboplatin and paclitaxel was efficacious in second-line SCLC with acceptable toxicity[106], but whether this combination is superior to CDE in first-line treatment is unknown.

Brain metastases are very common in SCLC, probably because chemotherapeutic drugs are not able to cross the blood-brain barrier. Prophylactic cranial irradiation (PCI) decreases the risk of developing brain metastases and improves overall survival[92,107], at the cost of moderate acute and long-term toxicity, such as fatigue and neurocognitive defects. Therefore, PCI should be offered to all patients who achieve a response to first-line treatment, both patients with LD and ED.

Almost all patients will eventually develop a recurrence of SCLC, either within 2-3 months (refractory disease), or after 3 months (sensitive disease). Not surprisingly, a shorter progression-free survival after first-line treatment is associated with a worse overall survival. Faced with a relapse, many patients, especially those in a good clinical condition, are again treated with chemotherapy. This treatment is

empirical, because few approved therapies for second-line and third-line treatment of SCLC exist; only recently the FDA approved oral topotecan as second-line treatment[108]. The use and efficacy of third-line chemotherapy has never been described. In this thesis, we will elaborate on the use of third-line chemotherapy.

Up to now, the new targeted drugs, despite their encouraging results in NSCLC, have not been shown to achieve similar results in SCLC. Trials studying imatinib, proteasome inhibitors, matrix metalloproteinase inhibitors, thalidomide, temsirolimus, and vaccine therapy all failed in yielding promising efficacy and survival data[91]. It is not clear whether new, efficacious drugs will come available in the near future.

Thymic epithelial tumors

The thymus gland is a small organ located in the anterior mediastinum. The thymus predominantly constitutes of epithelial cells and lymphoid cells. Before and during puberty, the thymus plays a role in the maturation and processing of lymphocytes. In older age, the thymus persists in an atrophic state, and its function is less clear. Thymomas and thymic carcinomas are rare tumors developing in the thymus. Despite the rarity of thymomas, they are the most common tumors to arise in the anterior mediastinum. Other lesions that may develop in the anterior mediastinum are lymphomas, germ cell tumors, and thyroid tumors.

Thymomas originate from thymic epithelial cells. Although these epithelial tumor cells lack cytologic characteristics of malignancy, thymomas may eventually behave as locally invasive tumors. Thymic carcinomas also arise from thymic epithelial cells, but they have both a malignant cellular appearance and behavior. Thymic epithelial tumors (the whole spectrum of thymomas and thymic carcinomas) are also composed of a variable proportion of non-neoplastic lymphocytes, often T-cells. Since 1999, the World Health Organization classification is the most widely used histological classification system for thymic neoplasms[109]. It divides thymic epithelial tumors into 6 subtypes, based on the predominant cell type and the ratio of epithelial and lymphoid cells. The WHO classification ranges from type A (medullary thymoma) via AB, B1, B2, and B3, to type C (thymic carcinoma) (Table 5).

Thymic epithelial tumors have a peak incidence in the sixth decade, and are equally distributed over both sexes. In the United States, the annual incidence of malignant thymomas is estimated at approximately 1.5/1,000,000[111]. The incidence of all thymic epithelial tumors, i.e. the whole clinicopathological spectrum, in the Netherlands is unknown. One third to two thirds of the thymomas are found in asymptomatic patients[112,113]. Patients generally present with symptoms due to the size of the tumor and its effects on adjacent organs (e.g. dyspnea, chest pain, cough, and the superior vena cava syndrome) or due to paraneoplastic effects. The most well known paraneoplastic syndrome is myasthenia gravis, occurring in up to 45% of patients with a thymoma[112]. Other less common syndromes are red-cell aplasia and hypogammaglobulinemia. In addition, second primary tumors are common in patients diagnosed with a thymic epithelial tumor[114,115].

Table 5. Description of WHO classification and Masaoka clinical staging of thymic epithelial tumors (adapted from[109,110]).

WHO classification	
A	medullary thymoma
AB	mixed thymoma
B1	predominantly cortical thymoma
B2	cortical thymoma
B3	well-differentiated thymic carcinoma
C	thymic carcinoma
Masaoka clinical stages	
I	macroscopically completely encapsulated lesion without capsular invasion
II	capsular invasion and/or invasion in surrounding fat or pleura
III	invasion in neighboring organs (lung, great vessels, pericardium)
IV	presence of pleural or pericardial dissemination (IV-a), and/or presence of lymphogenous or hematogenous metastases (IV-b)

Little is known about the diagnostic procedures for thymic epithelial tumors. One could assume that a provisional diagnosis is usually based on the appearance of the tumor on imaging tests, such as CT or MRI. Also PET scanning showed increased uptake in a thymus tumor[116]. The pathologic diagnosis can be established by transthoracic needle biopsy, incisional biopsy, cytological procedures, or primary resection, but the relative frequencies and sequence of these procedures are unknown.

Tumors of the thymus constitute a broad clinicopathological spectrum, ranging from benign, indolent, non-invasive tumors to highly metastatic, aggressive thymic carcinomas. Although the terms "benign" and "malignant" have been officially replaced by the WHO classification, these old-fashioned terms are unfortunately still often used in clinical practice. This might be explained by the fact that the clinical course (i.e. the malignancy) is not only determined by WHO classification, but also by other prognostic factors, such as disease stage[117-121]. Clinical disease stage of thymic epithelial tumors is assessed according to the Masaoka system[110]. This system is based on the presence of local invasion into the thymus capsule and neighboring organs, and on systemic involvement (Table 5). A higher WHO classification is associated with a higher disease stage. The third important factor which influences survival is, not surprisingly, treatment.

The primary treatment option is resection, resulting in superior survival compared to non-surgical treatment[112,122,123]. Most studies show that patients with a complete resection have a better survival than patients with an incomplete resection[117,118], but one has to realize that incomplete may range from microscopical tumor residue to large parts of the tumor left in the patient. In addition, tumor debulking and ad-

juvant treatment also resulted in reasonable results[123]. In summary, the most important question is what to do with tumors with questionable resectability.

The use of (neo-) adjuvant chemotherapy and/or radiotherapy is not standardized, and mostly based on small, phase II studies[122]. Thymomas are fairly sensitive to both radiotherapy and chemotherapy. For locally advanced, initially unresectable tumors, combined modality treatment with cisplatin-based chemotherapy and radiotherapy is recommended, to be followed by resection if a tumor response is observed. The use of adjuvant radiotherapy is somewhat controversial, but most retrospective studies suggest that radiotherapy is favorable after incomplete resection and for patients with higher disease stages[122]. If only chemotherapy is administered, cisplatin-based combination regimens resulted in the best results[122,123]. More recently, treatment with octreotide in combination with prednisone resulted in moderate response rates[124]. The use of targeted-agents, such as imatinib[125], in the treatment of thymic epithelial tumors is not frequently investigated.

As previously mentioned, survival is dependent on WHO class, disease stage, and treatment. Ten-year median overall survival is reported from up to 100% for type A to 20-40% for type C[117,121]. Ten-year median overall survival ranges from 80-100% for stage I to 20-40 for stage IV[112]. Because almost all studies only report on overall survival, it is unknown whether and which subtypes of thymic epithelial tumors are associated with thymoma-related mortality. In other words, do benign thymomas exist? In this thesis we looked into this problem.

Thymic epithelial tumors are rare, and the small numbers hamper the conduction of large, prospective trials. Therefore most clinical guidances are based on retrospective studies. Especially the optimal treatment strategy for locally advanced (stage III) tumors is not clear[126].

Aims of this thesis

To investigate whether the prognosis of thoracic tumors could be estimated more accurately and whether further improvements in the treatment of thoracic tumors are possible.

Outline of this thesis

1. It was investigated whether the incidence and stage distribution of thoracic tumors in the Netherlands has changed since 1989 (*chapter two*).
2. It was studied whether the prognosis of thoracic tumors can be estimated more accurately by using a model consisting of a combination of laboratory parameters and performance score instead of imaging techniques (*chapter three*) and by using the metabolic activity of tumors expressed as standard uptake value (SUV) (*chapter four*). In addition, the endobronchial distribution of promoter methylation and the value of methylation tests in order to better separate patients from healthy controls was studied (*chapter five*).
3. Less common thoracic tumors were explored, especially the incidence, diagnostic procedures, survival, and treatment of thymic epithelial tumors (*chapter six*), the problems associated with database linking for research of rare tumors (*chapter seven*), and the treatment of desmoid tumors (*chapter eight*).
4. It was studied whether further improvements in the therapy of thoracic tumors are possible, by evaluating the combination of cisplatin and irinotecan with radiotherapy for LD-SCLC (*chapter nine*), by testing whether the combination of carboplatin and paclitaxel is superior to CDE in ED-SCLC (*chapter ten*), and by investigating the efficacy of third-line chemotherapy for SCLC (*chapter eleven*).

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Epidemiology of thoracic tumors

Chapter 2

A recent overview of incidence and staging of pulmonary tumors in the Netherlands

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Submitted

Abstract

Introduction
The aim was to determine recent temporal changes in incidence and staging of pulmonary tumors in the Netherlands.

Methods
All tumors originating from trachea, bronchus, and lung recorded in the Netherlands Cancer Registry were included. Based on ICD-O morphology codes, five major subgroups were constructed: squamous carcinoma (SC), adenocarcinoma (AC), large-cell carcinoma (LC), small-cell lung cancer (SCLC), and other (including the uncommon tumors).

Results
Between 1989-2003, 134,894 tumors were included. In males, the age-adjusted incidence of SC and SCLC decreased (from 49.0 and 19.5 to 21.2 and 11.2/100,000, respectively; $p < 0.001$), AC remained stable (15.9/100,000) and LC increased (from 15.7 to 17.6/100,000; $p < 0.001$). In females, the incidence of SC, AC, LC, and SCLC increased (from 3.8, 4.9, 2.7, and 4.3 to 5.0, 9.5, 5.3, and 4.8/100,000, respectively; $p < 0.001$). Since 1996, a stage shift was observed, with fewer patients in stage I, and more patients in stage IV at diagnosis.
The incidence of adenosquamous carcinoma decreased (from 0.6 to 0.29/100,000; $p < 0.001$). The incidences of carcinoid tumors, sarcomatoid carcinomas, and primary pulmonary sarcomas were constant (0.44, 0.17, and 0.08/100,000, respectively).

Conclusion
The incidence of smoking-related tumors decreased in males (especially SC and SCLC) and increased in females (all subgroups). More patients presented with stage IV disease. Incidence of non-smoking-related, uncommon tumors remained constant.

2

Introduction

Lung cancer is the most common thoracic tumor, annually accounting for more than 350,000 deaths in Europe[1] and 160,000 in the United States[2]. In Europe, the age-adjusted incidence varies between 45-80/100,000 for males (highest in Eastern Europe) and 5-20/100,000 for females[1,3]. The age-adjusted incidence of lung cancer among American males is 50-80/100,000, and 20-55/100,000 among females[2,3].

Lung cancer is generally divided into small-cell lung cancer (SCLC) and non-small cell lung cancer (NSCLC), the latter subdivided into squamous carcinoma (SC), adenocarcinoma (AC), and large-cell carcinoma (LC). The major cause of lung cancer is tobacco smoke, and the association between lung cancer and smoking habits is firmly defined[4].

The epidemiology of lung cancer is changing world-wide[5]. The changes in incidence are associated with changes in smoking habits. The percentage of males who smoked has been declining since 1960 in most countries, including the Netherlands[6,7], but increasing in Eastern Europe[1]. The percentage of female-smokers increased until the 1970s, and thereafter declined in most countries. Currently, the percentage of males and females that smoke is approaching each other in the Netherlands (36% and 29% of all adults, respectively[8]). Observed changes in the distribution of the histologic subtypes of lung cancer have been linked to the introduction of the low-tar filter cigarette in the 1960s[9]. Filter cigarettes require deeper inhalation, resulting in a more peripheral particle deposit, and therefore a higher probability of the more peripheral occurring AC. Moreover, one could expect changes in the distribution of disease stages at presentation, due to improvements in imaging techniques.

Other tumors in the lungs are much less frequent than NSCLC and SCLC. The most well-known of these tumors are carcinoid tumors, sarcomatoid carcinomas, and primary pulmonary sarcomas, all deriving from orthotopic pulmonary tissue. The incidence, and especially the changes in incidence, of these rare tumors is not well-defined.

The last overview of the incidence of lung cancer in the Netherlands (data from 1989-1997) was presented in 2001[7]. In the present study, an update (1989-2003) of the incidence of all pulmonary tumors in the Netherlands is given, with particular focus on changes in disease stage and less common pulmonary tumors.

Methods

Netherlands Cancer Registry

Data were retrieved from the Netherlands Cancer Registry (NCR). The NCR receives data from nine regional cancer registries and has nation-wide coverage since

1989. Completeness of case ascertainment of the NCR is over 98%[10]. The database of the NCR contains among other items age, gender, year of diagnosis, tumor topography (primary site of origin), tumor morphology according to the International Classifications of Disease-Oncology (ICD-O)[11], and disease stage. Information on smoking history was not available.

This study was approved by the NCR scientific committee, and all procedures were performed according to NCR privacy regulations.

Patient selection

All patients diagnosed between January 1, 1989 and December 31, 2003 with a tumor located in trachea, bronchus, or lung (topography codes C.33 and C.34) were included. Tumors originating in the thymus, heart, mediastinum, or the pleura (C.37 and C.38) were not included. For incidence and disease stage evaluations, all tumors were categorized into five categories: squamous cell carcinoma (SC; ICD-O morphology codes 8050-8052, 8070-8078, 8083, 8084, 8094), adenocarcinoma (AC; 8140, 8200, 8230, 8250-8255 (thus including bronchio-alveolar carcinoma), 8260, 8263, 8323, 8333, 8430, 8470, 8480-8481, 8490, 8550, 8570), large-cell undifferentiated carcinoma (LC; 8010-8014, 8020-8021, 8046, 8082, 8090, 8123, 8246 (poorly differentiated), 8310, 8574), small-cell lung cancer (SCLC; 8041-8045), and other (remaining morphology codes). This last category encompasses the rarer pulmonary tumors, such as adenosquamous carcinoma (8560), carcinoid tumors (8240-8243, 8245, 8246 (well and moderately differentiated), 8249), sarcomatoid carcinomas (pleomorphic / giant cell / spindle carcinomas) (8022, 8030-8033, 8575, 8980, 8981, 8972), primary pulmonary sarcomas (8800-8921, 8930-8931, 8935, 8936, 8990, 8991, 9040-9044, 9120-9342, 9364-9372, 9540-9581) and tumors with unknown or other morphology codes. Lymphoma and leukaemia are not within the selection.

For NSCLC (SC, AC, and LC), disease stage was based on the Tumor-Node-Metastasis (TNM) classification. In the absence of pathological TNM stage, clinical TNM was used. SCLC was staged as limited or extensive disease.

Statistical analysis

The population at risk for each year was determined from data from Statistics Netherlands[8]. Incidence rates were calculated per 100,000 person years according to gender, histological subtype, and year of diagnosis. Incidence rates were age-standardized using the European Standard Population as reference[3]. Trends were estimated by calculating the Estimated Annual Percentage Change (EAPC). All reported p-values are two sided; the statistical significance level was set at a p-value < 0.05.

Results

Incidence

Between 1989 and 2003, 134,984 pulmonary tumors were diagnosed in the Netherlands, of which 30,600 (22.7%) occurred in females. Median age at diagnosis was 68.6 years (interquartile range, 60.9-72.2); median age was 69.4 years for men and 65.0 years for women ($p < 0.001$). The total number of pulmonary tumors slightly increased over time (approximately 9,000 annually, EAPC + 0.2%, $p = 0.04$); the proportion of females doubled from 15.2% in 1989 to 32.0% in 2003 ($p < 0.001$), while the proportion of males decreased. The proportion of SCLC as part of all pulmonary tumors decreased from 18.8% in 1989 to 16.8% in 2003 ($p = 0.0001$).

Among males, the incidence rate of all pulmonary tumors together substantially decreased from 109.2/100,000 in 1989 to 71.6/100,000 in 2003 (EAPC - 3.1%, $p < 0.001$). This is largely accounted for by the decrease in incidence of SC and SCLC (from 49.0 and 19.5 to 21.2 and 11.2/100,000, respectively, ($p < 0.001$), Figure 1). The incidence of SC in males decreased with a striking 57%, and does not seem to plateau yet. The incidence of AC remained constant at 15.9/100,000, the incidence of LC slightly increased from 15.7 to 17.6/100,000 ($p < 0.001$).

In females, the incidence of all pulmonary tumors together rose from 16.9 to 30.5/100,000 (EAPC + 4.3%, $p < 0.0001$), with all categories involved (from 3.8, 4.9, 2.7, and 4.3 to 5.0, 9.5, 5.3, and 4.8/100,000, for SC, AC, LC, and SCLC respectively (all $p < 0.001$), Figure 1).

Interestingly, the number of large cell neuro-endocrine tumors (morphology codes 8013, 8246 poorly differentiated, and 8574) showed a steep increase (Figure 2, EAPC + 38.3%, $p < 0.0001$).

Stage distribution

In NSCLC (SC, AC, and LC), the percentage of patients with stage I disease at presentation dropped in males, females, and in both sexes combined (Figure 3, EAPC - 2.7%, $p < 0.001$). The percentages of stages II and III remained stable at approximately 6 and 35%, $p = 0.28$ and $p = 0.20$, respectively. The percentage of patients with stage IV at diagnosis increased (EAPC + 3.9%, $p < 0.001$). These trends in staging mainly occurred after 1996, and since approximately the year 2000 stage IV was the most common. Interestingly, females generally presented with higher disease stages, for instance 44% with stage IV in 2003 versus only 37% of all males. This male/female difference in staging is present in all three major histologic subtypes. In addition, the percentage of NSCLC patients with unknown disease stage gradually decreased from 10.3 to 3.7% ($p < 0.001$).

Likewise, the incidence of stage I NSCLC significantly decreased from 12.0 to 9.9/100,000 (males and females combined, EAPC - 4.0%, $p < 0.001$), with a stable incidences of stages II (2.8/100,000 in 2003), a recent decrease in stage III (now 15.6/100,000) and an increase in incidence of stage IV to 12.5/100,000 in 2003

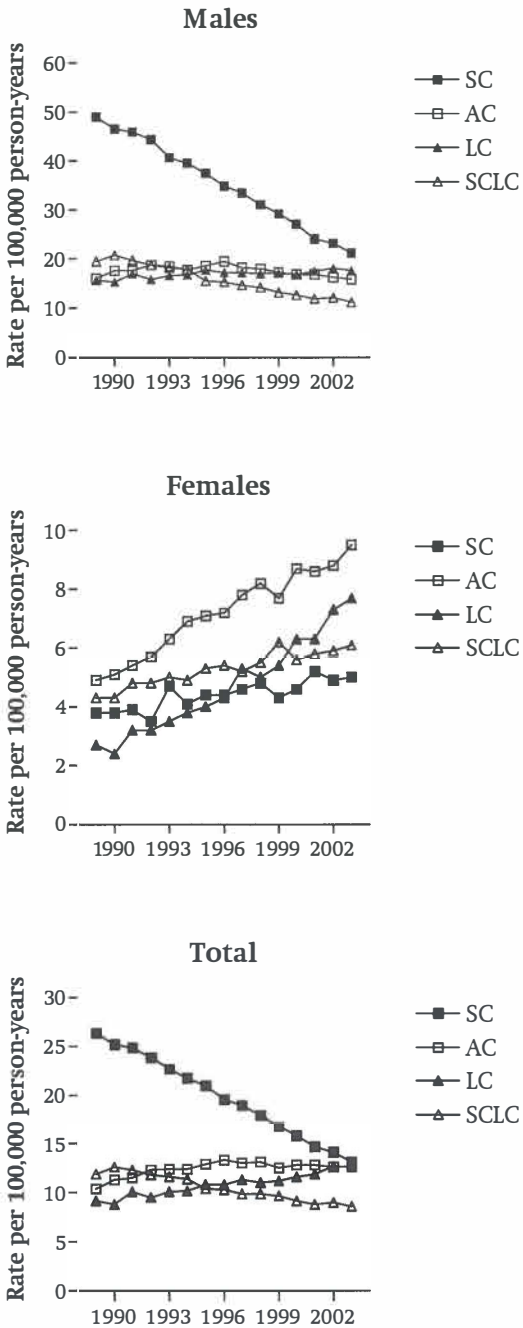

Figure 1.
Trends in incidence of squamous carcinoma (SC), adenocarcinoma (AC), large-cell carcinoma (LC), and small-cell lung cancer (SCLC) among males, females, and both sexes combined in the Netherlands from 1989-2003. The incidence is age-adjusted to the European Standard Population.

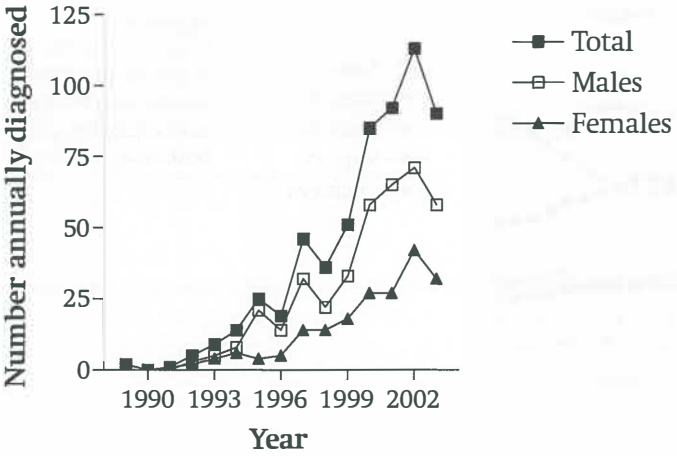

Figure 2.
Number of large-cell neuro-endocrine carcinoma annually diagnosed in the Netherlands. In 1999, a revision of the WHO classification of lung tumors was introduced[19] with large-cell neuroendocrine carcinoma now included as a variant of LC and not anymore among SCLC.

(EAPC + 2.4%, $p < 0.001$) (Figure 4).

Moreover, differences in staging between SC, AC, and LC were observed (Figure 5). The most remarkable difference was the percentage of patients with stage IV disease. In the later years of the study period, this percentage varied between 40 and 50% for AC and LC, compared to only 25% for SC (Figure 5). Stage I was more common in SC than in AC and especially LC. However, the stage shift observed among NSCLC (a decrease in stage I and increase in stage IV after 1996) was observed in all histologic subtypes.

After 1998, the percentage of patients with SCLC having ED at presentation gradually increased to 62% in 2003 (EAPC + 1.7%, $p < 0.001$), at a cost of a decrease in LD and unknown disease stage (Figure 6). This trend was similar for males and females.

Other tumors

The incidence of other pulmonary tumors (i.e. not SC, AC, LC, or SCLC) decreased in males (from 9.0 to 5.7/100,000, EAPC - 2.4%, $p < 0.001$), and increased among females, from 1.4 to 2.3/100,000 in 2003 (EAPC + 3.9%, $p < 0.0001$). The overall majority in the "other" group consists of unknown or unspecified tumors (77.5% in males and females combined).

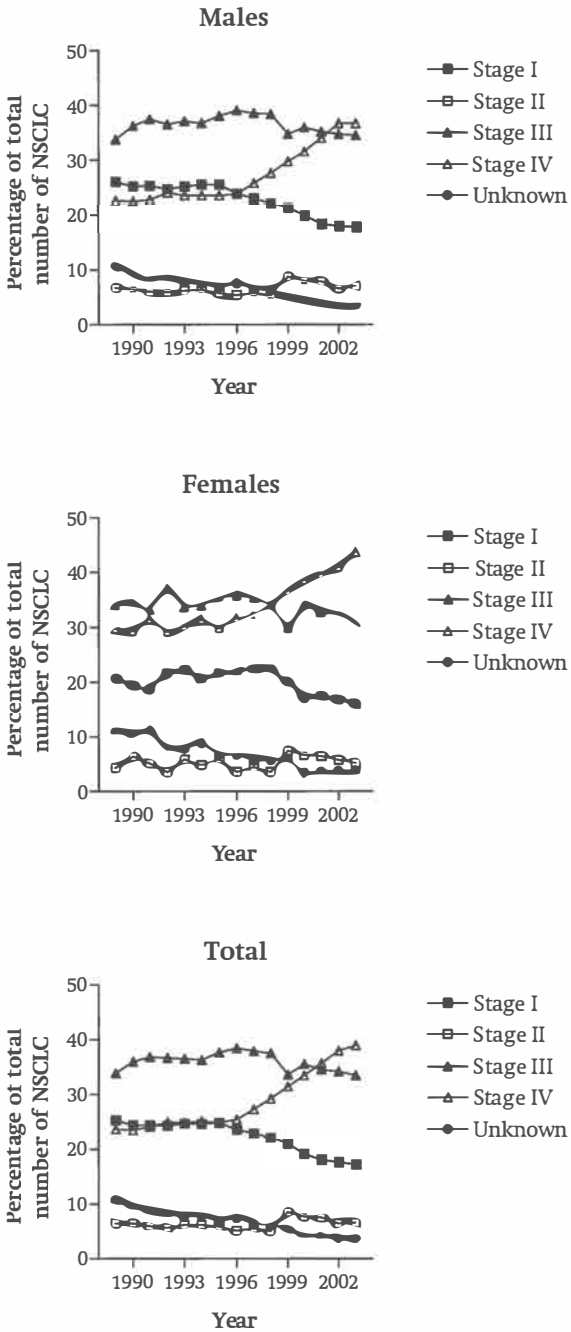

Figure 3.
Stage distribution at diagnosis of NSCLC (SC, AC, and LC) for males, females, and both sexes combined.

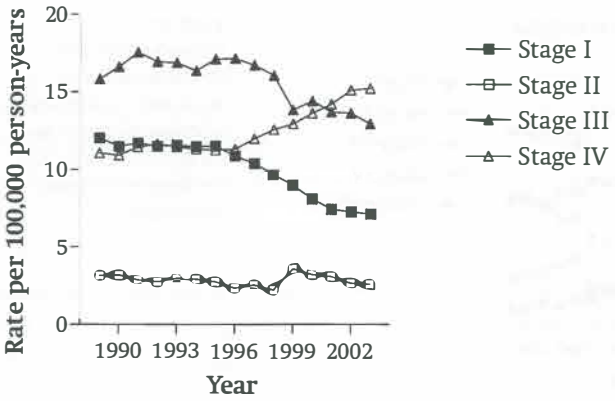

Figure 4.
Trends in incidence of the disease stage of NSCLC (SC, AC, and LC) in males and females combined in the Netherlands from 1989-2003. The incidence is age-adjusted to the European Standard Population.

Some groups can be discerned in this "other" group. Most common was adenosquamous carcinoma, accounting for 8.7% of all "other" tumors and 0.8% of all pulmonary tumors. The overall incidence of adenosquamous carcinoma decreased. This was caused by a decrease among males (EAPC - 7.2%, $p < 0.0001$), among females the incidence was constant.

The incidence of carcinoid tumors was constant at 0.44/100,000 (approximately 70 each year in the Netherlands, 0.8% of all pulmonary tumors), with a male: female ratio of 48%:52%. The large majority of carcinoid tumors (94%) was classified as "typical carcinoid". Nevertheless, the percentage atypical carcinoid tumors increased. Before 1998, maximally 1 was diagnosed each year, increasing to approximately 10 in the last years (EAPC + 23.6%, $p = 0.0001$).

The incidence of sarcomatoid carcinomas (pleomorhic, spindle cell, and giant-cell carcinomas) remained constant at 0.17/100,000 (approximately 25 annually, 0.3% of total). The male:female ratio of 75%:25% also did not change. Among the subgroups of the sarcomatoid carcinomas, the number of the spindle-cell carcinomas increased at the cost of giant-cell carcinomas in both sexes.

Finally, each year approximately 12 primary pulmonary sarcomas were diagnosed, without a change over time (annual incidence 0.08/100,000, EAPC - 0.6%, $p = 0.67$). Primary pulmonary sarcomas were twice as common among males as among females.

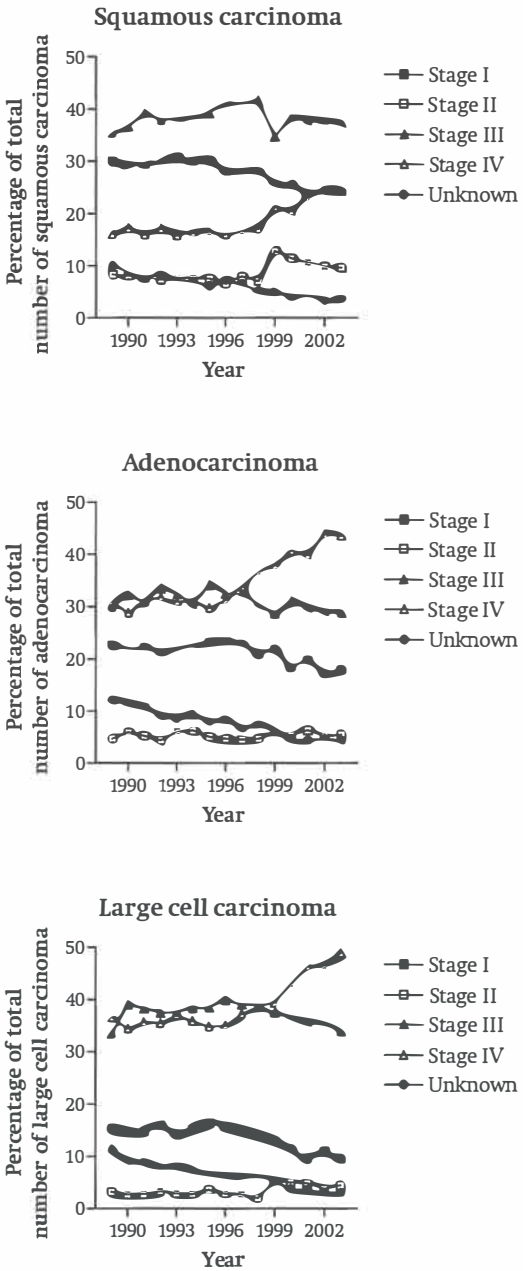

Figure 5.
Stage distribution at diagnosis for squamous carcinoma, adenocarcinoma, and large cell carcinoma (males and females combined).

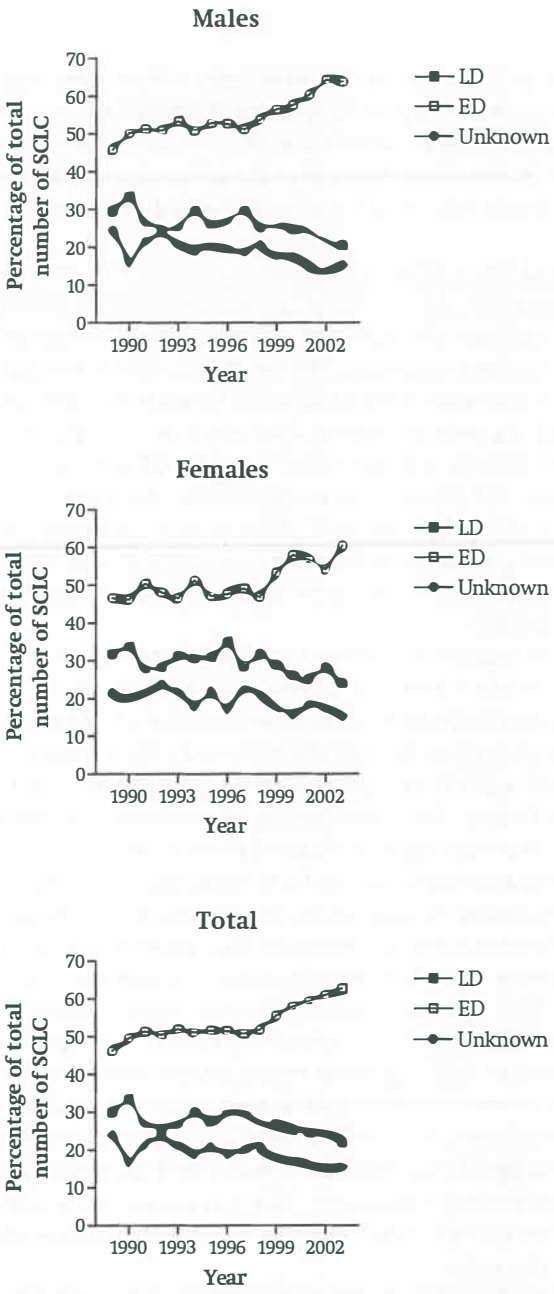

Figure 6.
Stage distribution for SCLC at diagnosis for males, females, and both sexes combined.

Discussion

During the 15-year study period, over 134,000 pulmonary tumors were diagnosed in the Netherlands. Since the last report[7], lung cancer incidence kept increasing in females and decreasing in males. Furthermore, AC is becoming the most common subtype of NSCLC. A remarkable increase in the percentage of patients with stage IV disease at diagnosis was noted since 1996, with a concomitant decrease in stage I patients.

The epidemiology of lung cancer is different for males and females. First, the age-adjusted incidence of lung cancer (SC, AC, LC, and SCLC) in males is still decreasing (to 66/100,000 in 2003). In contrast, the increasing incidence among women (to 28.3/100,000 in 2003) does not seem to plateau. The trends observed in the previous overview of lung cancer in the Netherlands (1989-1997)[7] therefore did continue. Second, female patients were 3-4 years younger at diagnosis than male patients. Third, the distribution of the histologic subtypes differs. In males, SC used to be by far the most common subtype, and although the incidence of SC decreases, SC is still the most common type in males, followed by LC, AC, and SCLC. In females, all four subtypes increased, with AC being by far the most common type, and SC the least common type. Several factors could explain the observed trends in incidence in lung cancer in males and females.

The main reason for these trends is the change in smoking habits. Since 1960, the percentage of males that smoked decreased, accompanied by an increase in females. Because the time between the onset of smoking and the onset of lung cancer is relatively long, this change in smoking has still effect. Currently, the incidence of lung cancer in the Netherlands is one of the higher in Europe, the highest incidence rates are observed in Eastern Europe[1]. This is paralleled by the smoking rates, which are high in the Netherlands, but even higher in Easter Europe[12,13]. With less smoking, the number of tumors most strongly associated with smoking (SC and SCLC[14]) tend to decrease, whereas the number of lung cancers less strongly associated with smoking such as AC (also the most common subtype in lung cancer in never-smokers[15]) tend to (relatively) increase. Apart from the percentage of smokers, it is likely that the introduction of the filter cigarettes, which were more popular among females than among males, is responsible for the relative increase in the proportion of AC, especially among females[9]. Filter cigarettes require deeper inhalation than non-filter cigarettes, causing a more peripheral particle deposition and therefore a predilection for peripheral occurring tumors (AC) in stead of more central tumors (SC). Finally, a meta-analysis suggested that females are more prone to develop lung cancer than males after a similar tobacco exposure[14]. This may explain that females are overrepresented among the younger ages[16], resulting in an overall younger age at diagnosis for females than for males.

Several international studies have described changes in epidemiology of NSCLC before[1,5,16,17]. Although the actual incidence numbers differ between countries as a

result of different smoking rates, the temporal changes in incidence and histologic distribution observed in the Netherlands occur world-wide. These trends occur later in Eastern Europe, because smoking cessation measures were started later[13]. Because in the US the decrease in smoking males and the increase in smoking females started earlier than in Europe[5,16], it could be that the current lung cancer status in the US reflects the status in Europe in the coming decade.

Despite an overall decrease in incidence, SCLC still represents 16.8% of all pulmonary tumors in the Netherlands, about one third of them occurring in women. This is in contrast with the 1:1 male:female ratio for SCLC in the US[18], and may explained by a narrower sex gap between male and female smoking rates in the US than in the Netherlands[18]. In addition, the 1999 revision of pathological classification[19] caused that many neuro-endocrine carcinomas that used to be classified as SCLC are now classified as (large-cell) neuro-endocrine carcinoma, a high grade variant of NSCLC. This is in line with the observed steep increase in the number of large-cell neuro-endocrine carcinoma after 1999 (Figure 2), and probably partly explains the decrease in SCLC. Moreover, the number of patients with ED at diagnosis has increased a little, and is currently at 62%, which is only a little more than in the US (57% in 2002)[18].

After 1996, a sudden increase in the relative proportion of patients in stage IV was observed, with a concomitant decrease in stage I patients. This trend seems to continue. It is unlikely that only patients in stage I are directly upstaged to stage IV, more plausible is that this upstaging occurs in all stages, with more patients in stage IV being the end-point. One reason for the stage shift probably is the introduction of positron-emission tomography (PET) scanning, which has become standard care in most hospitals in the Netherlands around the year 2000. PET scanning results in the detection of previously occult distant metastasis, thereby upgrading stage I, II, or III patients to stage IV[20]. The availability of better-quality CT scans has probably also encouraged upstaging. A second reason may be the increase in the proportion of adenocarcinomas, which are more prone to metastasize early in the course of the disease than the other subtypes of NSCLC[21]. We observed that over 40% of patients with AC present in stage IV, versus only 25% of patients with SC. Third, females present in higher disease stages (for all subtypes) than males, and the number of females with lung cancer is increasing. Our results suggest that currently a higher proportion of the patients is accurately staged compared to a decade ago. This might have implications for the frequently used historical stage-specific survival tables for lung cancer[22], which may represent an underestimation of stage-specific survival in the current times of modern staging techniques. Hypothetically, the observed stage shift could be reversed if CT-based lung cancer screening will be implemented, which may lead to an increase in the number of patients in stages I and II.

Our study specially focussed on the less common pulmonary tumors, and discerned four groups of uncommon tumors. Adenosquamous carcinomas, a rare subtype of NSCLC, constituted 0.8% of all pulmonary tumors, a little lower than the

reported rate of 2.1 to 4.5% for resected lung cancers[23-25]. The reason for the decrease in incidence of this smoking-related, poor-prognosis type of lung cancer probably lies in the decrease in smoking frequencies.

Carcinoid tumors constituted 0.8% of all pulmonary tumors, which is in line with other reports[26,27]. In our study, the incidence of carcinoid tumors did not change (0.44/100,000 for males and females together). Older studies suggest that the incidence of pulmonary carcinoid tumors is rising (study periods 1978-1997 and 1973-1991)[27,28], although their observed maximal incidence is not higher than that of the present study(1989-2003). This rise in incidence in the 1970s and 1980s can be attributed to the better availability of fiberoptic bronchoscopy, therefore it is questionable whether this observed increase is accurate. A current issue regarding carcinoid tumors are the changes in histological classification of the neuro-endocrine tumors, to which carcinoid tumors belong[19]. It is likely that tumors which used to be classified as SCLC are currently classified as large cell neuro-endocrine (as discussed before) or atypical carcinoids, of which the annually diagnosed number increased.

The sarcomatoid tumors are a heterogeneous group of smoking-related, poorly differentiated, aggressive tumors[29]. With a decrease in smoking frequencies, a decrease in incidence of these tumors is expected, but this was not observed, probably due to the low overall numbers.

The last subgroup of uncommon pulmonary tumors were the primary pulmonary sarcomas, representing approximately 0.1% of all pulmonary tumors, with a stable incidence. These rare tumors are not smoking-related, and mostly are angiosarcoma, leiomyosarcoma, rhabdomyosarcoma, or sarcomatoid mesothelioma[30]. Little is known about these sarcomas, but because no causal factor is identified, it is plausible that the incidence is stable.

In conclusion, this large nation-wide study confirmed the world-wide occurring changes in the incidence of lung cancer, showing an increase in lung cancer incidence in females, and a strong decrease in the number of SC. A remarkable stage shift was observed, with a still increasing proportion of stage IV disease at diagnosis, which could be explained by an increased availability of PET scanning. The incidence of less common, non-smoking related pulmonary tumors did not change.

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Prognostic factors in thoracic tumors

Chapter 3

Prognostic classification with laboratory parameters or imaging techniques in small-cell lung cancer

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Clin Lung Cancer 2007 May;8(6):376-81

Abstract

Introduction
Our aim in this study was to compare prognostic models based on laboratory tests with a model including imaging information in small-cell lung cancer (SCLC).

Methods
A retrospective analysis was performed on 156 consecutive patients. Three existing models based on laboratory tests and performance status (PS) and a model based on disease stage assessed by imaging techniques and PS were tested with Cox regression analysis.

Results
The three laboratory-based models and the imaging-based model were significant in predicting prognosis in our patient group, with hazard ratios of 1.6 to 3.0 for medium prognosis groups and 2.6 to 6.1 for poor prognosis groups compared with good prognosis groups.
Models based on laboratory tests appear to predict survival probabilities at least as well as a model with information from imaging techniques.

Conclusion
Prognostic models using PS and laboratory tests provide a similar estimation of survival of patients with SCLC as the combination of PS and disease stage assessed by imaging tests.

Introduction

Small-cell lung cancer (SCLC) is one of the most deadly solid tumors, with a rapid doubling time and a high risk of early metastasis. Currently, 14% of all lung cancers are SCLC, and the incidence is decreasing[1,2]. Traditionally, SCLC is staged as limited or extensive disease. The distinction between limited- and extensive-stage disease is generally based on imaging tests. At presentation, 30-40% of the patients have limited-stage disease[3]. The 5-year survival rate is about 15-25% with limited-stage disease and < 5% with extensive-stage disease[4]. In fact, disease stage is one of the strongest prognostic factors in SCLC.

In addition to disease stage, various other prognostic factors have been identified in large groups of patients[3,5]. A worse performance status (PS), elevated lactate dehydrogenase (LDH) and alkaline phosphatase (ALP) levels, and low serum sodium and albumin levels are well known factors that decrease life expectancy. One study found that a normal pretreatment leukocyte count was associated with prolonged survival[6]. Female sex was identified as a favorable prognostic factor[7]. The prognostic value of age is still controversial[8]. Together, laboratory, clinical, and radiologic parameters all have prognostic value.

In the past years, combinations of clinical and laboratory parameters have been used to classify patients with SCLC into prognostic groups. Two such prognostic models were proposed in British studies 20 years ago[9,10] and were validated in a large study afterward[11]. LDH, being highly correlated with survival[12], was combined with PS and serum sodium in a Japanese model[13]. These prognostic models based on clinical and laboratory parameters are advocated by some groups, whereas others use imaging tests for staging, for treatment decisions, and for prediction of survival. Laboratory and clinical parameters are less time-consuming and less expensive than imaging tests, but we do not know whether they predict survival better.

In the present study, we assessed the prognostic efficacy of three existing models based on laboratory tests and PS but without imaging information as well as a model combining imaging information, clinical information, and laboratory parameters. Secondly, we intended to compare all these models with each other to determine whether laboratory parameters or imaging information predict survival better.

Patient and methods

Patient selection

Between January 1996 and July 2004, all consecutive patients diagnosed with SCLC at University Medical Center Groningen in the Netherlands were studied. Approval of local institutional review board was not necessary because the present study involves the use of anonymized data from human subjects and explicitly does not involve the use of human subjects.

Evaluation

Patient records were assessed for the following baseline parameters: sex, age, date of diagnosis, and Eastern Cooperative Oncology Group (ECOG) performance score (PS). A complete blood count (hemoglobin, white blood cells (WBCs), and platelets), serum sodium, LDH, total protein, albumin, bilirubin, alanine aminotransferase (ALT), aspartate aminotransferase (AST), and ALP were obtained before the start of chemotherapy.

Disease stage (limited versus extensive) was defined according to the Veterans Affairs Lung Study Group (VALSG). In short, in limited-stage disease the tumor was confined to one hemithorax, with all tumor mass encompassed in a single radiation port. If the tumor extended into the contralateral hemithorax and/or in the presence of distant metastasis, it was classified as extensive-stage disease. In this study, computed tomography scans of thorax and upper abdomen, bone scans, and, if clinically indicated, needle aspirates of lymph nodes detected by physical examination or ultrasonography were used to separate limited- and extensive-stage disease.

Existing prognostic models

The Royal Marsden group used World Health Organization PS, serum albumin, and ALT[10]. Using these three variables, 3 patient groups could be distinguished. A good prognosis group (PS of 0 or 1, albumin ≥ 36 g/L, and a normal level of ALT), a medium prognosis group (up to 2 of the following characteristics: PS of 2, elevated ALT, and albumin 30-35 g/L) and a poor prognosis group including the remaining patients.

The London Group used Karnofsky PS, serum sodium, albumin, and ALP. Patients were designated into a good prognosis group (Karnofsky PS > 70, sodium > 136 g/L, albumin > 39 g/L, and ALP < 150% of upper limit of laboratory normal values) or poor prognosis group (Karnofsky PS < 40, or ALP more than three times upper limit of laboratory normal values, or sodium < 135 g/L and albumin < 38 g/L)[9]. A medium prognosis group was defined by exclusion from good or poor prognosis groups.

Kawahara et al. classified patient groups as good prognosis (normal LDH levels, ECOG PS of 0/1, and serum sodium of ≥ 136 g/L) and poor prognosis (LDH levels above normal and ECOG PS of 2 or 3)[13]. Medium prognosis was defined by exclusion from good or poor prognosis.

Statistical methods

Survival was calculated from date of diagnosis until date of death, loss to follow-up, or censored on August 1, 2004 (Kaplan-Meier method). We fitted separate Cox models for each of the three existing scoring systems. Prognostic categories are indicated with a hazard ratio (HR), a HR > 1 meaning an increased mortality. We also examined the effect of adding staging information from imaging tests to these models.

To construct a model including imaging information, we started with Cox univariate regression analysis to study the relation between survival time and 15 baseline variables. The variables WBC, platelets, and serum LDH were logarithmically transformed using base 2 logarithms so that for these variables HRs correspond to a two-fold increase on the original scale. Then, we built a best fitting model and simplified it into a three-category prognostic system (with categories good, medium, and poor). A p-value of ≤ 0.05 was considered significant.

To compare the performance of all models, we present the coefficient of explained variation (R^2; the higher R^2, the more variance of survival is explained by the model tested in survival analysis. R^2 is not a parameter for statistical significance, but for prognostic performance[14]) and the following two more intuitive descriptions of predictive abilities. Firstly, for each category of each scoring system, we present the percentage of patients with survival time above the median survival of the whole group. Secondly, for a pair of patients in different prognostic groups we calculated (from Cox model results) the probability that a patient in a better prognostic group will live longer than a patient in a worse prognostic group.

Results

Patient characteristics

Included were 156 consecutive patients. Median age at diagnosis was 63 years, 60% were men, and 40% had limited-stage disease. Median PS was 1 (range, 0-3). Patient characteristics are summarized in Table 1.

All 156 patients received first-line chemotherapy, with a median of 5 cycles (range, 1-6 cycles). One hundred patients (64%) received combination therapy with cyclophosphamide/doxorubicin/etoposide, and 44 (28%) combination therapy with carboplatin/paclitaxel. Twelve patients (8%) received other combinations. Not all patients with limited-stage disease received thoracic radiation therapy as part of the combination therapy because of deteriorated physical condition during treatment, early death, patient refusal, or other reasons. Overall tumor response rate to the first-line chemotherapy was 78%.

The median survival was 43 weeks (10 months; range, 1-324 weeks), with a 1-year survival rate of 39%. The median survival of patients with extensive- and limited-stage disease was 8 and 13.5 months, respectively.

Prognostic models

The three existing prognostic models were fitted with data from our patient group (Table 2). All three models were highly significant, and had R^2s between 0.08 and 0.13. Addition of the variable disease stage measured with imaging tests to any of the three existing models significantly improved the fit (p-values < 0.005; R^2 0.19

Table 1. Characteristics of 156 patients with SCLC before first-line chemotherapy.

Characteristic	Number of patients	Percentage of total number of patients	Median	Range
Gender male/female	93 / 63	60 / 40		
Disease stage limited/extensive	63 / 93	40 / 60		
Performance status 0 / 1 / 2 / 3	51 / 78 / 25 / 2	33 / 50 / 16 / 1		
Thoracic radiation therapy	28	18		
PCI / WBRT	12 / 36	8 / 23		
Age at first diagnosis (yrs)			63	37-86
Hemoglobin (mmol/L)			8.3	5.6-10.5
WBC count (10^9/L)			9.2	4.4-21.0
Platelets (10^9/L)			289	11-856
Serum sodium (mmol/L)			136	120-146
Serum LDH (U/L)			297	132-8343
Total serum protein (g/L)			68	33-89
Serum albumin (g/L)			39	17-50
Bilirubin (µmol/L)			11	2-468
Serum ALP (U/L)			98	33-1397
Serum AST (U/L)			27	8-373
Serum ALT (U/L)			26	5-722

Normal values are hemoglobin 7.5-10.5 mmol/L, WBC 4-10 x 10^9/L, platelets 150-350 x 10^9/L, serum sodium 132-144 mmol/L, LDH 114-235 U/L, total protein 65-79g/L, albumin 34-47 g/L, bilirubin 0-17 µmol/L, ALP 13-120 U/L, AST 0-40 U/L, ALT 0-30 U/L.
PCI prophylactic cranial irradiation, WBRT whole brain radiotherapy, WBC white blood cell count, LDH lactate dehydrogenase, ALP alkaline phosphatase, AST aspartate transaminase, ALT alanine transaminase.

for the London model and 0.18 for the other two models). These small increases in R^2 did not have much effect on median survival times.

Disease stage, PS, age at diagnosis, hemoglobin, WBC count, serum sodium, LDH, albumin, bilirubin, and ALP were significantly related to survival in univariate analysis. Sex, platelets, total serum protein, AST, and ALT were not significant.

In multivariate analysis of all baseline parameters using Cox's proportional hazard model, extensive-stage disease, higher PS, elevated WBC count, reduced platelet count, elevated serum LDH, and age > 70 years were independently significant adverse prognostic factors (Table 3). The R^2 of this model was 0.38. When the

Table 2. Prognostic classification of patients with SCLC according to three existing models based on laboratory tests and on a model based on imaging information (n=156).

Model	n	Median survival (weeks)	HR	95% CI for HR	P-value	R^{2*}
London Group						0.13
Good	33	69	1	-	-	
Medium	78	45	1.95	1.24-3.07	0.004	
Poor	45	25	3.21	1.94-5.31	< 0.001	
Royal Marsden						0.08
Good	61	58	1	-	-	
Medium	75	35	1.61	1.12-2.33	0.011	
Poor	20	19	2.57	1.53-4.32	< 0.001	
Kawahara						0.13
Good	20	71	1	-	-	
Medium	114	42	2.04	1.20-3.48	0.009	
Poor	22	19	4.79	2.45-9.34	< 0.001	
Model based on imaging information†						0.27
Good	27	92	1	-	-	
Medium	50	50	3.00	1.71- 5.25	< 0.001	
Poor	79	28	6.07	3.44-10.70	< 0.001	

HR Hazard Ratio.
*R^2 is the coefficient of explained variation and, thus, a parameter of prognostic performance.
†Good (limited-stage disease and PS of 0), Medium (limited-stage disease and PS of 1 or extensive-stage disease and PS of 0), Poor (limited-stage disease and PS of ≥ 2 or extensive-stage disease and PS of ≥ 1).

prognostic information (with survival as a continuous outcome variable) from this model including all variables was categorized in three groups (good, medium, and poor survival), the R^2 dropped to 0.28.

Subsequently, we constructed a model based on two important prognostic factors (disease stage by imaging techniques and PS) for classifying patients with SCLC. First, the HRs of the six possible combinations (limited- or extensive-stage disease, PS of 0, 1, or 2/3) were calculated (with limited-stage disease and PS of 0 as reference category), then groups with similar HRs were combined to form three prognostic groups. A good prognosis group was defined as those with limited-stage

Table 3. Multivariate Cox regression analysis for survival using baseline parameters in 156 patients with SCLC.

Characteristic	Hazard Ratio (HR)	95% CI for HR	P-value
ECOG PS			
0 (reference)	1	-	-
1	1.96	1.27-3.02	0.002
2 / 3	3.95	2.24-7.00	< 0.001
Disease stage (limited versus extensive)	1.71	1.13-2.58	0.010
^{2}Log WBC count*	2.05	1.34-3.15	0.001
^{2}Log platelets*	0.75	0.58-0.97	0.029
^{2}log serum LDH*	1.35	1.11-1.63	0.002
Age > 70 years	1.56	1.04-2.33	0.030

*WBC, platelets, and serum LDH are expressed as base 2 logarithms so that the given HR corresponds to a 2-fold increase of parameter value.

Table 4. Percentages of patients with a survival above overall median survival (43 weeks) for all categories of all four models.

Model	Prognostic Group	Number of patients in group	Percentage with a survival above median survival
London Group	Good	33	70
	Medium	78	49
	Poor	45	29
Royal Marsden	Good	61	67
	Medium	75	39
	Poor	20	20
Kawahara	Good	20	85
	Medium	114	46
	Poor	22	18
Model based on imaging and PS	Good	27	89
	Medium	50	54
	Poor	79	29

Table 5. Estimated probability that a patient with a higher prognostic score outlives a patient with a lower score.

	compared outcome categories		
Model	medium : poor	good : poor	good : medium
London Group	0.66	0.76	0.62
Royal Marsden	0.62	0.72	0.62
Kawahara	0.68	0.83	0.71
Model based on imaging and PS	0.75	0.86	0.67

A probability of 0.86 means that a patient in the good prognosis group has 86% chance of outliving a patient in the poor prognosis group.

disease and PS of 0. The medium prognosis group consisted of patients with limited-stage disease and PS of 1 or patients with extensive-stage disease and PS of 0. A poor prognosis group consisted of all remaining patients (limited-stage disease and PS of 2/3, or extensive-stage disease and PS of ≥ 1). Median survival of the good, medium, and poor group decreased from 92 weeks to 50 and 28 weeks, respectively ($p < 0.0001$; Table 2). The R^2 for this model, 0.27, is rather close to the R^2 based on the tertiles using all available information, and about twice that of the three existing prognostic models based on laboratory parameters (Table 2). Survival curves for the three existing models and the model based on imaging information are shown in Figure 1.

Percentages of patients with a survival above the overall median (43 weeks) per outcome category of each of the prognostic models show a gradual increase from poor to good prognosis, for all four models (Table 4). It should be noted that the minority of the patients in the scoring systems based on laboratory tests were placed in the poor prognosis group; this is in contrast to approximately 50% of such assignments to the poor prognosis group by the scoring system based on imaging.

The probability that, for two patients in different prognostic groups, the patient in a more favorable category outlives the other patient is presented in Table 5. The imaging-based model in comparing good versus poor estimates this probability as 86%. These probabilities are roughly equal for all four models.

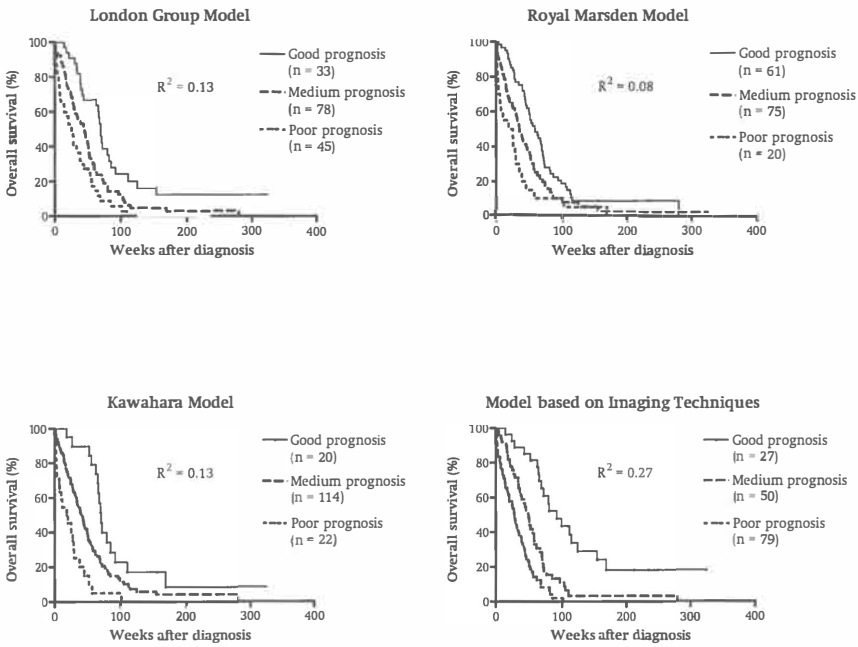

Figure 1.
Kaplan-Meier overall survival curves of the three models using laboratory tests and PS and of the prognostic model based on imaging and PS.
R^2 is the coefficient of explained variance and, thus, a parameter of prognostic performance.

Discussion

Prognostic models based on laboratory tests and performance status but not on information on disease stage obtained from imaging techniques are able to predict survival of patients with SCLC. Also a model based on the combination of disease stage by imaging tests and PS adequately predicts patient survival. Despite the fact that the explained variance (R^2) of this imaging-based model is higher than the explained variance of three existing models based on laboratory tests and PS, all four models appear to perform more or less equal in terms of predicting survival probabilities. Furthermore, adding disease stage by imaging tests to the laboratory tests models only marginally but significantly increased the explained variance of these models.

Three prognostic models only using PS and laboratory parameters have been proposed. The model constructed by the London Group, using PS, sodium, ALP, and albumin, has earlier been shown to be able to discern different prognostic groups, which also correlated well with radiologic tumor response[9]. Equally, the Royal Marsden scoring system (albumin, PS, and ALT) has proven to be useful for assessing survival[10]. Both studies conclude that staging information is not better than prognostic information from laboratory parameters. It should be noted that both studies are from the mid-1980s. At that time, staging did not include a computed tomography scan, and LDH was not routinely measured. As LDH is an independent prognostic factor, the results of Kawahara et al. are interesting. From LDH, PS, and serum sodium, they were able to construct a similarly potent predictive model[13]. The prognostic power of all three models has been confirmed in our patient group.

Prognostic models using combinations of disease stage and clinical or laboratory parameters have been studied before. Albain et al. identified four distinct prognostic subgroups, using disease stage (limited- or extensive-stage disease), age < or ≥ 70 years, normal or abnormal LDH, and presence of pleural effusion as parameters[15]. In a Canadian study, four prognostic classes were constructed[16]. They used up to eight variables: stage, LDH, mediastinal lymph nodes, WBC count, sex, ALP, PS, and liver metastases. Another classification into four prognostic groups was suggested based on stage, PS, sex, age, and neutrophil count[6]. These three previously mentioned models are more complex than the model reported here, which makes use of only two parameters, i.e. stage and PS. Another advantage is that both parameters are easily reproducible, with little interobserver variability in scoring ECOG PS[17]. It is important to realize that not only disease stage assessed by imaging techniques but also laboratory parameters such as serum sodium and LDH and PS have similar impact in predicting an individual patient's prognosis.

The comparison of predictive performances of the considered models is hampered by the lack of a gold standard for categorization. All four scoring systems use the following three outcome categories: poor, medium, and good. In order to describe the predictive potential of the various models in a clinically useful and comparable manner, we offer answers to the following questions: "Given a patient's prognostic category, what is his/her probability of surviving beyond the overall median?" and "Given two patients in different categories, what is the probability that the one in a more favorable category will outlive the other?" As can be derived from Tables 4 and 5, all four models are clinically equal in terms of assessing individual patient survival despite the fact that the R^2 of the imaging-based model is roughly twice the R^2s of the three existing models. Apparently, the less expensive and more accessible laboratory tests have an equal prognostic power compared with the more expensive imaging tests.

Staging with imaging techniques is not only a prognostic factor but is also important for treatment decisions, because both stages are treated differently. Limited-stage disease should currently be treated with platinum agent-based chemotherapy

with concurrent thoracic radiation therapy followed by prophylactic cranial irradiation in case of a complete response. Extensive-stage disease is treated with chemotherapy alone[1,4]. Not all patients were treated according to the previously mentioned current guidelines during our 9-year study period. This was because of deteriorated physical condition during treatment, early death, patient refusal, or other reasons.

In summary, prognostic models based on laboratory tests and PS provide a similar estimation of survival of patients with SCLC as the combination of PS and disease stage assessed by imaging tests. Although the present study confirms that imaging information is not necessary per se for prognostic purposes, it is not easy to imagine today's clinical practice of SCLC without imaging techniques, especially with respect to treatment decisions.

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Chapter 4

Prognostic value of different metabolic measurements with fluorine-18 fluorodeoxyglucose positron emission tomography in resectable non-small cell lung cancer: a two-center study

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J Thorac Oncol. 2010 Nov;5(11):1669-76.

Abstract

Introduction
Standard uptake value (SUV) is a quantitative measure for the preferential uptake of a radiopharmaceutical in a tumor compared with the homogeneous distribution in the body. SUV can be based on the maximum value of one pixel (SUVmax) or on the mean value in a region outlined by isodensity contours. The prognostic value of different SUVs in non-small cell lung cancer (NSCLC) is not established. We evaluated this value for SUVmax, SUV70%, and SUV50% among patients with resectable NSCLC.

Methods
All consecutive patients with resectable NSCLC who underwent an attenuation-corrected whole-body fluorine-18 fluorodeoxyglucose positron emission tomography scan from two university hospitals were selected. By adjusting the isocontour in the region of interest on the scan, SUVmax, SUV70%, and SUV50% of the primary tumor were calculated.

Results
Sixty-six patients (50 male, median age 63 years) were included. Of the tumors, 16 were pathological stage IA, 23 were IB, 4 were IIA, 14 were IIB, and 9 were IIIA. Median (range) values for SUVmax, SUV70%, and SUV50% were 6.4 (1.6-19.1), 5.1 (1.0-15.7), and 4.0 (0.9-13.4), respectively. SUVs were associated with survival.
Analysis of residuals of SUVmax as a continuous variable in a Cox's proportional hazard model for survival suggested no cut-off point and no indication of time-dependency. Patients with a SUV higher than the median value had a worse survival than patients with a SUV lower than median (hazard ratios for SUVmax, SUV70%, and SUV50% all were 2.9; $p = 0.02$).

Conclusion
SUVmax, SUV70%, and SUV50% measured with fluorine-18 fluorodeoxyglucose positron emission tomography have a similar prognostic value, and no "natural" cut-off point for SUVmax in resectable NSCLC was identified.

Introduction

Lung cancer is the leading cause of cancer-related death for both men and women in the Western world, accounting for more than 160,000 deaths annually in the United States alone[1]. In the recent years, fluorine-18 fluorodeoxyglucose (FDG) positron emission tomography (PET) has acquired a prominent place in staging non-small cell lung cancer (NSCLC)[2]. Staging by PET is superior to conventional staging with Computed Tomography (CT). This is largely explained by the detection of distant metastases and, to a lesser extent, by the detection of mediastinal metastases[3]. PET scan results are usually evaluated qualitatively, i.e. visual detection of increased FDG uptake in the primary tumor alone or as a visual estimation of the total tumor load[3], estimated with the tumor-node-metastasis (TNM) staging system[4].

Standard uptake values (SUVs) are used to quantitatively determine the increased FDG uptake in regions with increased metabolic activity. SUV is a measure for preferential FDG uptake in a tumor compared to a homogenous distribution in the body. SUVs are defined by drawing regions-of-interest (ROIs) over the tumor. The most commonly used SUV in the literature is SUVmax, based on the maximum value in a ROI. In addition, one can easily calculate other SUVs based on the mean value in a tumor area outlined by e.g. 70% and 50% isodensity contours. Our hypothesis was that such average SUVs better reflect the true metabolic nature of a tumor compared to SUVmax based on a single pixel value, and that such average SUVs therefore better predict the clinical outcome of a patient.

SUVs can be used to compare FDG uptake of tumors of different individuals or to measure response to chemotherapy[5]. SUVs can also be used to predict survival. Many studies observed that a higher SUVmax of the primary tumor is associated with a worse prognosis[6-15], but the prognostic value of the metabolic activity seems stronger in early disease than in advanced disease. Most authors were able to discern different prognostic groups in early stage NSCLC based on a cut-off level that was often arbitrarily chosen. There is not a good biological reason for a clear cut-off level of SUV values estimated in tumors.

In the present study, we evaluated the prognostic value of different SUVs in patients with early stage NSCLC in a two-center study. In addition, we explored whether it is possible to define an appropriate cut-off value for SUVs.

Patients and methods

Patients

All consecutive patients with pathological stage I to IIIA NSCLC from two University Medical Centers who underwent an attenuation-corrected whole-body FDG-PET scan before any treatment were included. Tumor staging was based on pre-

operative CT and PET scans, mediastinoscopy if indicated, and the resected tumor specimen and mediastinal lymph nodes. Gender, age at diagnosis, performance score, weight loss, histological subtype, tumor size, completeness of resection, and treatment with pre- or post-operative chemotherapy and/or radiotherapy were recorded. Survival analysis was censored on June 1, 2006.

To evaluate whether SUV also has prognostic value in advanced NSCLC, 33 subsequent patients with stage IV disease from University Medical Center Groningen were analyzed in an identical method during the same time period.

The present study was performed using anonymized data that were routinely collected. Therefore, consent was not specifically obtained and institutional review board approval was not necessary.

PET imaging

All PET scans were performed on Siemens ECAT scanners (Siemens/CTI, Knoxville, TN, USA) in two clinical PET Centers (University Medical Centers Groningen and Nijmegen) using the same acquisition protocol. The technical specifications of both Siemens ECAT scanners are similar, and the devices in both institutions were calibrated according to a standardized protocol[16]. Patients fasted for at least 6 hours before scanning. Sixty to ninety minutes after intravenous injection of 220-370 MBq FDG in the forearm, scanning was started using an interleaved protocol (emission-transmission) from mid-thigh to the skull. Measurements were performed consistently for every patient.

All scans were iteratively reconstructed using ordered subset expectation maximisation, and were interpreted by experienced nuclear medicine physicians.

SUV calculations

Regions-of-interest (ROI) were semi-automatically drawn around the region of focal FDG uptake in the primary tumor. Semi-automatically means that the tumor is three-dimensionally manually encircled, so that non-spherical tumors are also entirely within the ROI. SUV of the primary tumor was calculated by the following formula: SUV = activity concentration (Mbq/mL) / (injected dose [MBq] / body weight [g]). SUVmax was defined as the pixel with the highest FDG uptake within the ROI. SUV70% and SUV50% were calculated by adjusting the isodensity contour within the ROI by 70% or 50%, respectively. SUV70% is the mean SUV of all pixels with an activity of 70 to 100% of the pixel with the highest FDG uptake (SUVmax) within the ROI. SUV50% is similarly calculated by drawing the 50% isodensity contour in the ROI.

SUVs calculated from different scanners cannot generally be mutually exchanged, because of variations in scanning protocols and software settings. However, the difference between two scanners is highly consistent and constant, as was recently demonstrated for our institutions using anthropomorphic phantoms and equal settings and reconstructions[16]. The degree of the variation between our two

institutions is approximately 15%[16]. To overcome this inter-institutional variation, a correction factor between institutions may be calculated. In this study, we performed the measurements and analyses of SUV using the same protocol and reconstruction method in both hospitals. In our patient group, the median stage-specific SUV was not significantly different between both institutions. Therefore, we combined the retrospective clinical and raw SUV data of both institutions into one study.

Statistical methods

Spearman's correlation was calculated for the relation between the different SUVs mutually and between SUV and tumor size. SUVs of patients with different histology were compared using the Kruskall-Wallis test. SUVs of patients with different stages were compared using the Jonckheere test for ordered alternatives.

Overall survival was defined as the time between PET scan and date of death or last follow-up. Survival curves were constructed by means of the Kaplan Meier technique. Survival curves in different groups of patients were compared by means of the log-rank test.

Cox's proportional hazard regression analysis was used to evaluate the prognostic value of SUVmax, SUV70%, and SUV50%, and to adjust for other prognostic factors such as disease stage. The size of the effects was expressed as hazard ratio (HR) with a 95% confidence interval (CI). A HR greater than 1 indicates increased mortality. The existence of a natural SUV cut-off value and time dependency of SUV was evaluated by means of Martingale and Schoenfeld residuals[17,18]. P-values of less than 0.05 were considered statistically significant.

Results

Patient characteristics

A total of 87 patients were enrolled. Patients who were not treated by resection (n = 11), patients with incomplete SUV data (n = 6) and patients with insulin-dependent diabetes mellitus (n = 4) were excluded. Of the remaining 66 patients (50 male, 16 female) included, median age was 63 years (range, 38-79 years). Performance score was 0 for 20 patients (30%), 1 for 34 (52%), 2 for 4 (6%) and unknown for 8 patients (12%). Five patients (8%) had more than 10% weight loss. Seven patients (10%) received pre-operative chemotherapy consisting of three cycles of a platinum-based regimen.

Squamous cell carcinoma was found in 35 patients (53%), adenocarcinoma in 23 patients (35%), large-cell carcinoma in 7 patients (11%) and bronchoalveolar cell carcinoma in 1 patient (1%). Sixteen patients (24%) had pathological disease stage IA, 23 patients (35%) had stage IB, 4 patients (6%) had stage IIA, 14 patients (21%) had stage IIB, and 9 patients (14%) had stage IIIA. Median tumor size was 3.1 cm

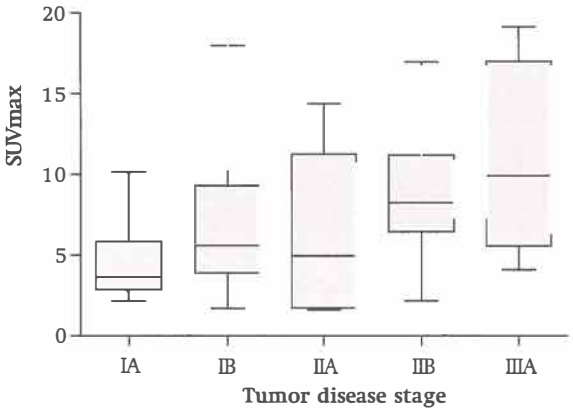

Figure 1.
Distribution of median SUVmax according to pathological tumor stage ($p < 0.001$).

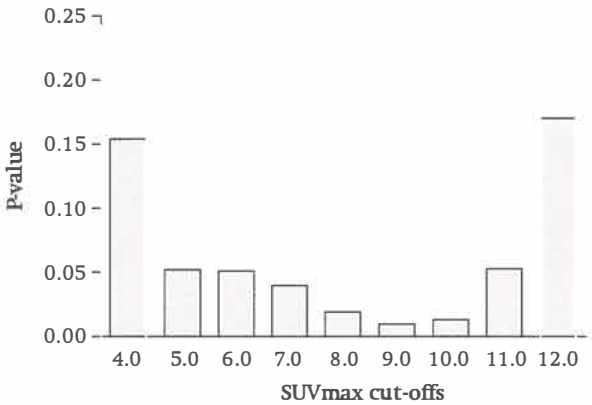

Figure 2.
Relationship between various cut-off levels of SUVmax and their discriminative value for overall survival estimated by univariate analysis. A p-value < 0.05 is considered significant.

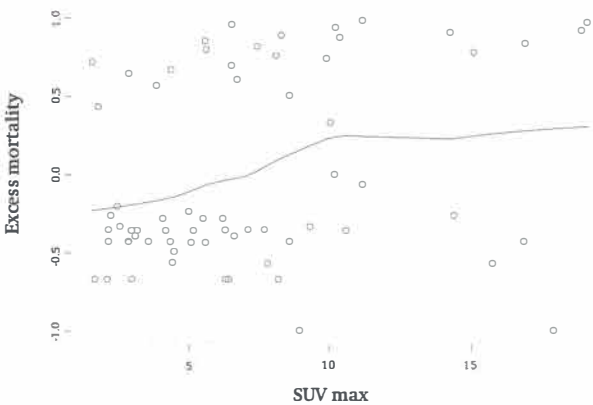

Figure 3.
Martingale residuals for a null model, suggesting that an increasing value of SUVmax is associated with an increased risk of death without showing a real cut-off value.

(range, 0.9-12 cm). At the time of analysis, 25 patients were deceased. The median overall survival of these patients was 1.7 years (range, 0.1-5.7 years). The median follow-up time for living patients was 3.2 years (range, 0.9-5.9 years).

Relation of SUV and patient characteristics

Median SUVmax was 6.4 (range, 1.6-19.1), median SUV70% was 5.1 (range, 1.0-15.7), and median SUV50% was 4.0 (range, 0.9-13.4). The distribution of SUVmax, SUV70, and SUV50 were skewed to the right. SUVmax, SUV70%, and SUV50% were highly correlated with each other; Spearman's correlation coefficients for SUVmax and SUV70% and SUV50%, and for SUV70% and SUV50% were 0.993, 0.990 and 0.997, respectively.

Median SUVs did not differ significantly among histological types of NSCLC; median SUVmax was 7.1 (range, 2-19) for patients with squamous cell carcinoma, 6.3 (range, 2-15) for patients with adenocarcinoma, and 5.6 (range, 3-19) for large cell carcinoma.

SUVmax, SUV70%, and SUV50% all increased with increasing disease stage (Jonckheere tests all $p<0.001$). Median SUVmax for patients with pathological stage IA, IB, IIA, IIB, and IIIA was 3.7, 5.6, 5.0, 8.2, and 9.9, respectively (Figure 1). A larger tumor size was correlated with a higher SUVmax (Spearman's correlation coefficient 0.565).

Exploring a cut-off point for SUV

SUVmax, SUV70%, and SUV50% as continuous variables were all significantly associated with survival ($p = 0.018$, $p = 0.012$, and $p = 0.011$, respectively). For the self-chosen cut-off points 7, 8, 9, and 10 SUVmax significantly predicted survival, as shown in Figure 2.

Analysis of Martingale and Schoenfeld residuals of SUVmax as a continuous variable suggests that an increasing value of SUVmax is associated with an increased risk of death without showing a real cut-off value (Figure 3). Because of this, the median values are chosen to dichotomize the data and present survival curves. This has the advantage of dividing the patients into groups of similar size.

SUV and prognosis

SUVmax, SUV70%, and SUV50% dichotomized at their median value predicted survival (HR 2.93 (95% CI 1.21-7.09), for all three SUVs). Patients with SUVmax higher than median value had a worse overall survival compared with patients with a lower SUVmax (Figure 4). All 66 patients had a tumor resection, 58 (88%) achieved a complete resection. SUVmax dichotomized at the median value predicted disease-free survival after complete resection ($p = 0.03$). Seven patients with an incomplete resection received post-operative thoracic radiotherapy.

Pathological disease stage ($p = 0.001$) and tumor size ($p = 0.007$) univariately predicted survival whereas gender, performance score, age older or younger than

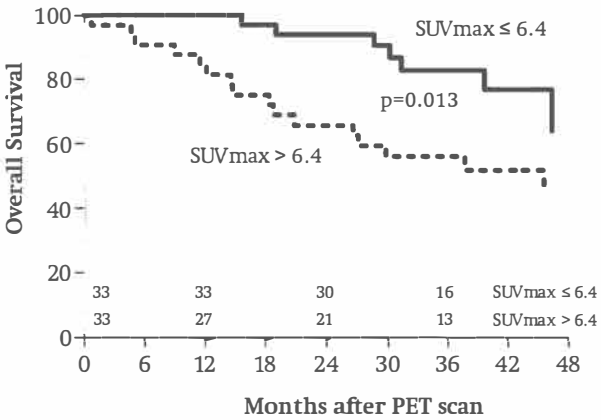

Figure 4.
Overall survival for 66 patients according to SUVmax, dichotomized at median value (6.4).

65 years, weight loss, pre-operative chemotherapy, and histological subtype did not predict survival.

Adjustment by stage in a multivariable analysis showed that SUVmax (and SUV70 or SUV50) provided additional prognostic information beyond pathological disease stage ($p = 0.051$, $p = 0.041$, or $p = 0.041$, respectively).

Prognostic value of SUV in advanced NSCLC

In contrast to the patients with early, resectable disease, no difference in overall survival was observed ($p = 0.949$) for 33 patients with stage IV NSCLC after dichotomizing at their median SUVmax (9.4).

Discussion

To our knowledge, this is the first clinical study to compare different SUVs in NSCLC. One pixel with maximum value within the whole primary tumor, 30% of the pixels with the highest metabolic activity, or even half of the pixels with the highest activity have similar prognostic impact in resectable NSCLC. Nevertheless, pathological TNM disease stage estimated with CT or PET remains the strongest independent prognostic factor. Another finding was that no "natural" cut-off point for SUVmax was observed in resectable NSCLC. In addition, the present study is the first two-center study evaluating the prognostic effect of SUV in NSCLC.

Methodological studies show that, provided that they are measured with the same equipment and reconstruction methods, SUVmax and average SUV within a ROI are closely correlated[19-21], as they were in the present study. Therefore, it does not seem surprising that they are equally able to predict prognosis. One could, how-

ever, argue that an average SUV better represents the true metabolic nature of a tumor and subsequently better reflects the clinical outcome of a patient than the metabolic information coming from a single pixel. However, our data suggest that SUVmax measured in the primary tumor is as effective as SUV70% and SUV50%, and SUVmax is the easiest SUV to calculate.

The accuracy of SUV can be influenced by several factors, of which ROI defining, image reconstruction, tracer administration, scanning time, and patient factors such as glucose metabolism and body weight are most well-known[20,22,23]. We diminished this variability of SUV by using the same scanning and reconstruction protocol in both institutions, because a previously performed study and results from this study showed that the differences between our institutions were highly consistent and constant[16]. In addition, the interobserver and intraobserver agreement in SUVs was found to be excellent[24]. These findings show that SUVmax is an easily reproducible and reliable prognostic parameter, permitted that the same scanning and reconstruction settings are used or a correction factor is applied between different institutions.

In contrast with several other reports[6,8,10-13], none of the SUVs could be identified as an independent prognostic factor in multivariate analysis. In their series of 100 patients with resected NSCLC, Downey et al. found that SUVmax greater than 9 (their observed median value) and primary tumor size larger than 3 cm and their interaction significantly predicted survival[12]. In our study, the SUVmax was associated with survival even when adjusting for stage of disease. In a large study with 315 patients planned for resection, SUVmax higher or lower than median value was the strongest prognostic factor, stronger than disease stage I/II versus III /IV, and even complete resection[13]. Similarly, in Sasaki et al.'s study of early stage NSCLC, SUVmax dichotomized at 5 and tumor size were independent predictors for survival, whereas nodal status and treatment were not[6]. Noteworthy, most of these studies use tumor size (T) without NM status. TNM disease stage is more powerful in predicting survival of resectable tumors than T status alone[4]. Therefore, the difference between these previous studies and our findings could be explained by the fact that we tested all separate TNM disease stages (IA to IIIA) instead of only T status in multivariate analysis. Interestingly, Veselle et al. recently reported that the correlation of SUVmax with tumor stage disappeared after correcting tumors smaller than 3 cm for partial volume effects[25]. It is indisputable that SUVmax has prognostic value, although the strength of its effect varies among the reported studies[26].

It is not clear which factor is most important in the prognosis of NSCLC. Two suitable candidates are the total tumor load in the body as expressed by TNM status and the biological behavior of the primary tumor, as expressed by SUV, or perhaps a combination of these two factors. For example, if tumor load is high as in advanced NSCLC, SUV may not have additional prognostic power, in contrast to cases with a low tumor load, such as early stage NSCLC, in which metabolic activity of a tumor may play an important role. Based on these assumptions, one would expect that

SUVmax would not be associated with survival in stage IV NSCLC. This is supported by our finding that SUVmax did not predict survival in 33 consecutive patients with stage IV NSCLC, in line with the observations of Lee et al. in advanced disease[27]. In addition, although median SUV generally increases with increasing disease stage, this increase is more pronounced for the lower disease stages, in contrast with the hardly noteworthy increase between the higher disease stages, such as IIIB and IV[13]. This is in agreement with the loss of prognostic value of SUV in the more advanced disease stages. Therefore, SUV seems to be most useful in early stage NSCLC.

Some groups have proposed data-driven cut-off values of SUV by calculating post hoc the discriminative value (expressed as p-value) for several self-chosen cut-off points. The cut-off point with the lowest p-value was considered the most suitable, regardless of the number of patients in the groups above and below the cut-off value[6,8-11]. By applying the same method, we also observed that SUV is a predictor for survival for several different cut-off values, but we question whether this methodology is appropriate. Firstly, this methodology may lead to overestimation because it is data-driven and because of the effects of multiple testing[28]. Secondly, there is no biological reason for a cut-off level phenomenon in tumors. SUV represents FDG uptake in a tumor, caused by the up-regulation of trans-membrane glucose receptors GLUT 1 and GLUT 3[29], and consequently is an indirect measurement for proliferation. The extent of this up-regulation represents as a continuous variable. In addition, SUVmax is highly correlated with Ki-67 expression, a marker of cell proliferation[30]. It is therefore plausible that no natural cut-off point for SUV was observed.

In conclusion, our two-center study showed that SUVmax, SUV70%, and SUV50% have similar prognostic impact in resectable NSCLC. In the absence of a biological cut-off point, the median SUVmax of the primary tumor, which divides the patients into groups of similar size, may be used. An interesting application of such a strong prognostic indicator as SUV is its use as stratification parameter in trials involving adjuvant chemotherapy.

Acknowledgements

The authors thank JWG van Putten, MD, PhD, chest physician, Martini Hospital, Groningen, the Netherlands, for providing patient data, ACL Grob, MD, Radboud University Nijmegen Medical Centre, Nijmegen, the Netherlands, for his help in collecting patient data, and R Boellaard, PhD, department of Nuclear Medicine, VU University Medical Center, Amsterdam, the Netherlands, for his helpful comments.

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Chapter 5

Promoter methylation is not randomly distributed in the bronchial epithelium but primarily occurs in tumor cells of patients with non-small cell lung cancer

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Submitted

Abstract

Introduction

Exposure to tobacco smoke may explain a random occurrence of methylation events throughout the bronchial epithelium of patients with NSCLC. The methylation status of the promoter region of 11 genes was assessed in brushes sampled of 3 well-defined endobronchial locations in patients with NSCLC and in brushes of controls.

Methods

Bronchial brushes were collected of 1) the tumor, 2) normal appearing bronchus 2-3 cm proximally of the tumor, and 3) the main bronchus of contralateral lung in patients with NSCLC, and of the main bronchus of controls. The methylation status of *RASSF1A*, *GATA4*, *GATA5*, *SFRP1*, *RARβ2*, *DAPK*, *MGMT*, *p16*, *p14*, *CHFR*, and *APC2* was determined in all samples using real-time methylation-specific PCR. Genes were considered methylated if the ratio of gene/*ACTB* PCR product levels was higher than gene-specific cut-off values (defined as the highest ratios observed in controls).

Results

Ten patients with NSCLC and 24 controls were included. Eight patients had one or more methylated genes in their tumor brush. Promoter methylation of genes in proximal or contralateral locations was much less frequent than in tumor brushes, and almost exclusively occurred in normal tissue if the same gene was also methylated in the tumor brush. No methylation of the promoter region was observed in controls.

Conclusion

Promoter methylation was observed in 80% of bronchial tumor brushes of NSCLC patients. Promoter methylation was almost exclusively observed in endobronchial tumor cells and was very uncommon or absent in normal appearing tissue samples and controls, which is in line with clonal field expansion.

Introduction

DNA-methylation is one of the most well-known epigenetic processes[1]. DNA-methylation refers to the binding of a methyl group to cytosine nucleotides in the DNA sequence. Methylated cytosines are found in relatively high contents in CpG islands. These CpG islands are distributed in a non-random manner across the humane genome, including the promoter regions of many tumor-suppressor genes. Aberrant DNA-methylation of CpG islands may lead to the transcriptional silencing of tumor suppressor genes, and is among the earliest and most frequent alterations in cancer. The varying levels of tumor-suppressor gene methylations among different malignancies can be measured with a sensitive methylation-specific polymerase chain reaction (MSP), which seems a promising tool for early cancer detection. Aberrant methylation is common in non-small cell lung cancer (NSCLC)[2].

Promoter methylation of multiple genes has not only been observed in NSCLC tumor tissue and NSCLC cell lines[3,4], but also in histologically tumor-free lung tissue of patients with resected NSCLC[5,6], lymph nodes[7,8], tumor brushes[9], serum[10], bronchoalveolar lavage (BAL)[11-14], and sputum[15,16] of patients with NSCLC. In addition, promoter methylation was also detected in sputum and bronchial brushes of heavy smokers[9,17,18]. The detection of promoter methylation in sputum or brushes of cancer-free heavy smokers proved to be a risk factor for the subsequent development of cancer[9,15,19], highlighting the potential of methylation assays as lung cancer screening tool. Evidence for a possible association between the presence of promoter methylation and smoking status and/or number of packyears is conflicting[12,13,17,18,20-23]. Altogether, methylation assays offer new possibilities in studying pathogenesis, diagnosis, and eventually treatment of NSCLC.

One could hypothesize that aberrant promoter methylation occurs randomly in bronchial epithelium that is exposed to tobacco smoke. Local recurrences or second primary tumors after first-line treatment may develop from premalignant lesions other than the lesion responsible for the first tumor. The presence of random promoter methylation throughout the lungs could therefore be a sign of the presence of random, multiple lesions with genetic damage caused by tobacco smoke.

In contrast with the occurrence of multiple, random lesions, one could as well hypothesize that molecular and subsequent histological abnormalities first arise at one, unique location. Synchronous abnormalities observed in other locations, and also subsequent local recurrences and second primary tumors, all originate from this primary tumor by means of clonal expansion[24]. This concurs with the field cancerization theory, originally proposed by Slaughter in 1953[25]. Obviously, the field cancerization theory was based on histological parameters, and not on methylation events. According to the field cancerization theory, promoter methylation detected in tissue sampled from other locations than the primary tumor originates from the primary tumor by either clonal expansion or cellular contamination. The clonal expansion theory predicts a decreasing number of promoter methylation events with

increasing distance from the primary tumor.

A bronchial brush is an excellent tool for obtaining a large sample of bronchial epithelial cells of well-defined locations in the bronchial tree. These brushes are widely used for the cytological diagnosis of pulmonary abnormalities, such as NSCLC. Bronchial brushes generally contain more than one million cells, and 80-90% of these cells are epithelial cells[26,27]. One study reported that 32% of former smokers had one or more genes with promoter methylation in epithelial cells obtained with a carinal bronchial brush[18]. Zochbauer et al. observed promoter methylation in up to 20% of bronchial brushes of smokers[17]. In another study, promoter methylation of *p16* was observed in 16% and 8% in bronchial brushes of patients with lung cancer versus smokers without lung cancer, respectively[9]. The sensitivity and specificity of detecting NSCLC in tumor brushes using methylation assays is not known.

To evaluate the distribution of promoter methylation, bronchial epithelial cells of three well-defined endobronchial locations in patients with NSCLC were collected, and the presence of promoter methylation of eleven genes *[RAS-association domain family 1, isoform A (RASSF1A), GATA binding protein 4 (GATA4), GATA binding protein 5 (GATA5), secreted frizzled-related protein 1 (SFRP1), retinoic acid receptor beta-2 (RARβ2), death-associated protein kinase (DAPK), O⁶-methylguanine-DNA-methyltransferase (MGMT), cyclin-dependent kinase inhibitor 2A (CDKN2A/p16 and p14), checkpoint with forkhead and ring finger domains (CHFR), and adenomatous polyposis coli 2 (APC2)]* at those three locations in the bronchial tree was quantitatively determined. Also, the sensitivity and specificity of detecting NSCLC in a tumor brush compared to brushes of controls using this panel of eleven genes associated with NSCLC were defined.

Patients and methods

Patients

Patients who were scheduled for a diagnostic bronchoscopy during the work-up for suspected lung cancer were asked to participate in the study. Ten patients were included (6 females, 4 males), with a median age of 54 years (range, 40-70). Six patients were current smokers; four patients were former smokers 1 to 12 years since quitting. Median number of packyears was 33 (range, 20-60). Five patients had adenocarcinoma, four had large cell carcinoma and one patient had a neuro-endocrine carcinoma. At diagnosis, one patient had stage IA disease, two had stage IIIB, and seven patients had stage IV disease.

Controls were patients who underwent a bronchoscopy for other reasons than suspected lung cancer. Twenty-four controls (9 females, 15 males) were included, with a median age of 53 (range, 23-69). Nine controls were never smokers, 7 former smokers (1-39 years since quitting) and 8 were current smokers. Median number of packyears was 6 (range, 0-70). Twenty controls had asthma; the remaining four con-

trols had chronic obstructive pulmonary disease (COPD).

The study protocol was approved by the medical ethics committee of the University Medical Center Groningen. All patients and controls gave informed consent.

Sample collection

Flexible fiberoptic bronchoscopy (Olympus, Tokyo, Japan) via nasal introduction was performed under local or general anaesthesia. In controls, one bronchial brush was obtained from the normal appearing main bronchus. In patients, brushes were sampled of three well-defined endobronchial locations. One brush was collected from the tumor, a second brush from the normal appearing main bronchus 2-3 cm proximal from the tumor, and a third from the normal appearing main bronchus of the contralateral lung. To avoid contamination of the bronchoscope with tumor cells, the contralateral brush was collected first, followed by the proximal brush, and finally the tumor brush. All brushes were sheathed cytology brushes (Cellebrity Endoscopic Cytology Brush, Boston Scientific, Boston, USA).

After sampling, brushes were stored in a solution of 50% ethanol and 2% polyethylene glycol at room temperature until further processing.

DNA isolation and modification

Cells attached on the bronchial brush were collected by centrifugation at 960 relative centrifugal forces for 10 minutes and by an additional washing with PBS and a centrifugation step. Then, genomic DNA was extracted using the classical SDS/protein K digestion and phenol-chloroform extraction, and resuspended in 50 mL Lo TE (3 mM Tris, 0.2 mM EDTA, pH 8.0). The DNA was quantified using the Picogreen dsDNA quantitation kit (Molecular Probes, Invitrogen, Breda, the Netherlands) following the manufacturer's recommendations. The average yield of DNA was 19.2 µg (range, 0.5-87.7). Up to 1.5 µg of genomic DNA was modified using sodium bisulfite to deaminate selectively unmethylated cytosine residues to uracil, while 5-methyl cytosine residues are not modified. The bisulfite modification was performed using the EZ DNA Methylation Kit™ (Zymo Research, Orange, CA, USA), which includes successively steps of conversion, desalting and desulfonation. At the end of the procedure, the modified DNA was eluted in 50 mL of 1 mM Tris-HCl, pH 8.0, and then stored at -80 °C until used for real-time MSP.

Real-time MSP

Briefly, for each gene of interest and *beta-actin (ACTB)*, methylation was determined in real-time MSP using bisulfite modified DNA as previously described by Herman et al.[28]. *ACTB* was used as an independent reference gene. The promoter methylation status of *RASSF1A*, *GATA4*, *GATA5*, *SFRP1*, *RARβ2*, *DAPK*, *MGMT*, *p16*, *p14*, *CHFR*, and *APC2* was calculated as the ratio of the gene of interest and *ACTB* according to the following equation: (copies methylated gene/copies *ACTB*) * 1000. This ratio is a measure for the relative level of methylation in an individual sample.

Statistical analysis and cut-off value

Promoter methylation was considered present if the ratio was above a certain cut-off value. The relative level of methylation varies significantly among the 11 genes, and therefore cut-off points were studied for each gene apart.

The gene specific cut-off value was defined as the highest ratio observed in controls (visualized in scatter plots). In this way, the specificity of a single gene was set at 100%.

The overall sensitivity, specificity, and accuracy of our panel of genes were calculated, i.e. the ability to detect lung cancer based on the presence of one or more genes with promoter methylation in patients and controls using our observed cut-off value.

The presence of promoter methylation at the tumor, proximal, and contralateral locations was visualized. Also, the overall level of methylation was determined using the methylation index (MI)[29]. The MI is defined as the ratio of number of genes with promoter methylation to the number of genes tested * 100%.

Associations between promoter methylation and demographic and clinical characteristics of patients and controls were calculated using chi-square and Mann-Whitney tests, where appropriate. A p-value of < 0.05 was considered statistically significant.

Results

Exploring cut-off values using normal controls and tumor brushes

In epithelial cells from bronchial brushes of 24 controls and 10 patients with NSCLC, the ratios of expression of 11 methylated genes as compared to the expression of *ACTB* ranged from 0 to 71 (Table 1). The mean ratio of methylated genes versus *ACTB* was higher in patients than in controls for all eleven genes except *DAPK* and *p14* (Table 1). Also for *DAPK* and *p14*, the highest ratio observed in controls was higher than in patients with NSCLC, for the other nine genes at least one ratio in patients was higher than the highest ratio observed in controls (Figure 1).

Sensitivity and specificity of promoter methylation assays on epithelial cells obtained by bronchial brushes

Eight out of ten patients with NSCLC had at least one gene with promoter methylation in the tumor brush and none of the 24 controls using our cut-off value, resulting in an overall sensitivity of 80%, specificity of 100%, and an accuracy of 94% (Figure 2). One patient had 5 genes with promoter methylation, one patient 4, one patient 3, three patients 2, and two patients only one. Promoter methylation was most frequent in *APC2* and *RASSF1A* (in 5 and 4 patients, respectively). For *DAPK* and *p14*, no promoter methylation was observed in these 10 patients.

Table 1. Promoter methylation status of eleven genes in endobronchial epithelial cells from 10 patients with NSCLC and 24 controls.

Gene	Mean ratio gene/ACTB *1000 in patients (range)	Mean ratio gene/ACTB *1000 in controls (range)	Cut-off value (based on 100% specificity)
RASSF1A	70.8 (0-393)	0.15 (0-2.5)	10
GATA4	21.9 (0-217)	0.06 (0-0.8)	1
GATA5	1.1 (0-11)	0.07 (0-0.3)	0.5
SFRP1	1.7 (0-17)	0.09 (0-0.6)	5
RARβ2	1.1 (0-6)	0	0.5
DAPK	0.08 (0-0.5)	0.14 (0-0.9)	2
MGMT	13.9 (0-138)	0.12 (0-1.2)	5
p16	0.63 (0-4.6)	0.02 (0-0.1)	1
p14	0.02 (0-0.2)	0.04 (0-0.6)	1
CHFR	55.0 (0-549)	0	0.5
APC2	5.4 (0-30)	0.65 (0-2.3)	2.5

Distribution of promoter methylation in bronchial epithelium

Overall, we identified in 10 NSCLC patients using a panel of 11 different genes a MI of only 18% (20/110) (Figure 2) in tumor cells and 2.7% in normal epithelial cells of NSCLC patients. Epithelial cells obtained from the proximal and contralateral brush did not differ in the number of genes with promoter methylation. No random pattern of promoter methylation in normal bronchial epithelium at the proximal and contralateral localizations was observed. The highest overall number of methylated genes was observed in patient 8. His *p16/ACTB* ratios * 1000 for tumor, proximal, and contralateral brushes were 4.6, 6.0, and 1.3, respectively, above the cut-off value of 1. Patient 8's *APC2/ACTB* ratios * 1000 were 11.2, 12.0, and 3.7, respectively, with a cut-off value of 2.5. *APC2* was also methylated in the proximal brush of patient 1 (3.4) and in the contralateral brush of patient 6 (3.2).

Correlation between promoter methylation and clinical characteristics

The presence of promoter methylation in tumor brushes was not associated with exposure to smoking ($p = 0.53$ for number of packyears) or acute smoking effects ($p = 0.2$ for current versus former smokers). In this study, age and gender were also not associated with the presence of methylation ($p = 0.53$ and $p = 0.75$, respectively). Finally, the histological subtype of NSCLC was not associated with methylation ($p = 0.29$). The two patients without any promoter methylation were a 51-years old female, a current smoker with 35 packyears, and a 69-years old male, a current smoker with 40 packyears, both with adenocarcinoma of the lung.

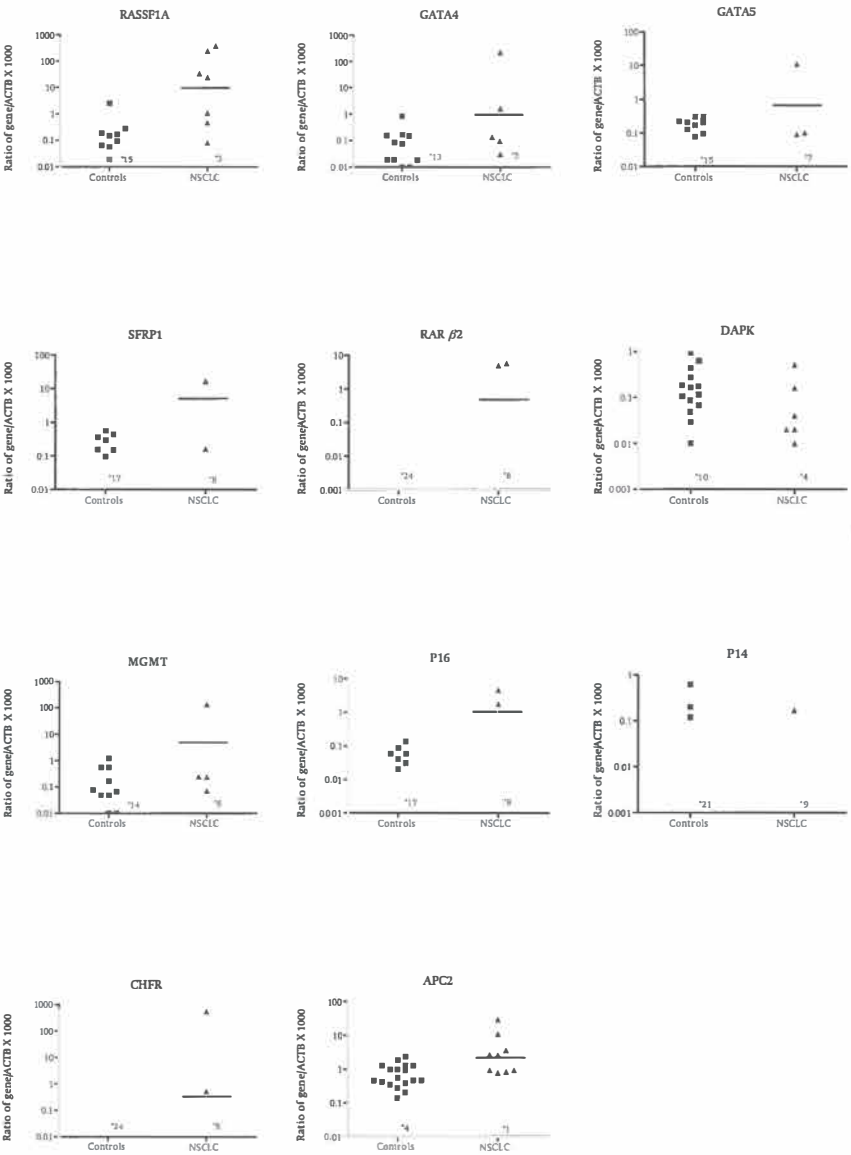

Figure 1.
Scatter plots of methylation ratios of 11 genes/ACTB levels * 1000 (^{10}log scale) in endobronchial brushes of 24 controls and 10 NSCLC patients. The bar in the NSCLC column represents the cut-off value based on 100% specificity.
* number of patients or controls with undetectable levels of the methylated allele.

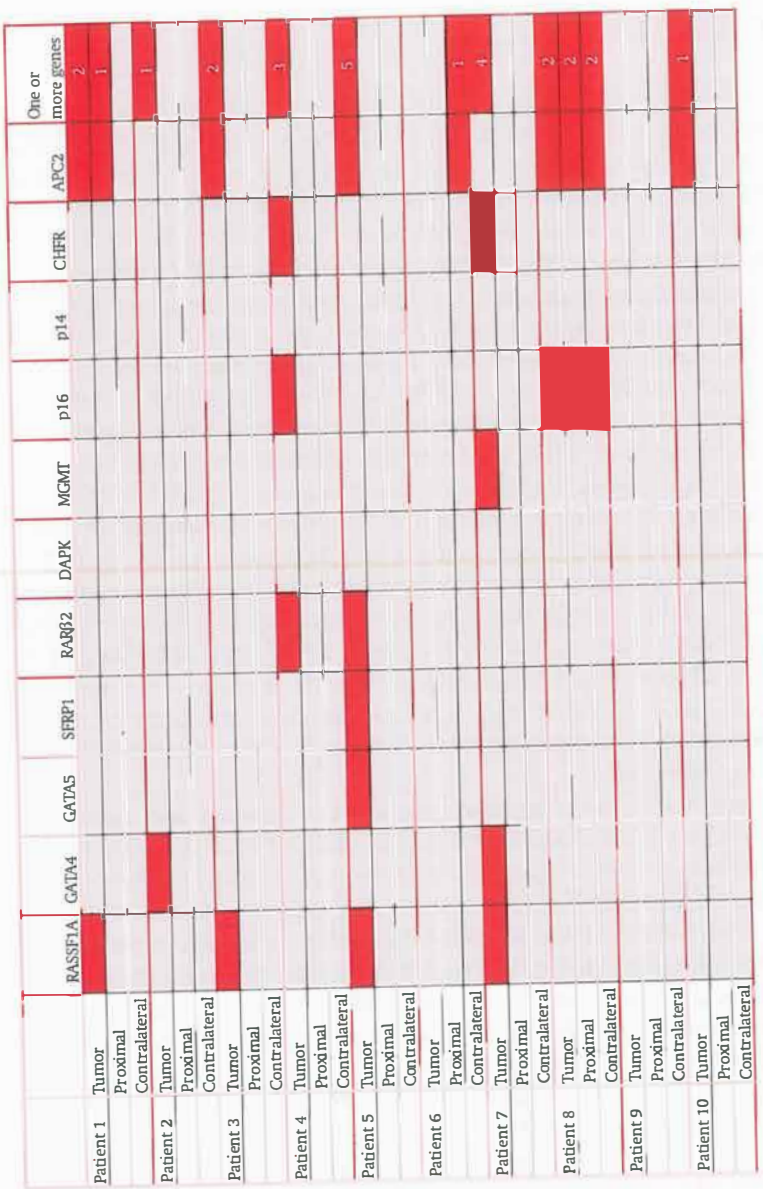

Figure 2.
Distribution of gene promoter methylation at three endobronchial locations (tumor, 2-3 cm proximal of tumor, contralateral lung) in 10 patients using a cut-offvalue based on 100% specificity in 24 controls. Light red denotes unmethylated genes, red methylated genes. The right column provides a summary with the number of methylated genes at a single location.

Discussion

The present exploratory study is the first study to evaluate the presence of promoter methylation at different endobronchial locations in patients with NSCLC. Promoter methylation was observed in tumor brushes of up to 80% of patients with NSCLC, whereas it was uncommon in proximal and contralateral brushes, and almost exclusively present if the same gene was also methylated in the tumor brush.

The quantitative results of a methylation-specific real-time PCR are often dichotomized for comparative purposes[11,30-33]. Different methods for defining the optimal cut-off values for dichotomization exist. One could define cut-off values based on methylation frequencies of genes in tumor tissue. These frequencies can be derived from previously published studies[30], and the cut-off value is adjusted to match the expected methylation frequencies. Essential for this method is that consistency is observed in independently established promoter methylation frequencies of tumor tissues for individual genes[30]. Yet, the methylation frequencies in exfoliative samples are generally lower than those in tumor tissue[17]. Also it is known that median methylation ratios were observed to be higher in tumor tissue than in other tissues[11,33]. This is partially explained by the fact that brushes and sputum contain relatively less bronchial epithelial tumor cells than tumor tissue due to contamination with other cells. Therefore, we chose to define a specific cut-off value for brushes, based on the ratios observed in controls, with specificity set at 100%. This means that cut-off points specifically determined for brushes are more appropriate for the use of methylation assays in the early detection of cancer in exfoliative samples than tissue-based cut-off values.

The overall frequencies of promoter methylation in proximal and contralateral brushes were substantially lower than in tumor brushes (Figure 2). In the present study, no random pattern of distribution of promoter methylation in the proximal and contralateral endobronchial locations was observed; methylation at these locations was in all but one case only present if the tumor brush also contained promoter methylation of the same gene. This observation favors the idea that promoter methylation is primarily present in tumor cells, and subsequently may spread from the primary tumor to adjacent and even further locations in the bronchial epithelium, as was predicted with the theory of clonal field expansion[24]. Exposure to tobacco smoke causes genetic damage to the entire bronchial epithelium, as was recently demonstrated by the construction of a biomarker panel that was able to predict the presence of lung cancer in normal airway epithelium of smokers with suspected lung cancer[34]. This panel has no overlap with the genes we tested. In our study, smoking did not result in a random development of promoter methylation in the entire bronchial epithelium in patients with lung cancer. The absence of methylated genes in our controls, of whom many had a long history of exposure to tobacco smoke, is in line with this finding. In contrast, other studies report the detection of promoter methylation in exfoliative bronchial material in 5-15% of heavy smokers

without NSCLC (Table 2). It is also reported that the detection of promoter methylation precedes the development and/or diagnosis of NSCLC, and that therefore methylation assays can be used as a screening tool for lung cancer[9,15,19]. Our results may lead to the conclusion that the use of methylation tests as screening tool for lung cancer is highly dependent on the presence of tumor cells in the sample to be screened, such as sputum or bronchial aspirates.

We did not observe any association between smoking history, age, gender, or even tumor type, in agreement with most studies[11-13,17,18,20,23,31,35]. In contrast, some studies reported that the frequency of methylation is higher in current smokers versus former or never smokers[12,14,21,36], in males versus females[5], or is dependent on tumor histology[36,37]. Our results, especially of the proximal and contralateral brushes, shows that methylation is much more pronounced in tumor cells compared to non-tumor cells from the same individual. This finding suggests that the tumor field effect may be limited in terms of distance from the tumor and/or percentage of affected cells in the vicinity of the tumor. Our results are not necessarily discrepant results of other groups observing methylation in smokers without lung cancer and cancer-free tissue. The difference may be due to the use of cut-off values resulting in low false-positivity rates of our assays, differences in specimens (Table 2), or other undefined factors.

To our knowledge only one study reported on promoter methylation assays on brushes of patients with lung cancer[9]. In brushes from either main bronchus or tumor of patients a 16% methylation frequency was observed for *p16*, dropping to 8% for chronic smokers without lung cancer. Using quantitative methylation-specific PCR, methylation frequencies of 10-30% were reported for several genes in sputum, BAL fluid, and brushes of heavy smokers without lung cancer (Table 2). The observed frequencies in studies using qualitative, real-time MSP are generally lower (Table 2). Real-time MSP has not been performed on bronchial epithelial cells collected by sheathed brushes before. In our study, single gene methylation frequencies of tumor brushes ranged from 0-50%, with an overall methylation index of 18%. Apart from technical differences, the reported methylation frequencies in other studies may be due to patient selection (for instance presence of sputum atypia[17] or controls with a history of cancer[18]). In addition, our strategy differed from others, in that we set our design and test condition in such a way to have the highest specificity for lung cancer.

In summary, promoter methylation was almost exclusively observed in endobronchial tumor cells and was very infrequent or absent in cancer-free samples. Whether the promoter methylation observed by other groups in cancer-free tissue and in controls without lung cancer, in contrast with our results, is a result of contamination with tumor cells or is due to methodological issues such as the high specificity of real-time MSP is not known.

Table 2. Summary of studies with promoter methylation in bronchial exfoliative material in cases without lung cancer.

Study	Method	Material (number of patients)	Smoking status (number of patients)	Genes (methylation frequency)	Remarks
Belinsky 1998[38]	Qualitative MSP	Sputum (33)	Smokers (33)	*p16* (24%)	3 of 8 positive controls were diagnosed with NSCLC
Palmisano 2000[15]	Nested two-stage qualitative MSP	Sputum (123)	Smoke and/or radon exposure (123)	*p16* (12-19%) *MGMT* (16-36%)	Methylation present in 100% of sputum of controls who developed lung cancer in follow-up
Kersting 2000[9]	Semi-nested two stage MSP	Sputum (25)	Smokers (25)	*p16* (16%)	Induced sputum
		BAL (25)	Smokers (25)	*p16* (12%)	10mL
		Brush (25)	Smokers (25)	*p16* (8%)	Main bronchus
Belinsky 2002[20]	Nested two- stage qualitative MSP	Sputum (66)	Current and former smokers	*p16* (35%) *DAPK* (25%) *MGMT* (16%) *RASSF1A* (3%)	Uninduced sputum
		Bronchial epithelial cells (41)	Current and former smokers	*p16* (44%) *DAPK* (4%)	Cells harvested by brush of normal appearing bifurcations
Soria 2002[18]	Qualitative MSP	Brushes(100)	Former smokers (100)	*p16* (17%) *DAPK* (17%) *GSTP-1* (6%)	Brushes of carina. 17% history of previous cancer
Zochbauer 2003[17]	Qualitative MSP	Sputum (103)	Smokers (73), never smokers (30)	*RARβ2* (13-27%) *CDH13* (7-10%) *p16* (3-5%) *RASSF1A* (0-4%)	Selected on sputum atypia
		Bronchial brushes (87)	Smokers (87)	*RARβ2* (20%) *CDH13* (7%) *p16* (7%) *RASSF1A* (6%)	Apical segment right upper lobe
		BAL fluid (43)	Smokers (43)	*RARβ2* (9%) *CDH13* (5%) *p16* (0%) *RASSF1A* (5%)	30ml, with incubation
Topaloglu 2004[11]	Fluorescence based real-time (quantitative) MSP	BAL fluid (10)	Smoking status unknown	*CDH1* (30%) *RASSF1A* (30%) *APC* (30%) *MGMT, p16, GSTP-1, RARβ2. ARF* all 0%	120mL. Use of cut-off of gene/*ACTB* ratio. Ratios in controls lower than in patients

Kim 2004[12]	Qualitative MSP	BAL fluid (127)	Current smokers (110), and never smokers (17)	*p16* (6%) *RARβ2* (13%) *H-CAD* (3%) *RASSF1A* (4%) *FHIT* (28%)	10mL, *FHIT* associated with smoking
Grote 2004[31] and 2005[13]	Fluorescence based real-time (quantitative) MSP	BAL fluid (64)	Current and former smokers	*APC* (1.5%), *p16* (0%) *RARβ2* (13%) *SEMA3B* (90%!)	Use of cut-off of gene/MYOD1 ratio.
Destro 2004[39]	Qualitative MSP	Sputum (100)	Heavy smokers (100)	*p16* (4%)	Uninduced sputum
Schmiemann 2005[14] Partially same patients as [13,31]	Fluorescence based real-time (quantitative) MSP	BAL fluid (102)	Current, former, and never smokers	*APC* (1%) *p16* (0%) *RARβ2* (21%) *RASSF1A* (0%)	Use of predefined cut-off values [13,31]
Russo 2005[40]	Qualitative MSP	Brushes (22)	Smokers (22)	*DAPK* (35%) *ECAD* (27%) *MGMT* (11%) *p16* (5%)	Location brush unknown. Five never smokers no methylation
Belinsky 2005 [41]	Nested two-stage qualitative MSP	Sputum (118)	Smokers (118)	*p16* (25%) *MGMT* (14%) *RASSF1A* (7%) *DAPK* (18%) *H-CAD* (29%) *PAX5* (9-12%)	Induced sputum
Cirincione 2006[42]	Nested qualitative MSP	Sputum (112)	Heavy smokers (112)	*RARβ2* (51%) *p16* (18%) *RASSF1A* (1%)	Uninduced sputum
Belinksy 2006 [19]	Nested two-stage qualitative MSP	Sputum (92)	Current and former smokers	14 genes, frequencies (7%-77%)	Uninduced sputum. Sensitivity and specificity of 64% to detect NSCLC
Shivapurkar 2007 [33]	Fluorescence based real-time (quantitative) MSP	Sputum (25)	Smoking status unknown	*3-OST-2* (12%) *RASSF1A* (8%) *p16* (8%) *APC* (4%)	Uninduced sputum. Use of cut-off of gene/*MYOD1* ratio.

MSP Methylation-specific PCR, BAL Bronchoalveolar lavage.

Acknowledgements

The authors thank Alexandre Meunier for his technical assistance.

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Uncommon thoracic tumors: thymic epithelial tumors and desmoid tumors

Chapter 6

Thymic epithelial tumors: a population-based study of the incidence, diagnostic procedures, and therapy

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Eur J Cancer, in press

Abstract

Introduction
Thymic epithelial tumors are uncommon, and therefore the diagnostic and therapeutic approaches are not standardized. The purpose of this population-based study was to determine the incidence of the whole spectrum of thymomas and thymic carcinomas and to evaluate the diagnostic procedures, therapy, and survival of these tumors.

Methods
Pathological and clinical data from the Netherlands National Pathological Archives and the Netherlands Cancer Registry were evaluated. Excess mortality compared to the Netherlands standard population was estimated by relative survival analysis.

Results
Between 1994-2003, 537 thymic epithelial tumors were diagnosed. Median age was 59 years (interquartile range, 48-70), 51% were females. The incidence of all thymic epithelial tumors was 3.2/1,000,000, and 2.2/1,000,000 for thymomas (WHO types A-B2).
Primary resection as the first procedure for obtaining a diagnosis was performed in 56% of cases, and was associated with smaller tumors ($p < 0.001$) and younger age ($p = 0.006$).
Survival data were available for 232 cases. Not only thymic carcinomas (type C) but also thymomas (types B1-B3) were associated with excess mortality. Cases that underwent resection (78%) had a better survival than non-operated cases (median survival > 10 years versus 1.1 years, $p < 0.001$). Among the surgically treated cases ($n = 180$), the completeness of resection did not predict survival ($p = 0.53$).

Conclusion
Thymic epithelial tumors are rare. Excess mortality was observed in the majority of tumors. Surgery offers the best perspectives, even if the resection is incomplete.

Introduction

Thymomas and thymic carcinomas are rare tumors but nevertheless are the most common neoplasms that arise from the thymus in the anterior mediastinum[1]. Thymomas originate from thymic epithelial cells. Although in thymomas epithelial cells lack cytological atypia, thymomas may behave as locally invasive tumors and can therefore be considered as potentially malignant. Whether they are truly malignant is still a subject of debate. Thymic carcinomas also arise from thymic epithelial cells, but they have both a malignant cellular appearance and behavior. One third to two thirds of the thymomas are found in asymptomatic patients[1]. The most well-known paraneoplastic syndrome is myasthenia gravis, occurring in up to 45% of patients with a thymoma[1,2].

A provisional diagnosis of a thymic tumor is based on clinical features and anatomical appearance on computed tomography (CT)[2]. A definitive diagnosis of a thymic epithelial tumor is subsequently established by examination of tissue obtained through transthoracic needle or surgical biopsy, or after primary resection. Thymomas are also incidentally found during thoracic surgery for other reasons or during autopsy[3]. How often cytological aspirates or biopsy procedures precede resection and lead to accurate classification is unknown.

Thymic epithelial tumors are characterized by histological subtype and stage of disease. Since 1999, the World Health Organization classification is the most widely used histological classification system[4]; the terms benign and malignant thymoma have fallen out of fashion. Thymic epithelial tumors are divided into six types, based on the predominant cell type. The Masaoka staging system is the most widely used clinical staging system for thymic epithelial tumors[5]. This system is based on the presence of local invasion in the thymus capsule and neighboring organs, and on systemic expansion.

At present, the primary choice of treatment is resection[1]. The use of chemotherapy and/or radiotherapy, with or without surgery, is not standardized[6]. Several studies report that survival is strongly dependent on achieving a complete resection, histological classification, and tumor stage[7-11].

Our aim was to determine the incidence of all thymic epithelial tumors, and to evaluate the diagnostic procedures and the therapeutic interventions for these tumors in the Dutch population during a 10-year period.

Patients and methods

Data on cancer patients were anonymously collected by two nationwide databases. The Netherlands National Pathological Archives (PALGA) database registers all cytopathological and histopathological reports, and contains basic patient

characteristics and diagnostic terms in line with the systematized nomenclature of medicine terminology.

The Netherlands Cancer Registry (NCR) records clinical and surgical data of all newly-diagnosed malignancies, with the exception of basal-cell skin cancer, and contains basic patient characteristics and clinical information such as tumor location, tumor stage, treatment, and survival. Vital statistics were collected either directly from the patient's medical record or through linkage of NCR with the municipal population registries. Information from the PALGA and NCR databases were merged over a period from 1994 to 2003.

All procedures were performed according to Dutch privacy regulations. Because the study involved anonymized data that were routinely collected and explicitly did not involve the use of human subjects, consent was not specifically obtained and Institutional Review Board approval was not necessary.

Selection of cases from PALGA

An initial screening was performed in PALGA using the key words: "thymoma, benign"; "thymoma, malignant"; "thymoma, lymphocyte-rich"; "thymus, neoplasm"; "thymus, benign"; and "thymus, malignant". Between January 1, 1994, and December 31, 2003, 1244 hits matched the keywords. These 1244 hits originated from 750 cases, because in many cases more than one pathological specimen was recorded of the same tumor.

Of these 750 cases, 151 (20.1%) were excluded from the analysis because of other diagnoses, of which malignant lymphoma (49 cases, 6%) and carcinoid tumor of the thymus (31 cases, 4%) were the most common. Another 38 cases (5.1%) were excluded, because their thymic epithelial tumor was originally diagnosed before 1994. In 24 cases (3.2%), a definitive diagnosis was inconclusive or unknown. These categories were all excluded, leaving a total of 537 primary thymomas and thymic carcinomas.

Selection of cases from NCR

Over the same period, a total of 269 cases with a malignancy located in the thymus region (ICD code C.37.9) were collected from the NCR database. Cases were matched with PALGA cases using date of birth, gender, and, if necessary, date of diagnosis.

Two hundred thirty-two (86.2%) of the 269 cases with a thymus tumor according to NCR could be merged with PALGA data. Of these 232 cases, six cases had a histological diagnosis other than a thymic epithelial tumor according to NCR. In these cases the PALGA classification prevailed. Of the 37 unmatched cases, 26 had a histological diagnosis other than a thymic epithelial tumor according to NCR, mostly carcinoid (17 cases) or neuro-endocrine carcinomas (6 cases) of the thymus.

Tumor classification and staging

All tumors were histopathologically classified according to WHO criteria[4]. Older classifications were reclassified according to WHO by two independent observers (JLGB and WKdJ). Some neoplasms are composed of combinations of the various types, and are therefore considered combined thymomas (e.g. B2 + B3). These mixed presentations were classified according to the most aggressive type, which determines the prognosis[7]. For clinical staging the Masaoka system was used[5].

Diagnostic approach

For each case the diagnostic procedures resulting in a definitive diagnosis of "thymoma" or "thymic carcinoma" were recorded. Diagnostic procedures were categorized as transthoracic fine needle aspirates for cytology, transthoracic needle biopsies, surgical biopsies, primary resection for histology, or incidental findings at autopsy or surgery for other reasons. The completeness of a resection was recorded, as well as the dimensions and weight of the resected specimen. All resections were checked and only considered to be complete if no microscopical and/or macroscopical tumor residue was present.

Statistical analyses

The present study is a population-based study, encompassing the entire Dutch population of approximately 16 million inhabitants. The population at risk for each year was determined from data from Statistics Netherlands[12]. Incidence rates were age-standardized using the European Standard Population as reference[13]. Trends in incidence rates were studied by calculating the Estimated Annual Percentage Change (EAPC).

Differences between groups were assessed by the chi-square or Kruskall-Wallis test for categorical variables and the Mann-Whitney test for continuous variables.

Survival was calculated from the date of diagnosis until death or until end of follow-up and was estimated using the Kaplan-Meier method. Survival differences in subgroups were compared with the log-rank test.

Relative survival, the ratio of the observed to the expected survival, was considered as an estimator of the excess risk of death. The expected survival was calculated using age, gender, and period matched mortality rates based on Dutch life expectancy tables[12]. Multivariate relative survival analysis was based on the estimation of the ratio of excess mortality rates[14]. All reported p-values are two-sided, the statistical significance level was set at a p-value < 0.05.

Results

Characteristics

Of the 537 cases from the PALGA database, 275 (51%) were females. Median age at time of definitive diagnosis was 59 years (range, 1-94; interquartile range, 48-70). Myasthenia gravis was present in 78 cases (15%). In 13 cases other paraneoplastic syndromes were observed (7 cases with pure red cell aplasia or aplastic anemia, 6 cases with rarer syndromes such as hypogammaglobulinemia). The median largest tumor diameter was 7.5 cm (range, 0.1-22 cm; n = 298), with a median weight of 213 grams (range, 20-2100 grams; n = 70).

Incidence of thymoma and thymic carcinoma

During the 10-year period, 54 thymic epithelial tumors were diagnosed each year. The average annual incidence of all thymic epithelial tumors was 3.4/1,000,000 and the age-standardized incidence was 3.2/1,000,000 (Table 1). Incidence was 2.2/1,000,000 for thymoma (types A, AB, B1, and B2) and only 0.3/1,000,000 for type C thymic carcinoma. Incidence rates for males and females were not different.

The incidence of thymic epithelial tumors did not increase over the 10-year period (3.2%, $p = 0.12$), but by excluding the first year of observation (1994), there was an increase of 6.0%, $p = 0.002$.

Tumor classification and staging

WHO types AB, B1, and B2 tumors were most common (Table 2). Thymic carcinoma (type C) was observed in 56 cases (10.4%). Cases with WHO types A and AB tumors were on average 5 years older than those with types B1 and higher ($p < 0.001$). The majority of cases had low Masaoka disease stages. Cases with thymic epithelial tumors of more malignant WHO types more often had a higher Masaoka stage at diagnosis than those with thymomas of less malignant WHO types (Table 2, $p < 0.001$).

Diagnostic approach

Primary resection as first definitive diagnostic approach was used in 302 cases (56%) (Table 3). Primary resection as first diagnostic procedure was more common in younger cases and cases with smaller tumors ($p = 0.002$ and $p < 0.001$, respectively). The percentage of primary resection as first diagnostic procedure gradually decreased with increasing Masaoka stage (76%, 75%, 46%, and 23% from stages I to IV, respectively). In cases that were diagnosed with thymic carcinoma, the most frequently used first diagnostic procedure was a needle biopsy (20 of 56 patients, 36%).

A pre-operative pathological diagnosis was available in 24% of all cases that underwent a resection. A transthoracic needle biopsy was the most frequently used procedure to obtain a diagnosis before resection (in 64% of cases with a pre-operative diagnosis), followed by surgical biopsy (25%) and needle aspiration for cytology

Table 1. Incidence of thymoma and thymic carcinoma (number, observed and age-standardized incidence, and annual change of incidence) in the Netherlands 1994-2003.

Year of diagnosis	Male		Female		Total		
	Number	Age-standardized incidence*	Number	Age-standardized incidence*	Number	Observed incidence†	Age-standardized incidence*
1994	29	3.83	31	3.67	60	3.91	3.79
1995	21	2.84	19	2.16	40	2.59	2.47
1996	19	2.48	19	2.18	38	2.45	2.33
1997	27	3.65	23	2.82	50	3.21	3.20
1998	21	2.70	31	3.43	52	3.32	3.13
1999	25	3.17	22	2.38	47	2.98	2.81
2000	28	3.50	27	2.93	55	3.47	3.21
2001	23	2.83	42	4.93	65	4.07	3.88
2002	34	4.05	32	3.66	66	4.10	3.84
2003	35	4.13	29	3.23	64	3.95	3.66
Total	262	3.32	275	3.14	537	3.41	3.23
EAPC‡	-	+ 2.3% (p = 0.266)	-	+ 3.9% (p = 0.196)	-	-	+ 3.2% (p = 0.120)

*Age-standardized incidence (based on European Standard Population[13]) per 1,000,000 person years.
†Observed incidence per 1,000,000 person years.
‡EAPC, Estimated Annual Percentage Change.

Table 2. WHO classification of thymic epithelial tumors distributed according to Masaoka clinical stages (n=537).

	I	II	III	IV	Unknown	Total
A	20	15	6	2	20	63
AB	55	28	3	4	25	115
B1	28	20	9	5	25	87
B2[a)]	19	27	19	15	17	97
B3[b)]	12	12	11	13	15	63
C[c)]	0	6	13	18	19	56
Unknown	8	14	7	5	22	56
Total	142	122	68	62	143	537

[a)]including 3 combined B1 + B2 thymomas, [b)]including 8 combined B2 + B3 thymomas
[c)]including 1 combined B3 + C thymoma.
Classification is not equally distributed among tumor stages ($p < 0.001$).
Description of WHO classification[4]: type A medullary thymoma, type AB mixed thymoma, type B1 predominantly cortical thymoma, type B2 cortical thymoma, type B3 well-differentiated thymic carcinoma, type C thymic carcinoma.
Description of Masaoka clinical stages[3]: stage I macroscopically completely encapsulated lesion without capsular invasion, stage II capsular invasion and/or invasion in surrounding fat or pleura, stage III invasion in neighboring organs (lung, great vessels, pericardium), stage IV presence of pleural or pericardial dissemination, and/or presence of lymphogenous or hematogenous metastases.

Table 3. Procedures leading to a definitive diagnosis of thymic epithelial tumors (n=537).

First procedure	Number of cases	Percentage of total number of cases	Followed by resection	Percentage of total number of resections
Needle aspiration (cytology)	19	3.5	11	2.6
Needle biopsy (histology)	130	24.2	65	15.5
Surgical biopsy	52	9.7	26	6.2
Primary resection	302	56.2	302	72.1
Incidental finding during surgery for other indications	15	2.8	15	3.6
Incidental finding at autopsy	16	3.0		
Other	1	0.2		
Unknown	2	0.4		
Total	537	100.0	419	100.0

(11%). Transthoracic needle biopsy was also the most frequently used method to obtain a diagnosis in cases that were not treated by resection.

Median time between preoperative diagnosis and subsequent resection was 37 days (interquartile range, 21-84). The type of diagnostic procedure did not influence this time interval ($p = 0.58$).

Small changes in diagnostic procedures were observed when comparing the first five years of the 10-year study period to the last five years. The most important difference was a decrease in frequency of incidental finding of thymus tumors (both during autopsy and during thoracic surgery for other indications) from 10% to 2.4%.

Myasthenia gravis

Primary resection was more common as first diagnostic procedure in cases with myasthenia gravis than in those without myasthenia gravis (90% and 51% respectively, $p < 0.001$). Cases with myasthenia gravis were younger and had smaller masses than those without myasthenia gravis ($p = 0.001$ and $p = 0.004$, respectively). Only three of the 78 thymic epithelial tumors in cases with myasthenia gravis were not resected. Two of those three tumors were found at autopsy, the reason for withholding resection in the third case was unknown.

Survival

Survival data of 232 cases (43.2% of 537 cases) were available. Gender and age distribution were not significantly different from the complete study population. The 2-, 5-, and 10-year overall survival of all these thymic epithelial tumors combined was 75%, 69%, and 40%, respectively.

WHO classification, Masaoka disease stage, resection, and age (distributed into three groups) were significant prognostic factors of overall and relative 5-year survival (Table 4). Year of diagnosis (1994-1998 versus 1999-2003), gender, and myasthenia gravis were not prognostic for survival (Table 4). Some adjoining histological types and disease stages had similar observed 5-year survival rates and could therefore be clustered (Figure 1). Excess mortality was present in all WHO types except types A and AB (Table 4).

Resection was the most important independent prognostic factor for survival ($p < 0.001$). When resection, being related to disease stage, was excluded from multivariate analysis, WHO classification, Masaoka disease stage, and age were independent prognostic factors ($p < 0.001$, $p = 0.010$, and $p < 0.001$, respectively).

Treatment

The majority of cases of which we had survival data ($n = 180$) was treated with surgery, or surgery combined with chemotherapy and/or radiotherapy. The 10-year survival of surgically treated cases was 52%; those not surgically treated ($n = 52$) had a median survival of 1.1 years (Figure 1).

Resection was complete in 74 cases (41%), incomplete in 96 cases (53%) and

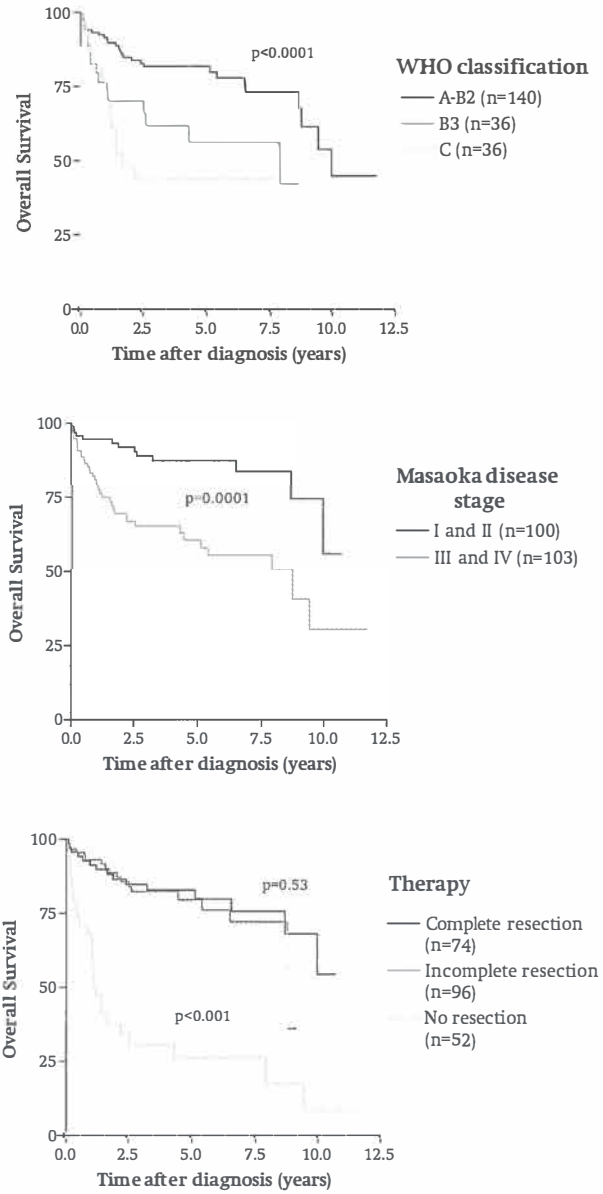

Figure 1.
Overall survival curves of cases with thymic epithelial tumors distributed according to WHO classification (clusters A-B2, B3, and C) or Masaoka disease stage (clusters I-II and III-IV). Tumors with unknown classification (n = 20) or disease stage (n = 29) are not included, respectively. Overall survival curves according to treatment are displayed in the lower panel. Cases with a resection with unknown completeness (n = 10) are excluded. No resection versus resection $p < 0.001$, and complete resection versus incomplete resection $p = 0.53$.

Table 4. Univariate overall and relative 5-year survival with 95% confidence intervals.

	Number	Overall 5-year survival in % (95% CI)	P-value*	Relative 5-year survival† in % (95% CI)	P-value‡
Time of diagnosis			0.4463		0.1582
1994-1998	100	70.2 (61.9-79.6)		78.3 (66.7-87.4)	
1999-2003	132	69.8 (54.8-77.5)		74.0 (63.1-82.6)	
Gender			0.6816		0.8309
Male	122	70.3 (60.0-78.4)		78.4 (66.8-87.5)	
Female	110	67.2 (56.2-76.1)		71.7 (59.8-81.3)	
Age			0.0004		0.0135
< 50	69	73.8 (60.2-83.5)		74.7 (61.0-84.4)	
50-69	116	74.8 (63.7-82.9)		79.8 (67.8-88.6)	
≥ 70	47	46.8 (31.3-60.9)		64.4 (43.5-83.2)	
WHO classification			0.0001		0.0004
A	19	87.8 (59.4-96.9)		100 (69.9-111.9)	
AB	33	83.0 (63.8-92.6)		92.8 (71.2-103.6)	
B1	34	81.7 (61.0-92.1)		86.5 (64.7-97.4)	
B2	54	81.9 (66.6-90.6)		85.9 (69.8-95.2)	
B3	36	53.1 (32.8-69.8)		56.9 (35.5-74.5)	
C	36	37.8 (20.5-55.0)		42.6 (23.7-61.0)	
Unknown	20	59.7 (33.0-78.7)		70.9 (39.1-93.5)	
Masaoka stage			< 0.0001		0.0028
I	25	82.9 (60.5-93.3)		91.2 (65.8-103.2)	
II	75	87.8 (75.7-94.1)		95.3 (82.2-102.1)	
III	53	57.6 (39.6-72.1)		63.2 (43.7-78.7)	
IV	50	55.6 (38.6-69.5)		59.7 (41.4-74.8)	
Unknown	29	49.9 (29.4-67.3)		56.3 (33.2-76.2)	
Tumor resected			< 0.0001		< 0.0001
Yes	180	80.1 (72.5-85.9)		87.7 (79.3-94.1)	
No	52	27.6 (14.1-42.9)		29.9 (15.3-46.3)	
Myasthenia gravis			0.1161		0.2280
Yes	27	82.3 (59.1-93.1)		88.9 (65.0-100.0)	
No	205	66.9 (58.9-73.7)		73.1 (64.4-80.6)	

*Log-rank test.
†Relative survival, the ratio of the observed to the expected survival, was considered as an estimator of the excess risk of death compared to the standard population.
‡Poisson regression analysis of relative survival rates, adjusted for follow-up time.

unknown in 10 cases (6%). Cases with a complete resection did not have a better survival than those with an incomplete resection (p = 0.53) (Figure 1). Cases with an incomplete resection more often received (neo)adjuvant therapy than those with a complete resection (64% versus 39%, p = 0.002).

Discussion

The present population-based study is the first to determine the incidence of all thymic epithelial tumors. This study points out that in the Netherlands primary resection is the most frequently used procedure to obtain a definitive diagnosis of a thymus tumor. The majority of the thymic epithelial tumors is associated with excess mortality compared to the standard population. Remarkably, contradictory to previous publications, the completeness of resection was not predictive for overall survival.

The design of this population-based study had some limitations. First, it is a retrospective study; therefore the conclusions should be interpreted with caution. For example, the low prevalence of MG in our population could be a result of under-reporting of co-existent diseases. However, one should realize that all large studies on thymic epithelial tumors were retrospective. Second, detailed information on the magnitude of incompleteness of resections was not available in all cases. Incomplete resections ranged from debulked large tumors to microscopical tumor residues. Third, the occurrence of second primary tumors, which are common for thymomas[15] and may influence survival time, was not recorded.

The incidence for all thymic epithelial tumors in the Netherlands was 3.2/1,000,000. We observed that slightly less than half of all thymic tumors are collected by the Netherlands Cancer Registry, resulting in an incidence of only 1.5/1,000,000 for thymomas considered as malignant in that registry. In the United States, the Surveillance, Epidemiology and End Results (SEER) program collects cancer incidence and survival data, thereby covering approximately 26% of the US population. Based on SEER data, an incidence of 1.5/1,000,000 (similar to NCR) for malignant thymomas was reported[16] without information about WHO types. Therefore, approximately 50% of all thymic epithelial tumors escape registration in cancer registries, probably because they are considered as benign tumors. Our data provide an increasing trend in the incidence of thymic epithelial tumors. This may be explained by the larger availability of CT-scans and more medical indications for performing CT scans leading to a decrease in incidental findings of thymomas. Overall, thymomas and thymic carcinomas are very uncommon, and literature about diagnostic approaches and treatment is sparse.

Primary resection, i.e. resection without a pre-operative tissue diagnosis, was performed in the majority of cases and was associated with smaller tumors, younger

age, and the presence of myasthenia gravis. Pre-operative tissue diagnosis was obtained in only 24% of cases, which is less than was reported in the literature[3,17]. In cases that had a primary resection, especially in those with smaller masses or myasthenia gravis, a resectable thymic mass as assessed by CT-scan was considered sufficient to proceed to thoracotomy without further diagnostics. A CT-scan is very useful to assess the resectability of a localized tumor in the anterior mediastinum, but cannot differentiate between the different histological subtypes[18]. The presumed risk of dissemination of disease following a needle procedure may also favor immediate thoracic surgery without a pre-operative diagnosis. There is considerable disagreement in the literature about the risk of implantation metastases during transthoracic procedures[2]. In an analysis evaluating more than 68,000 transthoracic needle biopsies for different malignancies, the incidence of needle-track metastasis was only 0.012%[19]. Therefore, despite two case reports that describe implantation metastases[20,21], the risk of seeding appears to be low.

Only 3.5% of all thymomas were diagnosed with a cytological procedure. The accuracy of cytology in diagnosing thymomas is limited, as this depends on the acquisition of both epithelial and lymphoid elements in one sample[22]. Cytology may provide some evidence for a thymic origin of the tumor but sampling error and tumor heterogeneity preclude proper typing and staging of the tumor. Therefore, histology is recommended.

Our results are in line with studies from Germany, Japan, China, and Italy in histological subtype frequencies of thymomas and thymic carcinomas[7,9-11,23,24]. Types AB and B2 were the most frequent, and type C thymic carcinoma represented about 5-15% of the disease spectrum. An other similarity between our findings and earlier studies[7,9,11,23-26] is that adjoining histological types and disease stages have comparable survival rates. Combining types A, AB, B1, and B2, as was recently proposed[27], results in better separation of survival curves (Figure 1). This is in line with the low reproducibility of the WHO classification between pathologists (interobserver agreement of only 0.49 for the B subtypes using κ statistics[10]) and the large heterogeneity of thymomas making classification prone to sampling errors[28]. The low reproducibility is also caused by the fact that most pathologists see only one or two thymic epithelial tumors per year due to the low incidence.

In this study, as opposed to most other studies that report only on overall survival, we showed that the majority of thymomas is associated with a decrease in life expectancy because excess mortality was observed in WHO types B1 up to C and for Masaoka stages III and IV.

Resection is by far the best treatment option for patients with a thymic epithelial tumor[7,8,23], although it is possible that not only tumor-related factors but also patient-related factors such as a worse performance status or co-morbid illnesses played a role in the decision not to operate, explaining the very poor survival for those patients without a resection. Contrary to earlier findings[1,7,8,23], cases with a complete resection did not have a better survival than those with an incomplete

resection. This may be due to the population-based design of our study compared to the single-center design of most other studies. Also, in this study, more patients had an incomplete resection than in most other studies[7,8,11], possibly due to our strict criteria for defining completeness. Patients with an incomplete resection received more (neo)adjuvant chemotherapy and/or radiotherapy than patients with a complete resection. It has been reported that postoperative adjuvant therapy decreases the recurrence rate in incompletely resected thymomas[7]. In addition, high overall survival rates were observed in patients with initially unresectable thymomas, who were treated with resection and postoperative radiotherapy after induction chemotherapy[29,30]. In conclusion, the present study points out that surgery offers the best perspectives for patients with thymic epithelial tumors, even if the resection is incomplete.

Acknowledgements

This study was supported by the Netherlands National Pathological Archives (PALGA) and the Netherlands Cancer Registry (NCR).

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Chapter 7

Thymomen en thymuscarcinomen in Nederland: een koppeling tussen het Pathologisch-Anatomisch Landelijk Geautomatiseerd Archief (PALGA) en de Nederlandse Kankerregistratie (NKR)

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Abstract

Introduction
Thymomas and thymic carcinomas are rare neoplasms and can be considered as orphan diseases. WHO classification of thymomas consists of a histopathological spectrum from relatively benign to more malignant subtypes.
We studied the linkage of two nation-wide registries and assessed the overlap and differences between both registries.

Methods
PALGA collects all diagnoses of all cytopathological and histopathological reports in the Netherlands together with basic patient characteristics. NCR collects patient and tumor characteristics and survival data of all newly diagnosed cancer cases. We searched PALGA for thymoma and thymus neoplasms and NCR for ICD code C.37.9 (thymus) for the period 1994-2003. These two selections were merged using date of birth, gender, and, if necessary, incidence date.

Results
PALGA identified 537 thymic epithelial tumors. NCR identified 269 cases, of which 232 could be matched with cases in the PALGA selection. This means that only 43% of PALGA cases were present in NCR database.
The percentage of benign histology (WHO types A, AB) is higher in unmatched cases than in matched cases. NCR only registers 61% of all histologically malignant tumors (types B3 and C).

Conclusion
Registration of epithelial thymus tumors is different between PALGA and NCR; less than half of all tumors are registered in NCR.
The institution of a national thymus tumor panel would standardize the diagnosis and NCR registration of these rare tumors.

Samenvatting

Inleiding

Epidemiologische gegevens van zeldzame tumoren zoals thymomen en thymuscarcinomen kunnen bestudeerd worden met de nationale databanken Pathologisch Anatomisch Landelijk Geautomatiseerd Archief (PALGA) en de Nederlandse Kankerregistratie (NKR).

De weesziekte epitheliale thymustumor werd als voorbeeld genomen om de mate van overlap en de verschillen tussen deze twee databanken te onderzoeken.

Methoden

PALGA verzamelt alle cyto- en histopathologische diagnosen in Nederland. De NKR bevat patiënten- en tumorgegevens van alle nieuw gediagnosticeerde kankergevallen. Tussen 1994 en 2003 werd PALGA doorzocht op thymomen en thymustumoren en de NKR op ICD-O topografie code C.37.9 (thymus). Beide zoekresultaten werden gekoppeld op basis van geboortedatum, geslacht en eventueel de incidentiedatum.

Resultaten

In PALGA werden 537 epitheliale thymustumoren gevonden. De NKR bevatte 269 gevallen, waarvan er 232 gekoppeld werden aan PALGA patiënten. De NKR bevat dus slechts 43% van de PALGA patiënten.

Het percentage met goedaardigere histologie (WHO histologische types A en AB) was hoger in de groep die alleen in PALGA voorkwam dan in de groep in beide registraties. De NKR bevat slechts 61% van de histologisch meest maligne tumoren (types B3 en C).

Conclusie

De registratiegraad van epitheliale thymustumoren is verschillend tussen PALGA en de NKR, ruim de helft wordt niet opgenomen in de NKR.

De oprichting van een nationaal (multidisciplinair) thymomenpanel, in navolging van het mesotheliomenpanel, kan hieraan bijdragen door de diagnose en de aanmelding bij de NKR te standaardiseren.

Inleiding

Het koppelen van gegevens van twee of meer databanken is een veelgebruikte methode in medisch-wetenschappelijk onderzoek. Zo'n koppeling vindt meestal plaats op basis van demografische gegevens als geboortedatum en geslacht, of idealiter met unieke gegevens als het Sofi-nummer. De nauwkeurigheid van de koppeling (in het Engels "record linkage") is vanzelfsprekend belangrijk voor de waarde van de uitkomst. Een koppeling kan mislukken door foutieve data-invoer of door abusievelijke koppeling van verschillende patiënten met toevalligerwijs dezelfde geboortedatum[1]. Bovendien kunnen patiënten simpelweg wel in de ene maar niet in de andere databank opgenomen zijn, bijvoorbeeld vanwege andere inclusiecriteria. Er is niet veel bekend over de nauwkeurigheid en de mate van overlap tussen Pathologisch-Anatomisch Landelijk Geautomatiseerd Archief (PALGA) en de Nederlandse Kankerregistratie (NKR). Om hiervan een beeld te krijgen hebben we gekeken naar de registratie van een zeldzame tumor met een breed klinisch spectrum.

Het thymuscarcinoom en thymoom is een weesziekte (minder dan 5 per 10.000 inwoners). Deze tumoren ontstaan uit de epitheliale cellen van de thymus in het voorste mediastinum. Deze epitheliale thymustumoren zijn erg zeldzaam, met een gecombineerde jaarlijkse incidentie van 3,2/1.000.000 in Nederland[2]. Thymomen komen op alle leeftijden voor, met een piek tussen het vijftigste en zestigste levensjaar. Thymomen zijn vaak geassocieerd met auto-immuun aandoeningen, waarvan myasthenia gravis de meest voorkomende is[3]. De behandeling is in eerste instantie gericht op resectie van de tumor.

Thymomen en thymus carcinomen worden pathologisch geclassificeerd volgens de recente World Health Organization (WHO) criteria[4]. Deze indeling is gebaseerd op het cytologische aspect van de epitheliale cellen en de verhouding tussen lymfocyten en epitheliale cellen. De zes subtypes lopen uiteen van type A (het medullaire of spoelvormige thymoom) tot type C (thymus carcinoom), met een breed spectrum aan maligniteitsgradering. Voor de klinische stadiëring van thymomen wordt gebruik gemaakt van de Masaoka indeling[5]. Deze stadiëring is gebaseerd op de mate van ingroei in het thymuskapsel en omgevende structuren en metastasering. Thymomen met Masaoka stadium I worden ook wel niet-invasieve thymomen genoemd, tumoren met stadium II en hoger zijn de zogenaamde invasieve thymomen. Het gevolg van dit grote bereik in classificatie en invasieve groei, gekoppeld aan het zeldzame voorkomen, is dat de termen "benigne" en "maligne" niet eenduidig en eensluidend worden gebruikt voor de karakterisering van thymomen.

Wanneer men PALGA beschouwt als bron voor de incidentie is het van belang te weten hoeveel van de epitheliale thymustumoren ook in de klinische databank van de NKR worden opgenomen. Het doel van deze studie was dan ook om de overlap en verschillen tussen deze twee landelijke databanken te bepalen wat betreft de registratie van epitheliale thymustumoren.

Data en methoden

Twee landelijke databanken verzamelen gegevens over kankerpatiënten in Nederland. PALGA ontvangt sinds 1990 de verslagen van elk cytologische en histologische onderzoek in Nederland. In deze databank worden voor elke verrichting enkele geanonimiseerde patiëntgegevens, een uittreksel met de conclusie van het verslag, en één of meer diagnostische trefwoorden geregistreerd[6]. Deze trefwoorden zijn in overeenstemming met de Systematized Nomenclature of Medicine (SNOMED) terminologie.

De NKR registreert sinds 1989 gegevens van elke nieuw gediagnosticeerde maligniteit, met uitzondering van basaalcelhuidkanker. De NKR krijgt zijn gegevens van de negen regionale kankerregistraties, die samen het hele land bestrijken. De NKR ontvangt regelmatig van PALGA een lijst met nieuw gediagnosticeerde maligniteiten. Tevens ontvangt de NKR de (klinische) ontslagdiagnosen van alle in Nederlandse ziekenhuizen opgenomen patiënten van de Landelijke Medische Registratie (LMR). Op deze manier worden vrijwel alle nieuwe kankergevallen opgespoord. Vervolgens verzamelt getraind personeel gegevens van de aldus aangemelde maligniteiten. Informatie over de diagnose, stadiëring, behandeling en overleving wordt opgezocht in de klinische en poliklinische dossiers. Voor topografie en morfologie van de tumoren wordt gebruik gemaakt van de International Classification of Diseases-Oncology (ICD-O)[7].

De selecties en koppeling van gegevens uit beide databanken werden uitgevoerd met in acht neming van de privacy reglementen en na goedkeuring door de wetenschappelijke adviescommissies van beide registraties. Omdat deze studie alleen gebruik maakt van geanonimiseerde gegevens die routinematig werden verzameld, werd er geen specifiek informed consent verkregen en was toetsing bij een Medisch-Ethische Toetsingscommissie niet nodig.

Selectie uit PALGA

Om alle nieuw gediagnosticeerde thymomen en thymuscarcinomen te selecteren werd in PALGA gezocht tussen 1 januari 1994 en 31 december 2003 op de volgende trefwoorden: "thymus*alle benigniteiten", "thymoom", "thymus*alle kanker", "thymus*alle dubieus maligne", "benigne thymoom", "benigne thymoom lymfocytair" en "lymfocytair thymoom". Binnen het aantal treffers werd gekeken hoeveel verslagen van dezelfde patiënten afkomstig waren, aangezien van sommige patiënten verslagen van twee of meer verschillende procedures voor dezelfde tumor gevonden werden. Van deze patiënten werd via een zogenaamde intermediaire procedure het volledige geanonimiseerde verslag opgevraagd bij de pathologie laboratoria.

Van alle patiënten werden leeftijd, geslacht, diagnose, incidentiedatum en, indien het een epitheliale thymustumor betrof, het Masaoka stadium en de WHO classificatie geregistreerd. Enkele thymomen bestonden uit componenten van meerdere subtypen, bijvoorbeeld B2 en B3. Deze gecombineerde thymomen werden steeds geclassificeerd naar de hoogste component, aangezien die de prognose bepaalt[8].

Selectie uit NKR

De selectie van patiënten uit het NKR bestand werd gedaan op locatie van de tumor, waarbij alle patiënten met een tumor in the thymusregio (ICD-O topografie code C.37.9) tussen 1994 en 2003 werden geïncludeerd.

Van alle patiënten werden leeftijd, geslacht, incidentiedatum en diagnose geregistreerd. De NKR gebruikt morfologie nummers van de ICD-O om de diagnose te coderen. De codes voor epitheliale thymustumoren lopen van 8580 (thymoma niet nader geclassificeerd) tot 8586 (thymus carcinoom, type C).

Samenvoeging van PALGA en NKR bestanden

Patiënten met een thymoom of thymus carcinoom volgens de diagnose in PALGA werden gekoppeld met de patiënten in het NKR bestand. Deze koppeling werd verricht door een onafhankelijke statisticus, die als enige ook de beschikking had over de geboortedatum. Patiënten uit beide bestanden werden gekoppeld met behulp van geboortedatum en geslacht en, indien nodig, de incidentiedatum.

Statistische analyses

Nadat de NKR en de PALGA bestanden waren samengevoegd werd berekend welk percentage patiënten gekoppeld kon worden (d.w.z. in beide bestanden voorkwam). Patiënten die alleen in PALGA of alleen in de NKR voorkwamen maar niet in beide bestanden werden nauwkeurig in kaart gebracht.

Om een indruk te krijgen van de betrouwbaarheid van de samenvoeging van twee bestanden werden de verschillen en overeenkomsten in WHO classificatie, Masaoka stadium en andere karakteristieken tussen gekoppelde en niet-gekoppelde patiënten geanalyseerd met behulp van χ^2- en Mann-Whitney testen. Binaire logistische regressie analyse werd uitgevoerd om de voorspellende waarde van variabelen voor opname in de NKR te bepalen. Een p-waarde $< 0{,}05$ werd beschouwd als statistisch significant.

Resultaten

PALGA selectie

De PALGA zoekopdracht resulteerde in 1244 treffers afkomstig van 750 patiënten. Van deze 750 patiënten hadden 151 een andere diagnose dan thymoom of thymuscarcinoom. Dit betrof een maligne lymfoom (n = 49), carcinoid van de thymus (n = 31), of een overige niet-epitheliale thymusafwijking (n=71). Verder waren er patiënten waarbij in de onderzoeksperiode sprake was van een recidief van een thymoom dat oorspronkelijk gediagnosticeerd was vòòr 1994 (n = 38) en patiënten waarbij de diagnose niet achterhaald kon worden (n = 24). Deze in totaal 213 patiënten (28,4%) werden geëxcludeerd, zodat er 537 patiënten met een thymoom of

thymus carcinoom overbleven. Van deze 537 patiënten was in 421 gevallen (78,4%) het volledige pathologie verslag beschikbaar.

NKR selectie

Van de 269 vanuit de NKR geselecteerde thymus tumoren hadden 237 een thymoom of thymuscarcinoom (ICD-O morfologie 8580-8586). Onder de 32 gevallen zonder thymoom-morfologie bevonden zich 14 typische en 4 atypische carcinoïd tumoren (morfologienummers 8240 en 8249, respectievelijk), 7 neuro-endocriene tumoren (nummer 8246) en 7 overigen (meest carcinomen).

In de NKR selectie bevonden zich geen maligne lymfomen. Maligne lymfomen hebben meestal meerdere locaties, waardoor de primaire topografie in NKR vaak een andere is dan thymusregio (C.37.9).

Koppeling

Van de 237 thymomen en thymuscarcinomen in de NKR selectie konden er 226 worden teruggevonden in de PALGA selectie en elf patiënten niet. Van deze elf "record linkage" fouten (4,6%) hadden acht patiënten een thymoom met een niet nader gespecificeerde morfologie (ICD-O nummer 8580) en drie een thymus carcinoom (8586).

Bovendien waren er nog zes patiënten die ook gekoppeld werden, maar waarbij de diagnose verschilde tussen PALGA en NKR (wel een thymoom of thymuscarcinoom volgens PALGA, maar een ICD-O morfologienummer anders dan 8580-8586; de NKR selectie was niet gebaseerd op ICD-O morfologie maar op topografie (thymusregio) codes). In vier van de zes patiënten betrof het een carcinoom dat uiteindelijk als type C thymuscarcinoom geclassificeerd werd. De PALGA diagnose werd beschouwd als de definitieve diagnose in deze zes gevallen. In totaal werden er dus 226 + 6 = 232 gevallen (43,2% van alle PALGA tumoren) gekoppeld tussen PALGA en de NKR.

Verschillen tussen gekoppelde en ongekoppelde patiënten

Er was een significant verschil in de WHO classificatie tussen patiënten in beide registraties (n = 232) en patiënten die alleen in PALGA maar niet in de NKR voorkwamen (n = 305) ($p < 0{,}001$). Patiënten die alleen in PALGA voorkwamen hadden significant vaker een thymoom met WHO classificatie A of AB dan patiënten die in beide bestanden voorkwamen (Tabel 1). Type B1 kwam in beide groepen evenveel voor, terwijl types B2, B3 en C veel frequenter waren onder de gekoppelde patiënten.

Desondanks waren 52 van de 178 (29,2%) histologisch meest benigne thymomen (A en AB) toch opgenomen in de NKR. Verder kwamen slechts 72 van de 119 (61%) patiënten met de histologisch meest maligne tumoren (B3 en C) voor in de NKR.

Ook waren er verschillen tussen de Masaoka stadia van de gekoppelde en de niet-gekoppelde tumoren ($p < 0{,}001$, Tabel 2). De bij NKR geregistreerde epitheliale

Tabel 1. WHO classificaties van alle epitheliale thymustumoren (n=537), tumoren die alleen in PALGA (ongekoppelde tumoren, n=305) en tumoren die zowel in PALGA als NKR (gekoppelde tumoren, n=232) voorkomen. In de rechter kolom de verschillen in WHO onderverdeling tussen beide groepen.

WHO classificatie	Totaal	Tumoren alleen in PALGA	Tumoren zowel in PALGA als NKR	
	Aantal patiënten (%)	Aantal patiënten (%)	Aantal patiënten (%)	Verschil in percentage
A	63 (11,7)	44 (14,4)	19 (8,2)	6,2
AB	115 (21,5)	82 (26,9)	33 (14,2)	12,7
B1	87 (16,2)	53 (17,4)	34 (14,7)	2,7
B2	97 (18,1)	43 (14,1)	54 (23,3)	-9,2
B3	63 (11,7)	27 (8,9)	36 (15,5)	-6,6
C	56 (10,4)	20 (6,5)	36 (15,5)	-9,0
Onbekend	56 (10,4)	36 (11,8)	20 (8,6)	3,2
Totaal	537 (100,0)	305 (100,0)	232 (100,0)	

Omschrijving WHO classificatie[4]: A medullair thymoom, AB gemengd thymoom, B1 overwegend corticaal thymoom, B2 corticaal thymoom, B3 goed gedifferentieerd thymus carcinoom, C thymuscarcinoom.

thymustumoren bevonden zich vaker in verder gevorderde Masaoka stadia. Ook bleek dat 12 van de 62 (19,3%) Masaoka stadium IV (aanwezigheid van pleurale en/of afstandsmetastasen) niet in de NKR registratie voorkwamen. Van deze 12 gevallen hadden acht patiënten WHO classificatie B3 of C.

Er werden geen significante verschillen gevonden tussen de beide groepen wat betreft leeftijd, geslachtsverdeling en tumorgrootte. Het percentage patiënten dat gekoppeld kon worden was in alle jaren gelijk. Er werd geen trend naar een hogere dan wel lagere dekkingsgraad van epitheliale thymustumoren in de NKR in het verloop van de tijd geconstateerd.

In multivariate logistische regressie waren alleen WHO classificatie en Masaoka ziekte stadium onafhankelijke voorspellers voor opname in de NKR (beiden $p < 0,001$), terwijl leeftijd opgesplitst in drie categorieën, periode 1994-1998 of 1999-2003, en tumorgrootte dat niet waren.

Tabel 2. Masaoka stadia van alle epitheliale thymustumoren (n=537), tumoren die alleen in PALGA (ongekoppelde tumoren, n=305) en tumoren die zowel in PALGA als NKR (gekoppelde tumoren, n=232) voorkomen. In de rechter kolom de verschillen in Masaoka stadia tussen beide groepen.

Masaoka stadium	Totaal	Tumoren alleen in PALGA	Tumoren zowel in PALGA als NKR	
	Aantal patiënten (%)	Aantal patiënten (%)	Aantal patiënten (%)	Verschil in percentage
I	142 (26,5)	117 (38,4)	25 (10,8)	27,6
II	122 (22,7)	47 (15,4)	75 (32,3)	-16.9
III	68 (12,7)	15 (4,9)	53 (22,8)	-17.9
IV	62 (11,5)	12 (3,9)	50 (21,6)	-17.7
Onbekend	143 (26,6)	114 (37,4)	29 (12,5)	24,9
Totaal	537 (100,0)	305 (100,0)	232 (100,0)	

Omschrijving Masaoka stadia[5]: I thymoom zonder kapsel invasie, II invasie in kapsel of omgevend vet, III ingroei in omliggende organen (longen, grote vaten, pericard), IV aanwezigheid van pleurale en/of pericardiale disseminatie of afstandsmetastasen.

Beschouwing

Epitheliale thymustumoren zijn zeldzaam; bij slechts circa 50 gevallen per jaar in Nederland komen pathologen en andere specialisten gemiddeld maar met 1 à 2 gevallen per jaar in aanraking. Slechts 43% van alle gediagnosticeerde epitheliale thymustumoren in PALGA wordt ook opgenomen in de NKR database. Hoewel de niet bij de NKR maar alleen in PALGA geregistreerde thymomen in meerderheid een relatief benigne histologie hebben is het opvallend dat ook een aanzienlijk deel van de histologisch meer maligne thymomen en thymuscarcinomen niet in de NKR voorkomen.

Sinds 1990 is PALGA landelijk dekkend en zijn alle klinisch-pathologische laboratoria erbij aangesloten. Ieder pathologisch onderzoek wordt na controle van de coderingen opgenomen in het PALGA register. Deze coderingen worden gemaakt aan de hand van de door de patholoog opgestelde diagnoseregel, maar deze diagnoseregel wordt niet gecontroleerd met de conclusie in het verslag. PALGA is dus een register van alle pathologie-uitslagen en niet van klinische diagnosen. Niettemin zijn de dekkingsgraad en de betrouwbaarheid van PALGA zeer hoog; recent bleek dat voor tumoren zoals het testiscarcinoom (een diagnose gesteld op strikt pathologische kenmerken en in principe ook allemaal opgenomen in de NKR) de incidentieverschillen tussen PALGA en NKR slechts 1-2% waren[9]. Ook uit onze studie blijkt dat het PALGA

register zeer compleet is, aangezien meer dan 95% van alle epitheliale thymustumoren van de NKR terug te vinden zijn in PALGA. PALGA kan dus beschouwd worden als gouden standaard voor het voorkomen van epitheliale thymustumoren.

Patiënten kunnen niet gekoppeld zijn doordat er fouten zijn geslopen in patiënten of tumor gegevens. Gezien de bovengenoemde 95% overlap zal de effectgrootte van deze "record linkage errors" niet meer dan vijf procent bedragen. Dit veronderstelde lage aantal coderingsfouten komt overeen met het feit dat thymomen in de NKR consistent gecodeerd werden in de thymusregio; wanneer gezocht werd op morfologiecodes 8580-8586 (thymoom) leverde dit 238 thymustumoren op, waarvan 237 in de thymusregio.

Bij gebrek aan een uniek patiënten identificatienummer moesten wij onze databasekoppeling baseren op geboortedatum, geslacht en eventueel incidentiedatum. De beschikbaarheid van deze onderscheidende karakteristieken en privacy vraagstukken maken deze eenvoudige "record linkage" zoals wij gedaan hebben bij uitstek geschikt voor relatief kleinere populaties. Voor grotere populaties moet men gebruik maken van meer of andere onderscheidende karakteristieken, zoals bijvoorbeeld postcode, naam, of sofi-nummer, eventueel in combinatie met software algoritmes zoals probabilistische record linkage[10].

Echter, de belangrijkste verklaring voor het feit dat slechts 43% van alle epitheliale thymustumoren in de NKR te vinden waren is waarschijnlijk de werkwijze, en vooral de inclusiecriteria, van de NKR. De NKR is gebaseerd op een combinatie van als maligniteit aangemerkte tumoren in pathologie uitslagen én klinische informatie vervat in de klinische diagnose van de LMR. Hierdoor ontlopen dus tumoren die niet als "maligne" geoormerkt worden in het pathologie verslag of de klinische diagnose registratie bij de NKR. Opgemerkt dient te worden dat het gebruik van de termen "benigne" en "maligne" voor een thymoom ouderwets is, hiervoor in de plaats is de WHO classificatie gekomen. Overigens kunnen ook tumoren zonder pathologisch bewezen diagnose in de NKR opgenomen worden, de klinische context kan dus ook een rol spelen bij de registratie. Met de gevolgde methode is het echter waarschijnlijk dat tumoren met een breed histopathologisch en klinisch spectrum zoals epitheliale thymustumoren niet consistent worden opgenomen in de NKR. Dit wordt geïllustreerd door bijvoorbeeld het feit dat 40% van de patiënten met een B3 of C WHO classificatie (histologisch dus het meest maligne) niet in de NKR te vinden zijn.

Concluderend, minder dan de helft van alle epitheliale thymustumoren wordt opgenomen in de NKR. Wij pleiten ervoor dat alle thymische epitheliale tumoren worden geregistreerd in de NKR, zodat niet alleen de pathologische maar ook de klinische gegevens van deze zeldzame tumoren met een groot klinisch spectrum algemeen beschikbaar zullen zijn.

De oprichting van een nationaal (multidisciplinair) thymomen panel, in navolging van het mesotheliomenpanel, kan hieraan bijdragen door de diagnose en de aanmelding bij de NKR te standaardiseren. Bovendien kan zo'n panel een goed uitgangspunt zijn voor landelijke trials met deze tumoren.

Referenties

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Chapter 8

A 20-year old male with thoracic pain and a lower thoracic mass. Diagnosis: intrathoracic desmoid tumor with microscopically incomplete resection

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Eur Respir J. 2005 Oct;26(4):740-3

Abstract

In this case report, a 20-year old male with a large intrathoracic desmoid tumor with a microscopically incomplete resection was presented. The differential diagnosis, the therapeutic options, and the ways of follow-up were discussed in detail.

Case report

A 20-year old Caucasian male construction worker had a history of a road traffic accident 3 years before presentation. A computed tomography (CT)-scan of the thoracic spine was carried out to exclude vertebral damage. No evidence of vertebral bone damage or other lesions was seen, and the patient recovered without sequelae.

A week before presentation, he noticed a stabbing pain in his right hemithorax, without dyspnea. The pain persisted, and the patient was referred, by his general practitioner, for a chest radiograph (Figure 1). Based on these results, the patient was referred to a general hospital for further diagnostic tests. A CT-scan of thorax and abdomen (not shown) revealed a large mass, which was interpreted to arise in the right upper abdomen, probably originating from the liver. A malignant tumor, or a metastatic lesion, was suspected and the patient was referred to University Medical Center Groningen (Groningen, the Netherlands).

The patient did not suffer from dyspnea, cough, or hemoptysis, and there was no history of fever, weight loss, fatigue, or excessive sweating. There were no neurological or gastro-intestinal complaints. The patient was a non-smoker, and did not use any medication.

On physical examination, a healthy appearing, hemodynamically stable young male of normal posture was seen. On percussion, a dull sound was found in the right lower zone of the chest. Auscultation revealed normal cardiac sounds without murmurs, and normal breathing sounds on the left side and upper right side of the chest. Abdominal examination revealed no palpable masses or other abnormalities. No palpable lymph nodes were present. Additional physical examination revealed no other abnormalities.

Laboratory tests only showed a slightly elevated serum alkaline phosphatase of 174 U/l (normal value, 13-120 U/l). Serum lactate dehydrogenase, α-fetoprotein, and β-human chorionic gonadotropin values were all normal; therefore, an extragonadal germ cell tumor was unlikely.

On revision of the CT-scan, there was doubt regarding the hepatic origin of the mass. Therefore, abdominal ultrasonography was performed. No focal lesions in the liver parenchyma were observed, and the liver blood flow appeared intact. In the right thoracic region, a mass was seen with variable echogenicity and rich vascularization. Due to the high degree of vascularization as seen on abdominal ultrasonography, no percutaneous biopsy was performed. A CT-angiography was performed: firstly, to narrow the differential diagnosis and secondly, to provide the thoracic surgeon with more detailed information about vascularization (Figure 2). At bronchoscopy, no endobronchial abnormalities were seen. Pathological examination of the bronchial lavage showed no signs of malignancy. A bone scan was normal.

Exploratory thoracotomy revealed a tumor originating from the right dorsal region, lateral from the vertebral column. A surgeon was able to remove the tumor

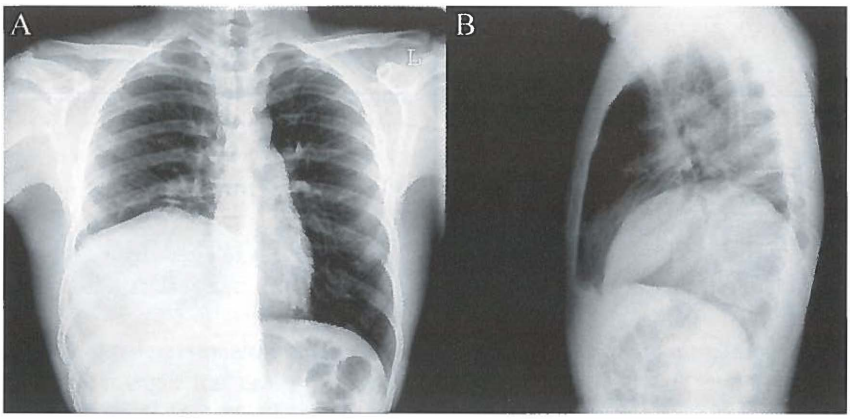

Figure 1.
Postero-anterior (A) and lateral (B) chest radiographs at presentation.

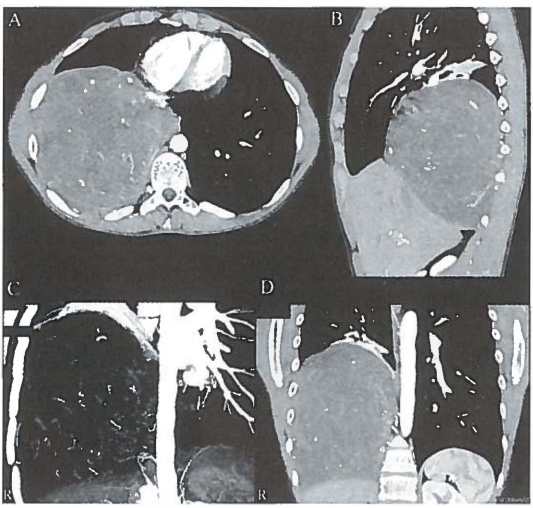

Figure 2.
Computed tomography-angiography of thorax and upper abdomen. Representative images are shown in transversal (A), sagital (B), and frontal (C and D) views. A computed reconstruction is shown in (C).

in toto. However, at pathological examination, the tumor extended into the surgical margins (Figures 3 and 4).

Before turning the page, interpret the chest radiographs (Figure 1), the CT-angiography (Figure 2), the macroscopical view after resection (Figure 3) and the histology (Figure 4), and suggest a diagnosis and follow-up regimen.

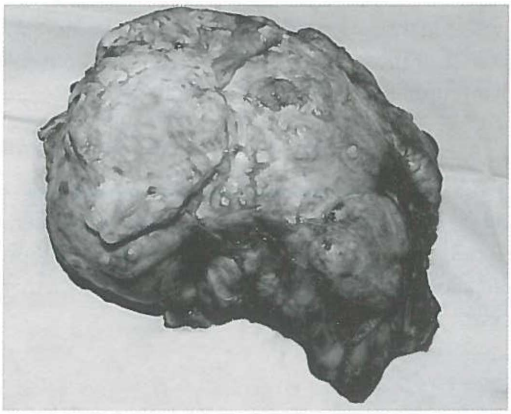

Figure 3.
Resection specimen.

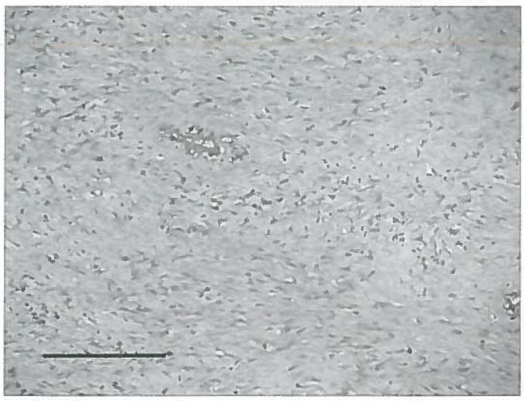

Figure 4.
Representative microphotograph of the tumor with hematoxylin-eosin staining. Scale bar = 200µm.

Interpretation

Chest radiograph at presentation

The chest radiograph showed loss of volume of the right lung (Figure 1). On the lateral view, the density extending from the right posterior ribs does not reach the sternum, suggesting a supradiaphragmatic lesion.

Computed tomography-angiography

In the right hemithorax, a relatively hypodense mass with large blood vessels was seen, compressing the normal lung and displacing the right hemidiaphragm

caudally (Figure 2). A relationship with the thoracic aorta could not be seen, nor any aberrant artery, suggesting a lung sequestration. Blood vessels originated from the intercostal arteries. No enlarged lymph nodes or suspect metastatic lesions were found.

Resection specimen

At examination, the tumor weighed 1,700 grams, with reconstructed dimensions of 19 x 17 x 6 cm with a resected peduncle of 6 x 4 cm, visible on the right lower side (Figure 3). The tumor had a lobular aspect, and was surrounded by a thin membrane with small fluid-filled cysts.

Histology

Microscopy evaluation was used to diagnose a mesenchymal tumor with small spindle cells with some variation in shape, but without atypia, mitotic figures, or other malignant characteristics in a fibrous background (Figure 4). The tumor extended into the surgical margins of the resected peduncle.

Using immunohistochemistry, tumor cells were only positive for vimentin, and lacked CD34, S-100, ALK-1, Bcl-2, cytokeratins, actin, and desmin staining. Few positive nuclei were seen with Ki-67 staining. The morphology, in combination with immunophenotype, is compatible with a desmoplastic fibroma of the pleura, also known as a desmoid tumor.

Diagnosis: Intrathoracic desmoid tumor with microscopically incomplete resection

Clinical course

Although the resection was microscopically incomplete, the patient was not treated with additional surgery or adjuvant radiotherapy (see Discussion). Instead, regular magnetic resonance imaging (MRI) examinations were applied to observe any local recurrence. During 18 months of follow-up, consecutive MRIs did not show any sign of recurrence. The patient has recovered without sequelae, and has caught up with his daily work.

Discussion

Desmoid tumors, also known as aggressive fibromatosis, are slowly growing fibroblastic neoplasms arising from fibroblastic stromal elements. Although desmoid tumors do not metastasize, they tend to be locally invasive. The etiology is not exactly known, but the association with Familial Adenomatous Polyposis Coli (FAP), as well as with previous trauma, has been extensively described[1,2].

Desmoid tumors are very rare; the incidence is between 2 and 4 per million[3]. The primary location for desmoids is extra-abdominal, with the limb girdle and extremities most commonly involved, followed by the chest wall[4]. Abdominal wall desmoids are mostly seen in females, especially during pregnancy[5]. Intra-abdominal desmoids are seen in correlation with FAP. Slightly more females than males are affected. The age at diagnosis is usually between 15-60 years.

This study reports a case of a large intrathoracic desmoid tumor. This type, and more so the pleural origin, is very rare, and has been reported in less than 20 cases[6]. There may be a relationship with the traumatic chest injury which the patient suffered three years prior to presentation.

The initial differential diagnosis of large, pedunculated, intrathoracic tumors includes both mostly benign and, less frequently, malignant lesions. The most frequent malignant pleural tumor is malignant mesothelioma which is, in general, a diffuse pleural proliferation and hardly ever presents as a pedunculated mass. Although in the past several entities were included in the group of mesotheliomas, at present the designation mesothelioma is used for neoplastic proliferation of mesothelial cells and not for proliferation of other cells of the pleura[7]. In this presentation, there were no indicators for malignancy, considering the complete lack of atypia and scarcity of mitoses. The morphology, together with negative cytokeratin stainings, makes a diagnosis of mesothelioma unlikely. Consequently, a malignant solitary fibrous tumor of the pleura, recently presented in an article in the European Respiratory Journal[8], could also be excluded on the basis of morphology and immunohistochemistry.

With respect to benign tumors to be considered in the differential diagnosis, a schwannoma of the paravertebral nerves could be excluded on clinicopathological grounds, as absence of relations to intervertebral structures and the negative S-100 staining[9]. A pulmonary sequestration was unlikely, due to anatomical presentation[10]. Also a solitary fibrous tumor (SFT), developing from the pleura, belongs to the differential diagnosis. Obsolete and confusing terms for SFT are localized or benign fibrous mesothelioma or benign localized fibroma[11]. A vascular peduncle may be present, especially in larger SFTs[12]. The morphology and the negative CD34 and Bcl-2 immunostaining made this diagnosis unlikely[13,14]. The morphology of the tumor, together with the distinctive immunohistochemical results, was compatible with a desmoid tumor.

The primary management of a desmoid tumor consists of complete resection. For patients deemed inoperable, primary radiotherapy treatment is a curative op-

tion. In two recent large series, no difference was observed in disease-free survival between microscopically positive and negative resection margins after primary resection[4,15]. However, some older and smaller studies showed a small decrease in disease-free survival time for microscopically positive margins as compared to disease-free resection margins[16]. Despite complete resection, the rate of local recurrence is approximately 30%[16]. Most local recurrences develop within two years after resection, but a time to recurrence of more than 10 years has also been described[15].

The use of radiotherapy as adjuvant therapy is, as yet, not substantiated. For incompletely resected tumors, the recurrence rate decreases from 39 to 25% after adjuvant radiotherapy, as was presented in a review by Nuyttens et al.[16]. Adjuvant radiotherapy was not a predictor for disease-free survival in other studies[4,15]. Moreover, radiation-related complications, such as radiation pneumonitis, soft tissue necrosis, and/or fibrosis, secondary malignancies, and skin problems are well known. The application of radiotherapy for incomplete resection, therefore, remains an issue for debate. The current authors chose not to let the patient be irradiated and to follow-up the patient clinically and radiologically.

Local recurrence can be treated with re-operation or local radiotherapy. In cases with incomplete resection the radiation dose should be at least 50 Gray to decrease the risk of local recurrence[17]. The disease-free survival for patients with recurrent disease is less than for primary desmoid tumors[15].

The benefit of additional surgery in case of positive margins is doubtful. Hence, gain in survival has not been demonstrated to be related to negative margins. Therefore, extensive surgery can be postponed until the presence of recurrent lesions[15].

Early detection of tumor relapse gives a higher chance of non-mutilating surgical re-resection. Therefore, follow-up should be performed with a sensitive imaging technique. MRI was preferred in the patient, as it is very specific for detecting slight differences of density between desmoid tumors and surrounding structures. Baseline MRI was chosen to be carried out at three months after the operation, because the clips left at operation needed to be firmly grown into the surrounding tissue. As desmoid tumors are slowly growing, three month intervals between MRIs were considered appropriate during the first two years, after which the intervals will become larger. At present, 18 months after diagnosis, the patient is in an excellent condition and there are no signs of relapse.

In conclusion, a patient with a large intrathoracic desmoid tumor, with microscopically incomplete resection, is presented. The differential diagnosis, the therapeutic options, and the ways of follow-up were discussed in detail.

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Treatment of thoracic tumors: small-cell lung cancer

Chapter 9

Irinotecan and cisplatin with concurrent thoracic radiotherapy in a once-every-three-weeks schedule in patients with limited-disease small-cell lung cancer: a phase I study

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Lung Cancer: Accepted

Abstract

Introduction

Irinotecan and cisplatin with concurrent radiotherapy is a powerful treatment combination for patients with limited-disease small-cell lung cancer (LD-SCLC).
The objective was to determine the dose-limiting toxicity (DLT) and maximum-tolerated dose (MTD) of irinotecan and cisplatin with concurrent thoracic radiotherapy (TRT) as a once-every-three-weeks schedule.

Methods

Patients with LD-SCLC received a fixed-dose of irinotecan (340 mg) and cisplatin (135 mg) at day 1 in cycles 1 and 4. During cycles 2 and 3, irinotecan and cisplatin were given in a dose-escalation schedule with concurrent TRT (once daily, total dose 45 Gray).

Results

No DLT was observed at first two levels (irinotecan 100 mg or 120 mg and cisplatin 100 mg at day 1 of cycles 2 and 3). In the first five patients, four episodes of grade III diarrhea/dehydration were observed at cycles 1 and 4. Therefore, from the sixth patient on, fixed-dose irinotecan at cycles 1 and 4 was reduced to 250 mg. At the subsequent level of irinotecan 140 mg and cisplatin 100 mg in cycles 2 and 3, two DLTs (severe esophagitis and late vertebral radiation toxicity) were observed in one patient. This level was considered the MTD.

Conclusion

Irinotecan and cisplatin in a once-every-three-weeks schedule is not recommended due to severe toxicity. Irinotecan may be more suited for intermittent weekly administration.

Introduction

Cisplatin and etoposide with concurrent thoracic radiotherapy (TRT) is considered the standard treatment for limited-disease small-cell lung cancer (LD-SCLC)[1-3]. For patients who achieve a response, treatment is completed with prophylactic cranial irradiation (PCI)[4]. Despite this intensive treatment, the 5-year survival rate of LD-SCLC is approximately 20%[2]. The introduction of new chemotherapeutic agents and radiotherapy regimens may improve the prognosis of patients with LD-SCLC.

Irinotecan (CPT-11) is a camptothecin derivative with increased water-solubility. Both irinotecan and its active metabolite SN-38 are powerful inhibitors of the topoisomerase I enzyme. In a Japanese study in extensive disease SCLC, the combination of irinotecan and cisplatin resulted in superior survival compared to cisplatin and etoposide[5]. These results, however, were not confirmed in two recent studies[6,7]. In LD-SCLC, the combination of irinotecan and cisplatin with TRT in different treatment regimens resulted in high response rates in recent phase I and II studies [8-14]. The most frequent toxic effects of irinotecan and its metabolites are myelosuppression and diarrhea, the latter present at grade III or higher in approximately 25% of all patients[15,16]. Diarrhea and associated dehydration is a frequent cause of hospitalization. The occurrence of esophagitis is a major concern if cisplatin and irinotecan are combined with TRT.

Irinotecan, combined with cisplatin, is usually administered in LD-SCLC at days 1, 8, and 15 in a four-weekly schedule[8-10,12]. The frequent omission of irinotecan at day 15, the long half life (48 hours[17]) of the active metabolite SN-38, and increased patient convenience all favor a schedule of once-every-three-weeks administration. In addition, the best-reported survival in trials with LD-SCLC are also reported in a three-weekly schedule with concurrent once-daily radiotherapy[3]. For ED-SCLC, a three-weekly schedule is common[6]. A phase I study in solid tumors defined the recommended dose at cisplatin 80 mg/m^2 and irinotecan 200 mg/m^2 in a three-weekly schedule[16].

In clinical oncology, most cytotoxic drug dose calculations are based on body-surface area (BSA). However, BSA was observed not to be predictive for cisplatin, irinotecan, or SN-38 clearance nor did BSA-based dose calculation reduce interpatient pharmacokinetic variability[18,19]. These findings provide a rationale for fixed-dosing of irinotecan and cisplatin.

The present phase I trial was designed to determine the feasibility and tolerability of irinotecan and cisplatin (fixed-dosing at cycles 1 and 4, dose escalating at cycles 2 and 3) in a once-every-three-weeks schedule with concurrent once-daily TRT in LD-SCLC.

Patients and methods

Patients

Major inclusion criteria were cytologically or histologically proven SCLC, disease confined to one hemithorax according to IASLC criteria without evidence of cytologically proven malignant pleural effusion, no prior chemotherapy and/or radiotherapy, age 18 years or older, ECOG performance score 0 or 1, adequate organ functions, and absence of diarrhea, bowel obstruction, inflammatory bowel disease or other serious medical conditions.

The study was conducted in two University Hospitals (Groningen and Rotterdam) and was approved by both local medical ethical committees; all patients gave written informed consent.

Study design

In this dose-escalation study, three patients were enrolled at each level (Table 1). If any of three patients in a cohort experienced dose-limiting toxicity (DLT), three more patients were to be included at that dose level. If no patients experienced DLT, dose escalation continued with the next level. If two or more patients in a cohort experienced DLT, lower doses were to be investigated until the recommended phase II dose was determined. A next cohort was started at least 14 weeks after completion of treatment of the previous cohort to determine late radiation toxicity.

Maximum tolerated dose (MTD) was defined as two or more patients in any cohort experiencing DLT. The recommended phase II dose was one dose level below MTD, with approximately 25% of patients having DLT.

The primary objective was to determine the DLT and MTD of cisplatin and irinotecan in combination with concurrent TRT and to determine a recommended phase II level.

Chemotherapy

Both irinotecan and cisplatin were administered in fixed-dosage schedules in cycles 1 and 4 (without TRT). Doses were fixed at BSA of 1.70 m^2. Doses of irinotecan and cisplatin in cycles 2 and 3 were escalated (Table 1).

Irinotecan (CPT-11, Aventis Pharma BV, Gouda, nowadays Pfizer BV, Capelle a/d IJssel, the Netherlands) was diluted in 250 mL of 0.9% NaCl and infused over 90 minutes. Irinotecan was followed by cisplatin dissolved in 100 mL 3% NaCl infused over 3 hours with forced diuresis.

Ondansetron and dexamethasone were routinely administered. If diarrhea occurred, it was treated with loperamide, antibiotics, and/or rehydration, depending on the severity.

Radiotherapy

Before the start of irradiation, a planning computed tomography (CT)-scan was

Table 1. Planned dose levels* in escalation schedule.

Dose level	Patients (n)	Irinotecan (mg)		Cisplatin (mg)	
		Cycles 1 and 4†	Cycles 2 and 3 (with TRT)	Cycles 1 and 4	Cycles 2 and 3 (with TRT)
1	3	340	100	135	100
2	3	340†	120	135	100
3	2	340†	140	135	100

*Doses are fixed, i.e. not based on actual BSA but on average BSA of 1.7 m^2.
†Dose of irinotecan was reduced from 340 mg to 250 mg in cycles 1 and 4 in last patient of level 2 and all patients at level 3 due to severe diarrhea (see text).

performed to assess the gross tumor volume in a three-dimensional way. In all dimensions this volume was enlarged with 0.5 cm to create the clinical target volume. Adding a margin of 1 to 1.5 cm to this volume to account for tumor motion and breathing resulted in the planning target volume. This planning target volume was irradiated with a total dose of 45 Gray in 25 fractions (five fractions per week) of 1.8 Gray. TRT started at day 1 of cycle 2, and continued for five consecutive weeks.

Radiation esophagitis was treated with antacids and antalgetics, or with enteral feeding in the presence of weight loss.

PCI (30 Gray in 15 doses of 2 Gray) was initiated 4 weeks after completion of chemotherapy.

Toxicity

Overall toxicity was scored according to the National Cancer Institute (NCI) Common Toxicity Criteria (CTC) version 2.0. Acute and late radiation toxicity was scored with EORTC/RTOG criteria. DLT was defined as one patient in any cohort having any of the following toxicities during cycles 2 and 3 (with concurrent TRT): grade III/IV non-hematological toxicity despite adequate medication (excluding grade III/IV nausea and vomiting), grade IV neutropenia lasting for more than five days or complicated by fever and/or platelets < 25 x 10^9/L, or grade IV esophagitis or grade III esophagitis lasting for more than two weeks.

Dose modification

Patients were treated with the next cycle if leukocytes were recovered to ≥ 3.0 x 10^9/L, neutrophils to ≥ 1.5 x 10^9/L, and platelets to ≥ 75 x 10^9/L. If these values were not met, treatment was delayed for one week, with a maximum of two weeks. In addition, diarrhea had to be recovered to grade 1 or less. If toxicity was not adequately recovered after two weeks of delay, patients went off-treatment.

If DLT was experienced at cycle 2, doses of cisplatin and irinotecan were reduced one dose level at cycle 3.

Dose of irinotecan at cycle 4 was reduced to 250 mg if neutropenic fever, grade IV neutropenia for more than five days, grade IV thrombocytopenia, or diarrhea grade III occurred at cycle 1. Cisplatin dose at cycle 4 was reduced to 100 mg in case of grade III nephrotoxicity.

Results

Patients

Between May 2003 and April 2005, eight patients were included and were evaluable for toxicity and efficacy. Four patients were male. Median age was 60 years (range, 49-70). Performance score was 0 and 1 in 3 and 5 patients, respectively. Median BSA was 1.98 m^2 (range, 1.57-2.15). One patient (level 2) had to be replaced due to a wrong dose at cycle 2.

Toxicity

No DLT was observed at levels 1 and 2 (Table 2). Irinotecan 120 mg and cisplatin 100 mg combined with TRT was well tolerated. The second patient at level 3 experienced 2 DLTs. The first DLT was esophagitis grade III, leading to more than 15% weight loss. She was hospitalized and got an enteral tube for feeding. She recovered slowly, and the 4th planned cycle of chemotherapy was skipped. The second DLT was grade IV late radiation toxicity (thoracic vertebral compression fracture 6 months after radiation therapy, treated with anti-analgesics). The same patient, and also one patient at level 2, experienced grade II late radiation pneumonitis. Hematological toxicity was not common (Table 2). Interestingly, the patient with the lowest BSA (1.57 m^2) did not experience severe toxicity; the patient with DLT had a BSA of 1.98 m^2.

The first 5 patients were treated in cycles 1 and 4 with irinotecan 340 mg and cisplatin 135 mg, according to protocol. Despite prophylactic measures, 3 out of 5 patients developed grade III diarrhea/dehydration, leading to two hospitalizations. In addition, hematological toxicity during cycle 1 and 4 was frequently observed (Table 2). This high toxicity was considered to be related to irinotecan and resulted in an amendment of the protocol. From the sixth patient, irinotecan was reduced to 250 mg at cycles 1 and 4. Also in the three patients treated with these lower doses, one episode of diarrhea/dehydration grade III resulting in hospitalization and one episode of neutropenia grade III was observed (Table 2).

Delivery of treatment

Except for cycle 4 in the last patient, all planned cycles were administered. Cycle 4 was delayed for 1 week in 4 patients, mainly due to hematological toxicity.

All patients received the complete concurrent thoracic radiotherapy of 45 Gray. Seven patients were treated with PCI.

Table 2. Toxicities (Common Toxicity Criteria version 2.0) occurring during cycles 2 and 3 (chemotherapy with concurrent TRT) according to dose level, and during cycles 1 and 4 (chemotherapy without TRT).

	Cycles 2 and 3			Cycles 1 and 4*	
	Dose level 1	Dose level 2	Dose level 3	Planned dose levels	Reduced dose levels
Dose-limiting toxicity	0 / 3	0 / 3	2 / 2†	-	-
Esophagitis grade II	0 / 3	0 / 3	0 / 2	0 / 5	0 / 3
Esophagitis grade III	0 / 3	0 / 3	1 / 2	0 / 5	0 / 3
Diarrhea/dehydration grade II	0 / 3	0 / 3	0 / 2	1 / 5	1 / 3
Diarrhea/dehydration grade III	0 / 3	0 / 3	0 / 2	3 / 5	1 / 3
Leukopenia grade III/IV	1 / 3	1 / 3	0 / 2	3 / 5	1 / 3
Thrombopenia grade III	0 / 3	0 / 3	0 / 2	2 / 5	0 / 3
Anemia grade III	1 / 3	0 / 3	0 / 2	1 / 5	0 / 3
Neutropenic fever	0 / 3	0 / 3	0 / 2	1 / 5	0 / 3
Fatigue grade III	0 / 3	0 / 3	0 / 2	1 / 5	1 / 3
Nausea grade II	1 / 3	0 / 3	0 / 2	1 / 5	1 / 3
Vomiting grade II	1 / 3	0 / 3	0 / 2	0 / 5	1 / 3
Vomiting grade III	0 / 3	0 / 3	0 / 2	2 / 5	0 / 3

*Fixed-dose of irinotecan was reduced from 340 mg to 250 mg after 5 patients due to severe diarrhea and dehydration.
†Dose-limiting toxicities were esophagitis CTC grade III lasting for more than two weeks and grade IV late radiation toxicity (thoracic vertebral compression fracture) .

Response and survival

Tumor response rate was 100% (4 complete and 4 partial responses). Median progression-free survival was 11.2 months (95% CI, 9.5-13.0). Median overall survival was 14.6 months (13.5-15.6), 1-year survival 75%, 2-year survival 38%.

Discussion

In the present study, a dose-escalation schedule of irinotecan and cisplatin during concurrent TRT in a once-every-three-weeks schedule for LD-SCLC was carried out. DLT (severe esophagitis and severe late radiation toxicity) was observed at fixed doses of irinotecan 140 mg and cisplatin 100 mg.

Toxicities observed at cycles 1 and 4 (cycles without TRT) were not considered DLTs. However, the observed toxicity at these cycles at the initial level of irinotecan 340 mg was severe and led to a dose reduction. Irinotecan 250 mg and cisplatin 135 mg once-every-three-weeks was better tolerated. Both diarrhea and hematological toxicity are well-known side-effects of irinotecan and its active metabolite SN-38[15,16],

Table 3. Phase I and II studies with irinotecan and cisplatin with concurrent TRT in LD-SCLC.

Phase (pts)	Country	Number of cycles	Schedule irinotecan*	Schedule cisplatin*	Total dose irinotecan (mg/m^2)	Total dose cisplatin (mg/m^2)	TRT (total dose)	Toxicity	Response rate	Median PFS (months)
I (17) [10]	Japan	4 cycles, q4w	40 mg/m^2 d1,8,15	60 mg/m^2 d1	480	240	Once daily, split schedule (60 Gy)	Mainly hematological toxicity and fatigue. Only mild esophagitis and diarrhea	93.8%	N/A
I (12) [8]	Germany	6 cycles q4w	60 mg/m^2 d1,8,15	20 mg/m^2 d1,2,3	1080	360	Once daily (54 Gy)	Grade II or III esophagitis present in all patients, overall toxicity well tolerated.	100%	12
I (36) [14]	U.S.	Unknown number, q3w	60 mg/m^2 d1,8	60 mg/m^2 d1	N/A	N/A	Twice daily (45 Gy) or once-daily (70 Gy)	At 45 Gy toxicity (esophagitis) was tolerable, at 70 Gy too toxic	N/A	N/A
II (20) [9]	Korea	6 cycles q4w	40 mg/m^2 d1,8,15 (60 mg/m^2 after TRT)	60 mg/m^2 d1	960	360	Once daily (50.4 Gy)	Mainly hematological toxicity and nausea/vomiting. Mild dysphagia	85%	12
II (33) [12]	Korea	6 cycles q4w	60 mg/m^2 d1,8,15	40 mg/m^2 d1,8	1080	480	Once daily (45-54 Gy)	82% had grade III-V hematological toxicity. Diarrhea and esophagitis well tolerated	87.9%	14.4
II (26) [13]	Spain	6 cycles q3w	60 mg/m^2 d1,8	60 mg/m^2 d1	720	360	Once daily (60 Gy)	Well tolerated schedule, mild esophagitis	84%	12
I (8) present study	Netherlands	4 cycles q3w	120 mg d1 (during TRT) 250 mg d1 (before and after TRT)	100 mg d1 (during TRT) 135 mg d1 (before and after TRT)	435†	276†	Once daily (45 Gy)	Esophagitis well tolerated, mainly hematological toxicity and diarrhea	100%	11.2

* In phase I studies, doses are the recommend doses for subsequent phase II studies.

† based on an average BSA of 1.70 m^2.

PFS progression-free survival, N/A not available, TRT thoracic radiotherapy.

although the relative contribution of both substances is not exactly clear. SN-38 is cleared in the liver by uridine diphosphate glycosyltransferase-1 family polypeptide A1 (UGT1A1). Polymorphisms in this enzyme may explain the interindividual differences in pharmacokinetics and toxicity[20,21].

Irinotecan and cisplatin with concurrent TRT in LD-SCLC was studied before. In recently published studies, irinotecan was administered at days 1, 8, and 15 of a four-weekly schedule[8-10,12] or at days 1 and 8 of a three-weekly schedule[13,14] (Table 3). In all four-weekly schedules, irinotecan was frequently omitted at day 15 due to hematological toxicity. It is difficult to compare all these studies with our results for several reasons.

First, in the present study irinotecan was administered once-every-three- weeks and not every week. Our regimen was based on the long half-life of SN-38, causing long duration of biologically active concentrations[17], and the increased patient convenience of a three-weekly schedule[16]. Considering an average BSA of 1.70 m^2, the total administered dose during TRT of both agents was higher in the other phase I and II studies than in the present study (Table 3). It seems that our once-every-three-weeks regimen, despite the lower total doses, is less well tolerated compared to weekly administration in three- or four- weekly schedules. Therefore, toxicity in this study seems schedule-dependent. At equal efficacy, it suggests that irinotecan is more suitable for intermittent administration in a three-weekly schedule. On the other hand, in a recent Norwegian study in ED-SCLC, irinotecan 175 mg/m^2 in combination with carboplatin (AUC 4) once-every-three-weeks was not associated with severe diarrhea[22].

A second difference between our study and other reports is that we used fixed-dosing. Previously performed pharmacokinetic studies found that interpatient variability in plasma levels of cisplatin, irinotecan, or SN-38 was not related to BSA, and therefore provided a rationale for fixed-dosing[18,19]. Our fixed-doses were based on an average BSA of 1.70 m^2, which was somewhat lower than the average BSA in our population (1.98 m^2). In our small population, we did not observe correlations between BSA and toxicities. Therefore, it is not likely that our results are influenced by the fixed-dosing principle.

In conclusion, a once-every-three-weeks schedule of irinotecan and cisplatin is not recommended due to the severe toxicity, despite the low total drug doses. Irinotecan may be more suited for intermittent weekly administration.

Acknowledgements

Irinotecan was provided by Aventis Pharma BV, Gouda, nowadays Pfizer BV, Capelle a/d IJssel, the Netherlands.

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Chapter 10

Phase III study of cyclophosphamide, doxorubicin, and etoposide compared with carboplatin and paclitaxel in patients with extensive disease small-cell lung cancer

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Eur J Cancer. 2007 Nov;43(16):2345-50

Abstract

The progression-free survival (PFS) of cyclophosphamide/doxorubicin/etoposide (CDE) and carboplatin/paclitaxel (CP) was compared in chemonaive patients with extensive disease small-cell lung cancer (ED-SCLC). A total of 203 patients were randomized to three-weekly CDE (n = 102) or CP (n = 101) for five cycles. Tumor response rates in CDE and CP were 60% and 61%. PFS of CP was 5.2 months, PFS of CDE 4.9 months ($p = 0.60$). The major difference in toxicity between CDE and CP was grade IV leukocytopenia in 64% and 9% of the patients ($p < 0.0001$), leading to febrile neutropenia in 30% and 4% of the patients ($p < 0.0001$), respectively. This was the reason for differences in the total number of hospital admissions (63 for CDE and 40 for CP, $p = 0.0025$)

This study failed to demonstrate any benefit in PFS with CP compared with CDE. CP was associated with significantly less hematological toxicity, leading to 37% less hospital admissions for febrile neutropenia.

Introduction

Until recently, cyclophosphamide, doxorubicin, and etoposide (CDE) was commonly used for patients with ED-SCLC in the European Union. This schedule was based on large studies conducted by the European Organization for Research and Treatment of Cancer (EORTC)-Lung Cancer group[1-3]. In these studies, a tumor response rate of 79%, a median time to progression of 5.8 months, and a median survival time of approximately 8 months were observed. The major toxicity of CDE is myelosuppression, which is associated with febrile leukopenia in about 30% of the patients[4].

In North America, the preferred treatment for SCLC is a platinum-containing combination[5]. Multiple agents have been combined with either cisplatin or carboplatin, such as etoposide[6], irinotecan or topotecan[7-9], or pemetrexed[10]. In phase II studies, carboplatin with paclitaxel (CP) was effective as first- and second-line treatment in ED-SCLC[11-13], but this doublet has never been compared to other regimens. Based on previously observed high response rates and mild toxicity for carboplatin (Area under the Curve (AUC) of 7) and paclitaxel (175 mg/m^2) as second-line treatment[11], a similar regimen was chosen as first-line treatment.

In the present phase III study, the efficacy of CP and CDE as first-line treatment for ED-SCLC was compared.

Patients and methods

Patients

Patients were included if they met all the following criteria: age over 18 years, histologically or cytologically proven ED-SCLC with measurable or evaluable lesions, no prior chemotherapy or radiotherapy except for symptomatic brain metastases, Eastern Cooperative Oncology Group (ECOG) performance score 0-2, adequate hematological, renal and hepatic functions (absolute neutrophil count (ANC) $\geq$ 2.0 x 10^9/L, platelet count $\geq$ 100 x 10^9/L, bilirubin $\leq$ 1.25 x upper normal limit, creatinine clearance according to Cockroft formula $\geq$ 60 ml/min). Patients were excluded if they had cardiac failure, pre-existent peripheral neuropathy, hypersensitivity to castor oil, uncontrolled infection, other malignant disease, or if they were pregnant or breast-feeding. Radiotherapy was allowed as long as at least one measurable lesion was outside the irradiated field. No other anti-cancer drugs were allowed. The protocol was approved by all local medical ethics committees, and all patients gave written informed consent.

Treatment and dose modifications

Cyclophosphamide (1000 mg/m^2) was administered intravenously on day 1, doxorubicin (45 mg/m^2) on day 1, and etoposide (100 mg/m^2) on days 1, 2, and 3.

Carboplatin (AUC 7 using the Calvert formula[14]) was administered intravenously on day 1 followed by paclitaxel (175 mg/m²) as a 3-hour infusion.

Cycles were repeated every 3 weeks, with a maximum of 5 cycles. Patients were retreated if leukocytes were ≥ 3 x 10^9/L and platelets were ≥ 100 x 10^9/L. Otherwise, treatment was delayed for one week. If the delay was more than two weeks, patients went off-study.

All drugs in both regimens were 25% reduced if ANC < 0.5 x 10^9/L and/or platelets < 50 x 10^9/L for two consecutive counts one week apart, and in case of febrile neutropenia or severe bleeding. If despite this dose reduction grade III or IV toxicity occurred, patients went off-treatment. In the event of grade III or IV non-hematological toxicity (excluding alopecia, nausea, and vomiting) both regimens were reduced by 25%. In case of grade III or IV neurological toxicity or a severe hypersensitivity reaction, patients went off-treatment.

Treatment was stopped for intolerable toxicity, treatment delay of more than two weeks, progressive disease, or on patient's request. The mean relative dose intensity was calculated by dividing the actual delivered dose (mg/m²/week) by the planned dose (mg/m²/week) for the number of cycles each patient received.

Evaluations

Before chemotherapy, patients were evaluated with a history, physical examination, ECOG performance status, complete blood cell count (CBC), electrolytes, liver enzymes, serum creatinine, and electrocardiography (ECG), which was all repeated before every next cycle, except for the ECG. On day 14 of each cycle and on clinical indications a CBC was performed in a similar way in both arms. Tumor evaluations were performed with computed tomography (CT)-scan of the chest and repeated after two cycles and at the end of treatment. Tumor response was defined according to the WHO criteria.

Follow-up after treatment was every 4-6 weeks with a CBC, liver enzymes, chest X-ray, or additional tests if clinically indicated. Toxicity was scored before each cycle according to the National Cancer Institute Common Toxicity Criteria (CTC), version 2.0.

Statistics

All patients were randomized by telephone at the Trial Office and checked for their eligibility. Stratification was performed according to institute and performance status (0-1 versus 2) using the minimization technique[15].

This study was powered to detect a 50% increase in median time to progression (i.e. from 5.8 to 8.7 months), using a two-sided 0.05 α-level test with 80% power. The primary objective was progression-free survival (PFS). Efficacy of chemotherapy is better evaluated by PFS than by overall survival, because subsequent second-line chemotherapy can subsequently prolong survival[16]. Secondary objectives were overall survival, tumor response rates, duration of response, and safety.

All analyses were performed on the intention-to-treat principle. Patients remained on study till death or loss of follow-up. The database was closed on July 1, 2006.

Progression-free survival was defined as the interval from the date of start of treatment to the date of progression or death from any cause. Overall survival was calculated from date of randomization till date of death or censored at end of study. Duration of response was defined as time from documentation of tumor response until progression.

Patient characteristics and toxicity scores were compared using Mann-Whitney U or chi-square tests, as appropriate. Differences in severity of hematological toxicity between the two arms were tested with the Cochran-Armitage trend test. Differences between Kaplan-Meier survival curves were tested with the log-rank test.

Results

Patient characteristics

From February 1999 till February 2005, 203 patients were randomized to either CDE (n = 102) or CP (n = 101). Two patients could not be evaluated for dose intensity and toxicity (one patient received one cycle CDE, but data are missing and one patient randomized to CP never started therapy). One patient randomized to CDE received five cycles of CP. Patient characteristics were well balanced in the two arms (Table 1).

Delivery of treatment

A total of 374 cycles were administered to patients randomized to treatment with CDE and 384 cycles to patients randomized to treatment with CP. Median number of cycles was 5 for both groups. This maximum number of cycles was achieved in 56% of patients treated with CDE and in 51% of patients treated with CP. At least 3 cycles of chemotherapy were administered to 72% of patients in the CDE group and 74% in the CP group. Reasons for treatment discontinuation were similar in both groups, with unacceptable toxicity, progressive disease, and non-treatment related death as most common reasons.

In the CDE group, 39 of 374 cycles (10%) were delayed, in the CP group 62 of 384 cycles (16%) were delayed (p = 0.027). In both treatment arms 20% of patients had a dose reduction of one or more drugs. The mean relative dose intensities for CDE were 93.9% for C, 91.7% for D and 94.2% for E, and for the CP schedule 93.1% for C and 93.3% for P. The average mean dose intensity was not different between CDE and CP (both 93%).

Table 1. Patient characteristics.

Characteristic	CDE (n = 102)	CP (n = 101)
Age (year)		
Median	61.7	62.7
Range	37-77	42-84
Sex		
Male	55 (54%)	63 (62%)
Female	47 (46%)	38 (38%)
ECOG performance score		
0	17 (17%)	21 (21%)
1	66 (65%)	57 (56%)
2	19 (19%)	22 (22%)
Missing	0	1 (1%)
Prior radiotherapy	6 (6%)	1 (1%)
Any comorbidity	50 (49%)	48 (48%)
Pulmonary	9 (9%)	16 (16%)
Cardiac	21 (21%)	24 (24%)
Diabetes mellitus	14 (14%)	6 (6%)

Tumor response and survival

Forty-four patients (22%) could not be evaluated for tumor response (23 in CDE group, 21 in CP group), because of early end of treatment due to toxicity (n = 31), non-treatment related death (n = 5), or other reasons (n = 8). Overall tumor response rate was similar for the CDE group (60%; 95% CI, 50-69) and the CP group (61%; 95% CI, 51-71) (Table 2).

No difference in efficacy between CDE and CP schedules was observed in patients with age over 65 versus younger patients, patients with performance score ≥ 2 versus patients with performance score ≤ 1, or between males and females.

Median PFS for patients treated with CDE was 4.9 months (95% CI, 3.5-5.7), that is not different from those treated with CP (5.2 months; 95% CI, 4.8-5.7) (Figure 1). Median duration of tumor response for complete or partial responders was not different between both treatment groups (6.5 months for CDE (n = 61) and 5.6 months for CP (n = 62), p = 0.425). Overall survival for patients with CDE was 6.8 months (95% CI, 5.3-8.9; 1-year survival 24%) and for CP 6.7 months (95% CI, 5.9-8.7; 1-year survival 26%) (Figure 2).

Table 2. Tumor response rate evaluated with CT-scans in ED-SCLC patients.

Response	CDE (n = 102)		CP (n = 101)	
	Number of patients	%	Number of patients	%
Complete response	13	13	14	14
Partial response	48	47	48	47
Overall response (CR + PR)	61	60	62	61
Stable disease	6	6	8	8
Progressive disease	12	12	10	10
No evaluation*	23	22	21	21

*Reasons for no evaluation were early end of treatment due to toxicity (n = 31), non-treatment related death (n = 5), or other (n = 8).

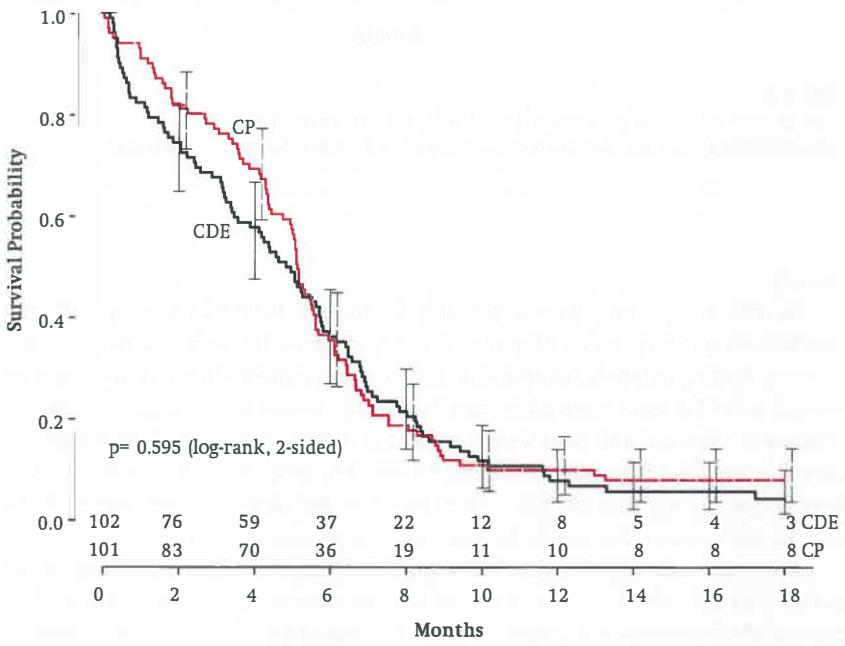

Figure 1.
Progression-free survival in 203 patients with ED-SCLC treated with CDE or CP.
CDE cyclophosphamide, doxorubicin, etoposide, CP carboplatin and paclitaxel.

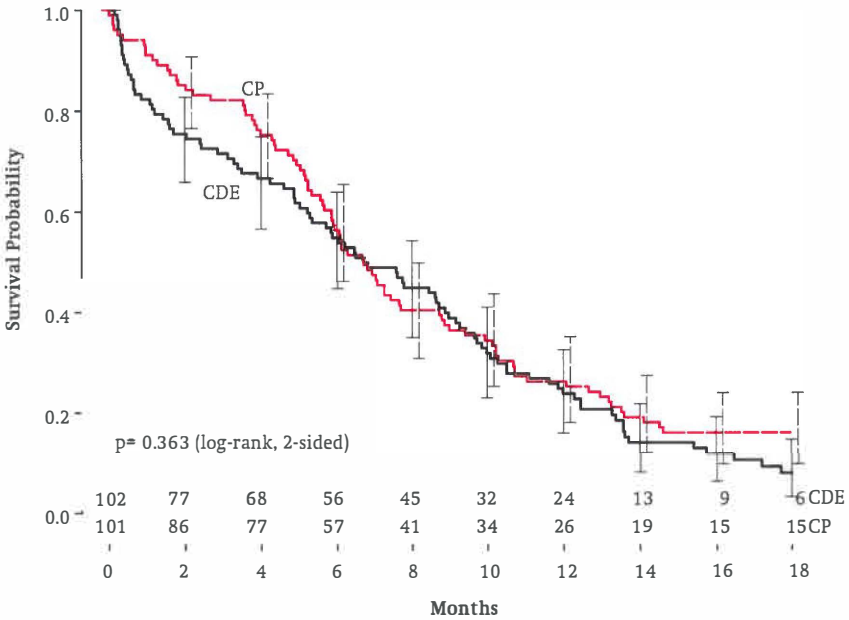

Figure 2.
Overall survival in 203 patients with ED-SCLC treated with CDE or CP.
CDE cyclophosphamide, doxorubicin, etoposide, CP carboplatin and paclitaxel.

Toxicity

Overall, 201 patients were assessable for toxicity. Patients receiving CDE had significantly more episodes of grade IV leukocytopenia (65 versus 9 patients, $p < 0.0001$), leading to febrile neutropenia in 31 versus 4 patients ($p < 0.0001$). Patients treated with CDE were more often hospitalized (63 versus 40 patients, $p = 0.0025$). Infections, dyspnea, and pain were the most common reasons for hospitalization. Seven patients in CP group had very mild hypersensitivity reactions which were easily managed during infusion. Most other toxicities (hematological and non-hematological) were equally distributed between the two groups (Table 3).

Treatment-related death occurred in eight patients treated with CDE and in one patient treated with CP ($p = 0.035$), in all but two patients occurring during the first cycle of chemotherapy. Neutropenic sepsis was the cause of death in all patients.

Table 3. Grade III and IV hematological and non-hematological toxicity in ED-SCLC patients.

Toxicity		CDE (n = 101)	CP (n = 100)
Hematological			
Anemia	Grade III	20	9
	Grade IV	3	3
Thrombocytopenia	Grade III	27	20
	Grade IV	2	2
Leukopenia*	Grade III	26	24
	Grade IV†	65	9
Neutropenia*	Grade III	12	23
	Grade IV†	37	16
Non-hematological			
Hemorrhage		5	1
Cardiac toxicity		3	5
Fatigue		13	9
Myalgia		0	5
Anorexia		6	3
Diarrhea		4	3
Nausea		6	4
Vomiting		3	5
Infection†		28	3
Neurotoxicity		9	6
Pulmonary toxicity		14	18
Alopecia (grade II)		50	47
Other toxicity		19	15
Hypersensitivity reactions (any grade)†		0	7
Febrile neutropenia†		31	4

*Leukopenia and neutropenia were more common and more severe in CDE group ($p < 0.0001$ and $p = 0.016$ respectively, Cochran-Armitage trend test).
†$P < 0.01$ (Chi-square-test).

Discussion

In the present phase III study comparing CP versus CDE as first-line treatment for ED-SCLC, CP did not result in a longer PFS and overall survival compared to CDE. In both groups about half of the patients completed the five planned cycles of treatment, but the toxicity profile of CDE was worse, reflected by a higher number of neutropenic fever episodes and hospitalizations.

CP has never been directly compared with the CDE regimen in ED-SCLC. Our results in patients treated with CDE were consistent with results observed in previously reported studies (tumor response rates 52-73%, progression-free survival 5.8 months, and overall survival 7.6-8.7 months)[1-3,17]. CP as doublet regimen has been studied in several phase II studies, with slightly different treatment regimens as compared to our regimen. Thomas and colleagues treated patients with carboplatin AUC 6 and paclitaxel 200 mg/m^2 every three weeks[12], with a response rate of 65%, and a median overall survival of 8.7 months. With carboplatin AUC 6 and paclitaxel 175 mg/m^2 every 4 weeks, a response rate of 54%, a median time to progression of 5.7 months, and a median overall survival of 9.7 months were observed[13]. Both regimens had a tolerable toxicity profile. Using paclitaxel 200 mg/m^2 and carboplatin AUC 6 every three weeks, an overall response rate of 61% and a median survival of 11.8 months in patients with ED-SCLC was reported[18]. CP as second-line treatment yielded a response rate of 74% and a median time to progression of 4.8 months[11]. In summary, the results of the first comparative study further define the therapeutic performances of CDE and CP regimens, both resulting in a median progression-free survival of approximately 5 months and a response rate around 60% for ED-SCLC.

With CP and CDE showing an equal efficacy in ED-SCLC, toxicity and safety are major issues in treatment decisions. The toxicity profile of CP was significantly better than that of CDE. More treatment-related deaths were observed in the CDE group compared to the CP group. This was mainly caused by the higher number of grade IV leukocytopenia in CDE, resulting in more hospitalizations for treatment complications. The CP regimen did not result in more grade III and IV neurotoxicity than the CDE regimen. The 8% treatment-related deaths for CDE treatment is not significantly different from previously reported studies (2%-7%)[1-4,17,19], and one should realize that all patients had ED, which is regarded as a risk factor for treatment-related death[4,17]. Treatment-related death was most common during the first cycle of chemotherapy, as was previously described [4,19]. Standardized use of prophylactic antibiotics and/or granulocyte colony-stimulating factors might decrease this toxic death rate[19], as recommended by the 2006 ASCO and EORTC guidelines[20,21]. Despite a higher toxicity observed in the CDE treatment arm, the percentage of patients that completed the planned five cycles of chemotherapy was equal in both groups.

In conclusion, the present study failed to demonstrate any benefit in terms of PFS with CP compared to CDE. CP has a more favorable toxicity profile than CDE. Given the results of the present study, CDE should no longer be used as first-line treatment for ED-SCLC, with CP being a good alternative treatment combination.

Acknowledgements

Paclitaxel was provided free of charge by Bristol-Myers Squibb, Woerden, the Netherlands.

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Chapter 11

Third-line chemotherapy for small-cell lung cancer

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Lung Cancer. 2006 Jun;52(3):319-42.

Abstract

Introduction

Efficacy of third-line chemotherapy treatment for small-cell lung cancer (SCLC) is unknown. We present our experience with third-line chemotherapy for recurrent SCLC.

Methods

Between January 1996 and July 2004, all consecutive patients treated for SCLC were retrospectively studied. We recorded patient characteristics, treatment details for each subsequent regimen, response to chemotherapy, and survival.

Results

From 191 patients treated with chemotherapy, 35 patients (18%) received third-line chemotherapy. At the start of third-line therapy, median age was 58 years (range, 36-77), male/female 54/46%, and ECOG performance score was 0/1/2/3 in 15/64/12/9% of patients. Median time from diagnosis till start of third-line treatment was 15 months (range, 5-34). Tumor response to first-line, second-line, and third-line therapy was 91%, 51%, and 26%, respectively. Median survival time estimated from the start of third-line treatment was 5 months (range, 1-15). No toxic deaths were observed. Comparison of characteristics of patients who were treated with third-line chemotherapy with patients treated with maximally two lines of chemotherapy revealed that those who received third-line therapy were younger ($p < 0.001$), had a better performance score ($p < 0.001$), and had a better response to first-line treatment ($p = 0.004$).

Conclusion

Third-line chemotherapy is still active in one in four SCLC patients.

Introduction

Chemotherapy is the primary treatment option for patients with small-cell lung cancer (SCLC)[1], leading to a 5-year survival of about 20% in limited disease (LD), and less than 5% in extensive disease (ED). Although initial tumor response rate to chemotherapy is very high[1] (up to 96% for LD and up to 65% in ED), SCLC relapses in approximately 4 months in ED and 12 months in LD[2]. Relapses can be treated with second-line chemotherapy, with acceptable response rates[3], and eventually in most patients a second tumor relapse will occur.

The efficacy of third-line treatment in SCLC is yet unknown. Third-line therapy is defined as chemotherapy after two previous lines of chemotherapy. Literature on third-line chemotherapy is at most anecdotal[4,5]. From both articles it was not possible to extract information about the efficacy of third-line chemotherapy.

We can only speculate which patients are selected for third-line chemotherapy. It is plausible that younger patients in a good clinical condition may be more likely to receive third-line chemotherapy, as these characteristics are known favorable prognostic factors[6]. Furthermore, patients who have demonstrated good tumor responses to, and long disease-free intervals after the two previous lines may benefit more from a third-line treatment. Also, patients with progressive disease during previous lines of chemotherapy may be candidates for a fast switch to third-line chemotherapy. However, these patients have tumors that have previously proven resistance to chemotherapy, and they may already be beyond reach of further treatment.

The aim of our study was to analyze the efficacy of third-line chemotherapy for recurrent SCLC. Also the clinical characteristics of patients receiving third-line chemotherapy were described and they were compared with characteristics of patients who received only one or two lines of chemotherapy.

Patients and methods

Patients

Between January 1996 and July 2004, all consecutive patients diagnosed with SCLC and treated with chemotherapy at University Medical Center Groningen, the Netherlands, were included. SCLC was diagnosed by experienced pathologists and assessed according to WHO classification. The following baseline characteristics were recorded from the clinical file: sex, age at diagnosis, Eastern Cooperative Oncology Group (ECOG) performance score (PS), laboratory values (LDH, sodium, albumin, alkaline phosphatase (AF)), and disease stage. LD was defined as disease confined to one hemithorax, all other cases were considered ED, according to the Veterans' Affairs Lung Study Group (VALSG). At start of third-line chemotherapy patient characteristics were also recorded.

Date of start of chemotherapy, date of last cycle, chemotherapy regimen, number of cycles, tumor response to chemotherapy, and date of progression were recorded for first-line and subsequent lines of chemotherapy. Tumor response was evaluated following the World Health Organization criteria. Patients with a complete or partial response (CR or PR) were considered responders, patients with stable or progressive disease (SD or PD) were considered non-responders.

Application of radiotherapy was recorded, and classified as thoracic radiotherapy (for patients with LD), whole brain radiotherapy (WBRT) for cerebral metastases, or prophylactic cranial irradiation (PCI) after a complete response. Worst hematological toxicity was scored according to National Cancer Institute Common Toxicity Criteria (CTC), version 2.0.

Statistics

Characteristics of patients who received third-line chemotherapy are described. Their characteristics were compared with characteristics of those patients who only received one or two lines of chemotherapy using Mann-Whitney U tests. Overall survival and survival after start of third-line chemotherapy were calculated with the Kaplan-Meier method.

Results

Characteristics of third-line treatment

First-line chemotherapy was administered to 191 consecutive patients. Of these patients, 94 patients (49%) received second-line chemotherapy. Thirty-five patients (18%) were eventually treated with third-line chemotherapy (Figure 1). Their characteristics are shown in Table 1.

The combination of cyclophosphamide, doxorubicin and etoposide (CDE) was used as third-line chemotherapy regimen in 11 patients (31%). CDE was also used as first-line or second-line regimen in 10 of these 11 patients. The median interval between CDE lines was 8 months (range, 6-20). Carboplatin and paclitaxel, with or without ifosfamide, was used in 8 patients (22%) as third-line regimen. All had received CDE as first-line and/or second-line regimen. Cisplatin combinations were administered to 6 patients (17%). Less frequently used regimens were gemcitabine monotherapy (3 patients), gemcitabine combined with carboplatin (2 patients), teniposide (2 patients), and others. Again, all but one patient received CDE as first-line and/or second-line regimen. One patient was treated three times with CDE, with 20 and 6 months between the successive lines. Median number of cycles in third-line was 3 (range, 1-6). Toxicity for third-line chemotherapy was mainly hematological. No toxic deaths were observed.

Tumor response rates after first-line, second-line, and third-line chemotherapy

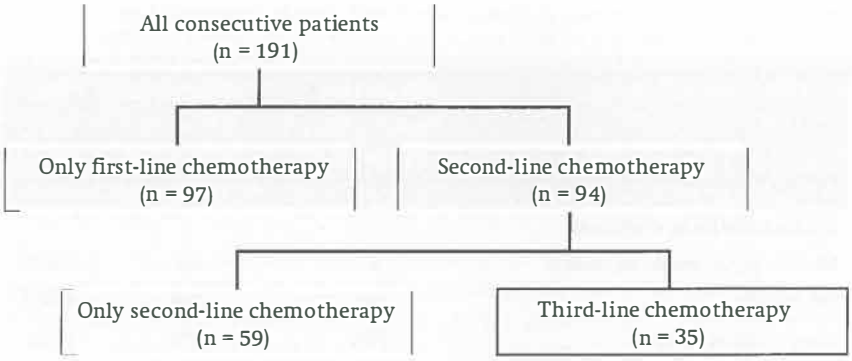

Figure 1.
Distribution of patients according to number of chemotherapy lines received. Eighteen percent of patients eventually received third-line chemotherapy.

Table 1. Patient characteristics (n = 35) at start of third-line chemotherapy.

Median age	58 years (range, 36-77)
Male / Female	54% / 46%
ECOG performance score 0 / 1 / 2 / 3	15% / 64% / 12% / 9%
Disease stage at diagnosis: limited / extensive	51% / 49%
Tumor response to first-line chemotherapy	91%
Tumor response to second-line chemotherapy	51%
Median time from diagnosis till start third-line	15 months (range, 5-34)

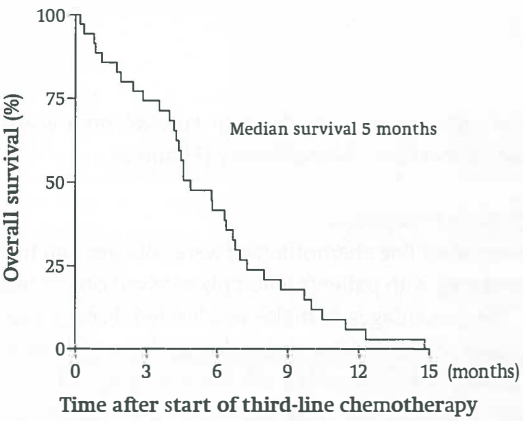

Figure 2.
Overall survival after start of third-line chemotherapy (n = 35).

Table 2. Patient characteristics of those who received third-line chemotherapy (n = 35) and those who only received one or two lines of chemotherapy (n = 156).

	Third-line chemotherapy patients	Patients with maximally two lines of chemotherapy	P-value
Characteristics at diagnosis			
Median age at diagnosis (years)	57	64	< 0.001
Sex (males)	54%	58%	0.662
Stage (limited disease)	51%	37%	0.121
ECOG performance score 0 / 1 / 2	71% / 29% / 0%	27% / 53% / 20%	
Median ECOG performance score	0	1	< 0.001
Mean serum LDH* (U/L)	270	599	0.005
Mean serum sodium* (mmol/L)	135	135	0.831
Mean serum albumin* (g/L)	40	38	0.059
Mean serum AF*(U/L)	94	150	0.011
Treatment characteristics			
CDE as first-line chemotherapy	77%	65%	0.160
Median number of first-line cycles	5	4	< 0.001
Responder to first-line chemotherapy	91%	67%	0.004
Thoracic radiotherapy	31%	17%	0.046
Prophylactic cranial irradiation	9%	8%	0.963
Brain radiotherapy for metastases	40%	18%	0.005

*Normal values: LDH (lactate dehydrogenase) 114-235 U/L, serum sodium 132-144 mmol/L, serum albumin 34-47 g/L, AF (alkaline phosphatase) 13-120 U/L.
CDE cyclofosfamide, doxorubicin, etoposide.

decreased from 91%, to 51% and 26%, respectively. Median survival time was 5 months (range, 1-15) after the start of third-line chemotherapy (Figure 2).

Comparison of patient characteristics at diagnosis

Patients who eventually received third-line chemotherapy were younger and had a better performance score as compared with patients who only received one or two lines of chemotherapy (Table 2). The percentages of males and limited disease were not different between the two groups. Patients who received three lines of chemotherapy also had a lower serum LDH and AF before first-line chemotherapy as compared with those patients who only received maximally two lines of chemotherapy.

Comparison of first-line treatment characteristics

There were also differences between patients who received third-line chemotherapy compared to patients who only received maximally two lines of chemotherapy regarding treatment characteristics. Patients treated with third-line chemotherapy underwent more cycles during first-line treatment and more often experienced a tumor response to first-line chemotherapy (Table 2). The choice of first-line chemotherapy regimen was similar in both groups. Thoracic radiotherapy and whole brain radiotherapy for metastases were more often encountered in patients treated with third-line chemotherapy.

Discussion

To our knowledge, this study is the first report describing in detail the administration of third-line chemotherapy in patients with SCLC. One out of six patients in our study population was treated with third-line chemotherapy. These patients were younger and had a better clinical condition at the start of first-line treatment compared with patients treated with only one or two lines. Furthermore, they were treated with a higher number of first-line cycles, demonstrated higher tumor response rates, and received more thoracic and brain radiotherapy.

The 26% tumor response rate of third-line chemotherapy, as observed in this study, was obviously lower than that of the first-line (91%) and second-line treatment (51%). This may be reflective of the development of resistance to chemotherapy. Tumor responses are still high in the study group as compared to the tumor response rates observed in patients with non-small cell lung cancer in second-line and third-line therapy. Therefore, it is surprising that many clinicians do not administer third-line chemotherapy in SCLC. This study also demonstrated a median survival of 5 months after starting third-line chemotherapy. Although we did not investigate the survival of patients who were not actively treated, it is commonly accepted that, at least at presentation of their disease, untreated SCLC patients will die as a result of their disease within 3 months[2]. In accordance, a median survival of 5.3 months after start of second-line chemotherapy compared to 2.2 months for best supportive care has been reported[7]. The authors partly attributed this survival benefit to the better performance status of patients treated with second-line chemotherapy, which may also play a role in our study. In a randomized study, O'Brien et al. observed a median survival of 3.1 months in patients treated with best supportive care alone[8]. Together, our 26% response rate and 5 months survival time may suggest that third-line chemotherapy is a feasible option for treating SCLC. However, it should be taken into account that this was a retrospective study, in which variable factors may have played a role in the selection of patients to undergo third-line chemotherapy.

In this study younger patients with a good clinical condition at diagnosis were

more likely to be eventually treated with third-line chemotherapy. Furthermore, patients with a good tumor response to previous lines and a longer disease-free survival (the observed median elapsed time from diagnosis until start of third-line chemotherapy was 15 months) were more likely to receive third-line chemotherapy. Patients with highly aggressive or therapy resistant tumors, characterized by an elevated serum LDH and unresponsiveness to previous lines, did not receive third-line chemotherapy more frequently. Apparently, the condition of these patients did contribute to the decision to abstain from further active treatment. Of course, patients fit enough to receive third-line chemotherapy also may refuse further treatment for a number of reasons, although no data to support this were available. The choice for a regimen is dependent on the former regimens, elapsed time between lines, comorbidity, and availability of new agents, and those regimens showed an acceptable toxicity during second-line and third-line treatment. We conclude that many factors may play a role in proceeding with third-line chemotherapy, which may also affect tumor response rates and survival.

In conclusion, this study demonstrated that third-line chemotherapy for multiple recurrent SCLC was administered to one out of six patients in our hospital, and was effective in one out of four patients. This considerable response rate warrants the use of third-line chemotherapy for selected patients in a good clinical condition. Future studies should take cost-effectiveness into account.

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Chapter 12

Summary and future prospectives

Thoracic tumors are a major burden of disease in many countries[1]. Non-small cell lung cancer and small-cell lung cancer are the most common thoracic malignancies, both responsible for a high number of cancer deaths. Thymic epithelial tumors are one of the most well-known of the less common thoracic tumors. In this thesis, we investigated new ways in estimating the prognosis of patients with thoracic tumors and explored improvements in therapy for these tumors.

Epidemiology of thoracic tumors

In **chapter two**, the database of the Netherlands Cancer Registry was used to determine the changes in the incidence of pulmonary tumors in the Netherlands between 1989 and 2003. NSCLC was by far the most common pulmonary tumor. The annual incidence of NSCLC among males decreased from 109 to 72/100,000, among females a significant increase from 17 to 31/100,000 was observed, and the relative number of females with NSCLC rose significantly (to 32% in 2003). The trends observed in an earlier study in the Netherlands[2] therefore did continue, meaning that the lung cancer epidemic in the Netherlands is far from over.

Regarding the histological subtypes, the relative proportion of adenocarcinoma increased, and nowadays accounts for 33% of all NSCLC. The incidence of SCLC slightly decreased to 11 and 6/100,000 in males and females, respectively. In 2003, SCLC accounted for 17% of all lung cancers. We observed that changes reported in many other countries also occur in more or less the same direction in the Netherlands, and that these trends are probably largely accounted for by the change in smoking habits and the differences in smoking frequencies between countries[3,4].

Since 1996, a remarkable stage shift in the disease stage at diagnosis of NSCLC was noted in the Dutch population. The relative proportion of patients in stage I decreased from 25% in 1989 to 18% in 2003, and stage IV increased from 25% to 37%. One likely explanation could be the introduction of PET scanning, leading to upstaging due to the detection of occult metastases[5]. Moreover, we observed that the incidence of less common, non-smoking related pulmonary tumors such as carcinoid tumors and primary pulmonary sarcomas did not change over time. In summary, chapter two, combined with data from chapter six and eight and other sources, provided an overview of the magnitude of the problem of thoracic tumors (Table 1).

Prognostic factors in thoracic tumors

In **chapter three**, it was demonstrated that prognostic models based on clinical and laboratory parameters were more or less equally able to predict the prognosis of patients with SCLC as a model based on imaging techniques. Despite the fact that the explained variance (a measure of statistical robustness) of the imaging-based model was higher than the explained variance of three existing models based on clinical and laboratory parameters, all four models resulted in similar survival curves (Figure 1, chapter three). Chapter three thus demonstrated that imaging is not per

Table 1. Overview of age-adjusted incidence (per 100,000) and annual numbers of thoracic tumors in the Netherlands (2003).

Tumor type	Males			Females			Total
	Incidence		Number	Incidence		Number	Number
NSCLC (total)[1]	54.7	↓	4680	22.2	↑	2092	6772
Squamous carcinoma[1]	21.2	↓	1817	5.0	↑	482	2299
Adenocarcinoma[1]	15.9	↔	1348	9.5	↑	883	2231
Large-cell carcinoma[1]	17.6	↑	1515	7.7	↑	727	2242
SCLC[1]	11.2	↓	952	6.1	↑	564	1516
Adenosquamous carcinoma[1]	0.46	↓	38	0.12	↔	11	49
Carcinoid tumors[1]	0.44	↔	37	0.43	↔	39	76
Sarcomatoid carcinomas[1]	0.35	↔	30	0.09	↔	9	39
Pulmonary sarcomas[1]	0.08	↔	7	0.01	↔	1	8
Thymic epithelial tumors[2]	0.32	↔	28	0.32	↔	34	62
Thoracic desmoid tumor[3]	<0.01		?	<0.01		?	?
Mesothelioma[4]	3.82	↔	327	0.45	↔	45	372
Oesophagus carcinoma[4]	11.9	↑	1017	3.8	↑	417	1434

[1]Chapter two; [2]Chapter six; [3]Chapter eight; [4]Netherlands Cancer Registry.
↑Incidence increases, ↓Incidence decreases, ↔ Stable incidence (between 1989 and 2003).

se necessary for estimating prognosis in SCLC and underlines the importance of clinical and laboratory parameters in assessing an individual patient's prognosis.

New imaging techniques have expanded the traditional radiological arsenal of chest X-ray and CT-scans. PET scanning made it possible to obtain information on the metabolic activity of tumors. In **chapter four**, we reported that the metabolic activity of a tumor, quantified in the standard uptake value (SUV), is a good marker in order to divide patients with resectable NSCLC into prognostic groups. Moreover, it was shown that the different types of SUV are correlated with each other and all have prognostic value. Because the prognostic information from the SUV based on the maximal value measured within a hotspot (SUVmax) was similar to the SUV based on the mean value within a hotspot, we recommend that SUVmax is used for prognostic purposes, because it is the easiest SUV to calculate.

To continue, there was no natural cut-off value for SUVmax in its use as prognostic parameter in resectable NSCLC, which is biologically plausible. In the absence of a natural cut-off point, we suggest that the median value of SUVmax (7 in our study) is used as cut-off value in order to divide patients into two prognostic groups. In this way, one could speculate that SUVmax can be used to stratify patients with resectable NSCLC. This could mean that patients with metabolically active tumors (i.e., those with a high SUVmax) will receive adjuvant chemotherapy and patients with

a lower SUVmax not. Based on these assumptions, a nation-wide study in which patients are selected for adjuvant chemotherapy or no adjuvant treatment based on the SUVmax of the primary tumor is currently enrolling patients in the Netherlands (NVALT-8 study). Because the settings of the PET scanners will be modified for this study, the estimated median SUVmax in the NVALT-8 study, and thus the cut-off, will be 10 instead of 7 as observed in chapter four.

Early detection of a tumor could be a good solution for improving the prognosis of patients with NSCLC. CT-based imaging of high-risk populations (defined as long-term smokers) has up to now not resulted in indisputable evidence in favor of this strategy[6-9]. Therefore, better methods for selecting high-risk patients are needed. High-risk patients could be identified by the detection of premalignant genetic or epigenetic abnormalities in long-term smokers, but therefore it is necessary to better understand the genetic and epigenetic abnormalities in the bronchial epithelium. The distribution of aberrant methylation, an epigenetic phenomenon associated with the development of lung cancer, throughout the lungs is not known. Also, the differences between lung cancer patients and controls with respect to the frequencies of aberrant methylation are not well established.

In **chapter five**, we demonstrated that promoter methylation of at least one gene is present in epithelial tumor cells obtained by bronchial brushes in 80% of all patients with NSCLC. Epithelial cells originating from other endobronchial locations (3 cm proximal from the tumor and from the contralateral lung) did hardly contain any promoter methylation. Finally, in bronchial brushes from controls without NSCLC no aberrant methylation was observed. Based on these results one may conclude that promoter methylation (at least of the eleven tested genes) only occurs in tumor cells and not in non-tumor epithelial cells. This is supported by the fact that we could not detect correlations between the presence of promoter methylation and smoking habits. Because our study was only a small, exploratory study, one has to be careful to extrapolate our results. The true value of methylation assays in screening and early detection of NSCLC remains to be resolved. Several studies reported a sensitivity of up to 60% for methylation tests in sputum to detect NSCLC[10-12]. Our findings contribute to defining the value of methylation assays in identifying patients with a high risk of lung cancer (to select individuals for CT screening), discriminating between patients with good or worse prognosis, or even as starting point for future treatment options of lung cancer using demethylating agents.

Uncommon thoracic tumors: thymic epithelial tumors and desmoid tumors

Thymic epithelial tumors are less common than lung cancer. In the population-based study presented in **chapter six**, we present several new finding regarding these tumors. First, we showed that the annual incidence of all thymic epithelial tumors, along the whole histopathological spectrum ranging from benign to malignant, is 3.2/1,000,000 in the Netherlands. This incidence was never determined before, and

Table 2. Overall and relative 5-year survival of 232 patients with thymic epithelial tumors in the Netherlands, according to WHO classification and Masaoka clinical stage.

	Number of patients	Overall 5-year survival (95% CI)	Relative 5-year survival (95% CI)
WHO classification			
A	19	87.8 (59.4-96.9)	100 (69.9-111.9)
AB	33	83.0 (63.8-92.6)	92.8 (71.2-103.6)
B1	34	81.7 (61.0-92.1)	86.5 (64.7-97.4)
B2	54	81.9 (66.6-90.6)	85.9 (69.8-95.2)
B3	36	53.1 (32.8-69.8)	56.9 (35.5-74.5)
C	36	37.8 (20.5-55.0)	42.6 (23.7-61.0)
Unknown classification	20	59.7 (33.0-78.7)	70.9 (39.1-93.5)
Masaoka clinical stages			
I	25	82.9 (60.5-93.3)	91.2 (65.8-103.2)
II	75	87.8 (75.7-94.1)	95.3 (82.2-102.1)
III	53	57.6 (39.6-72.1)	63.2 (43.7-78.7)
IV	50	55.6 (38.6-69.5)	59.7 (41.4-74.8)
Unknown stage	29	49.9 (29.4-67.3)	56.3 (33.2-76.2)

means that approximately 50 thymic epithelial tumors are diagnosed in the Netherlands each year. The incidence was constant during our 10-year study period.

Second, we established the diagnostic procedures for thymic epithelial tumors. More than half of all thymic epithelial tumors were not definitively diagnosed until after resection, meaning that the resection was performed without a pre-operative pathological diagnosis. Thus, common practice in the Netherlands is that a suspicion of a thymic epithelial tumor based on clinical and radiological information is sufficient to proceed to thoracotomy.

Third, we observed that most patients with a thymic epithelial tumor could die from their tumors, as assessed by a relative survival of less than 100% for subtypes B1 and higher and Masaoka clinical disease stage of III or more. The overall and relative survival of thymic epithelial tumors in the Netherlands is presented in Table 2. These findings question the supposed benign character of thymic epithelial tumors. Also because the term benign and malignant has fallen out of fashion regarding thymomas, it is recommended to describe the clinical characteristics of these tumors with the WHO classification and the Masaoka disease stage.

Fourth, a remarkable finding in chapter six was that we could not detect a difference in overall survival between patients with a complete resection and patients with an incomplete resection ($p = 0.53$). This is in contrast with most earlier studies[13,14] but not all[15]. The reasons could lie in the more frequent use of (neo-)adjuvant therapy in patients with an incomplete resection, or in the lack of knowledge about the extent of incompleteness (microscopical tumor residue or debulking). Overall,

our findings point out that surgery offers the best perspectives for patients with thymic epithelial tumors, even if the resection is incomplete. The low incidence of thymic epithelial tumors hampers the execution of large controlled trials with thymic epithelial tumors.

Nation-wide studies are dependent on good-functioning nation-wide registries, with record linkage being a quintessential element in population-based studies. For rare tumors such as thymic epithelial tumors, and for tumors with a broad histopathological and clinical spectrum, little is known about the difficulties and potential errors of record linkage. To investigate this issue, we studied the differences and overlap between the Dutch National Pathological Archives (PALGA) and the Netherlands Cancer Registry (NCR) with thymic epithelial tumors as example.

In **chapter seven**, we observed that only 43% of all PALGA thymic epithelial tumors are registered in the NCR. Reversely, almost all tumors found in NCR are also recorded in PALGA. Tumors with a more benign histology (WHO subtypes A and AB) were less likely to be entered into the NCR. Surprisingly, NCR contained only 61% of the histologically most malignant tumors (types B3 and C). It is likely that these differences are explained by registration procedures used by the databases, and only in a small part are caused by true linkage errors. For example, tumors are only entered in NCR if they are considered malignant, and as can be learned from the previous chapter, not all thymomas have a clinically malignant behavior. We proposed that the NCR should register all thymic epithelial tumors. This will support the institution of a nation-wide study group for thymic epithelial tumors, which may play a role in improving the diagnostic accuracy of these tumors and which may be functional in conducting trials.

In **chapter eight**, a young patient with a large intrathoracic desmoid tumor is presented. This is a slowly growing, locally invasive tumor originating from pleural fibroblastic cells. Other rarer thoracic tumors, such as a solitary fibrous tumor, were ruled out based on morphology and immunohistochemistry. Also the treatment choices for this tumor are described in this chapter. Choices are even more difficult for rarer tumors because of the paucity of literature and evidence on treatment in orphan diseases. We argued that post-operative radiotherapy, despite the incomplete resection, was not the best option due to the potential toxicity, and that regular MRI screening to detect a recurrence in an early stage was preferred.

Treatment of thoracic tumors: small-cell lung cancer

In **chapter nine**, we studied the optimal dosage regimen of the new cytotoxic agent irinotecan in combination with cisplatin and concurrent thoracic radiotherapy in patients with limited-disease SCLC. In this dose-escalating study, severe dose-limiting toxicity was observed at a dose of 140 mg irinotecan and 100 mg cisplatin once every three weeks, in combination with radiotherapy. However, also at lower

dosages and during the cycles without radiotherapy severe toxicity (diarrhea and neutropenia) was observed. Based on these results we concluded that irinotecan and cisplatin dosed once every three weeks is not recommended. Irinotecan may be more suited for a weekly administration. In addition, patient selection based on pharmacokinetics might help in selecting patients less prone to toxic side-effects of irinotecan[16,17].

In **chapter ten**, we determined that the combination of carboplatin and paclitaxel (which was a promising combination as second-line treatment[18]) was not superior in terms of progression-free survival compared to the former EORTC standard treatment with cyclophosphamide, doxorubicin, and etoposide in patients with ED-SCLC. Nevertheless, carboplatin and paclitaxel had significantly less hematological toxicity than CDE, leading to 37% less hospital admissions for febrile neutropenia. This phase III trial demonstrated that CDE should no longer be used as first-line treatment for ED-SCLC due to its higher toxicity. The tumor response rate and the progression free survival was not significantly different between the platinum-containing regimen (carboplatin and paclitaxel) and the non-platinum containing regimen (CDE), but one has to keep in mind that this study was not designed to test the non-inferiority of one treatment compared with the other. Until new (targeted) drugs are available, first-line treatment of ED-SCLC should consist of a platinum-containing regimen and not the CDE regimen anymore.

Tumor relapse is the key issue after first-line treatment of SCLC. Despite the fact that only one study demonstrated an improved survival after second-line treatment versus best supportive care[19], second-line treatment is frequently applied, with impressive response rates in phase II trials. In **chapter eleven**, we demonstrated that in our hospital second-line treatment was offered to half of all original patients with SCLC, and that less than 20% of all patients eventually were treated with third-line chemotherapy. The tumor response rate to third-line chemotherapy was still 26%, but substantially lower than response rates to first- and second-line treatment. Patients that were younger and in a good clinical condition were more likely to be treated with third-line treatment. Moreover, third-line treatment was generally well-tolerated. Our results warrant the use of third-line treatment in selected patients.

Future prospectives

With the start of the new millennium, it seems that the estimation of prognosis and the treatment for patients with thoracic malignancies are heading for new directions.

The increasing knowledge on the molecular and genetic basis of lung cancer will provide more insight in the pathogenesis of NSCLC. Apart from the traditional clinical prognostic markers, new genetic and molecular markers will provide ad-

ditional prognostic and predictive power. Based on the presence of markers, one could distribute NSCLC patients, and perhaps also patients with other thoracic tumors, into prognostic groups, and thereby selecting patients for appropriate, individualized treatment. For example, the presence of epidermal growth factor receptor mutations and/or overexpression seems to be predictive for tumor response and for overall survival[20,21].

Improvements in the sensitivity and specificity of promoter methylation tests may lead to its wide-spread use as a tool for early detection of lung cancer, for instance by testing sputum samples of people at high-risk for lung cancer. Positive results may lead to detection of tumors in curable stages, and may thus improve chances of survival.

The better understanding of the molecular pathogenesis of lung cancer also resulted in a cornucopia of new drugs, which enhances the process leading to individualized treatment. It is expected that these new treatments will improve the survival and quality of life of patients with thoracic tumors.

Despite these new developments on the molecular level, it remains questionable whether these tests will replace traditional prognostic factors in thoracic tumors or even pinpoint to certain specific treatments. Laboratory parameters and performance score are easily available indications for patient's prognosis and should be generally used. In addition, the metabolic activity of the tumor expressed as SUV might become more important by using it in an algorithm for deciding whether or not to administer adjuvant chemotherapy.

For thymic epithelial tumors the most important question will remain how to treat patients with locally advanced disease, especially the role of debulking surgery. Future studies should randomize patients with incomplete resection (after stratification for WHO classification) into adjuvant chemoradiotherapy or no adjuvant treatment. It is hoped that the institution of a nation-wide thymic epithelial tumor panel will make the conduction of such trials possible.

For SCLC, research will be focused not primarily on improving the already impressive response rate, but on ways to reduce the chances of tumor recurrence. With the current combination chemotherapy regimens, a therapeutic plateau seems to have been reached, and research should be focused on exploring the least toxic treatment regimens. In addition, the response rates observed in phase II studies in second-line treatment might result in an increase in approved second-line therapies. Finally, increasing insight in the pathogenesis of SCLC might reveal new pathways assessable for new drugs.

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Nederlandse samenvatting

(Summary in Dutch)

Inleiding

De borstkas (thorax) wordt omgeven door de ribben, en loopt van de sleutelbeenderen tot aan het middenrif. In de borstkas bevinden zich twee grote organen, het hart en de longen. Verder liggen er nog diverse andere organen en structuren in de borstkas, zoals de slokdarm, de longvliezen, en de zwezerik (thymus). In al deze organen kunnen tumoren, kwaadaardige en goedaardige, ontstaan. Dit proefschrift gaat over kwaadaardige tumoren die uitgaan van organen in de thorax, en dan met name longkanker en thymustumoren. Om een indruk te krijgen van de ernst en grootte van het probleem van de thoracale tumoren wordt in Tabel 1 een overzicht getoond van de tumoren die besproken worden in dit proefschrift. Voordat de onderwerpen van het proefschrift in detail worden weergegeven zal eerst in het kort de huidige kennis over deze tumoren worden besproken.

Longkanker

Longkanker komt erg veel voor. Jaarlijks wordt in Nederland bij ruim 9000 mensen longkanker vastgesteld (Tabel 1). Elk jaar overlijden ook bijna 9000 Nederlanders hieraan. Dit betekent dat in Nederland meer mensen overlijden aan longkanker dan aan borstkanker en darmkanker samen. Het aantal gevallen per jaar is de laatste jaren vrij stabiel in Nederland. In de hele Westerse wereld is longkanker een groot probleem, maar ook in Oost-Europa en in ontwikkelingslanden zoals China neemt het aantal gevallen van longkanker snel toe. Dit heeft voor een groot deel te maken met de rookgewoonten.

Voor haast geen enkele andere maligniteit is de relatie tussen veroorzaker en kwaal zo sterk als voor longkanker. Meer dan 90% van alle patiënten met longkanker heeft fors gerookt of rookt nog steeds. Omdat meer mannen roken of gerookt hebben dan vrouwen treft longkanker op dit moment nog 2 à 3 keer zoveel mannen als vrouwen. In Nederland rookt helaas nog steeds 1 op de 3 volwassenen, en ongeveer evenveel mannen als vrouwen. Hoewel het totaal aantal gevallen van longkanker nu elk jaar daalt, is de verwachting is dat het totaal aantal gevallen van longkanker in Nederland na 2010 weer zal stijgen doordat het percentage ouderen toe zal nemen.

De meest voorkomende klachten van patiënten met longkanker zijn benauwdheid, het ophoesten van slijm en/of bloed, pijn in de borstkas of op andere plaatsen en malaise. Geen enkele klacht is echter specifiek. Verder wordt soms longkanker vastgesteld bij patiënten zonder klachten, bijvoorbeeld wanneer beeldvorming plaats vindt om andere redenen of in het kader van een bevolkingsonderzoek naar longkanker (NELSON project). De gemiddelde leeftijd waarop longkanker ontstaat is ongeveer 62 jaar voor mannen, en iets lager bij vrouwen.

Tabel 1. Overzicht van de incidentie (het aantal nieuwe gevallen per 100.000 inwoners per jaar) en het totaal aantal nieuwe gevallen per jaar van thoracale tumoren in Nederland (2003). Ter vergelijking wordt ook het voorkomen van thoracale tumoren die niet worden besproken in dit proefschrift (longvlies- en slokdarmkanker) en het voorkomen van andere veel voorkomende tumoren (darm- en borstkanker) vermeld.

Tumor type	Mannen			Vrouwen			Totaal
	Incidentie		Aantal	Incidentie		Aantal	Aantal
Longkanker (totaal)[1]	71.6	↓	6126	30.5	↑	2888	9014
Niet-kleincellig longkanker[1]	54.7	↓	4680	22.2	↑	2092	6772
Plaveiselcelcarcinoom[1]	21.2	↓	1817	5.0	↑	482	2299
Adenocarcinoom[1]	15.9	↔	1348	9.5	↑	883	2231
Grootcellig carcinoom[1]	17.6	↑	1515	7.7	↑	727	2242
Kleincellig longkanker[1]	11.2	↓	952	6.1	↑	564	1516
Overige longtumoren[1]	5.7	↓	494	2.3	↑	232	726
Thymustumor[2]	0.32	↔	28	0.32	↔	34	62
Thoracale desmoid tumor[3]	< 0.01		?	< 0.01		?	?
Longvlieskanker[4]	3.82	↔	327	0.45	↔	45	372
Slokdarmkanker[4]	11.9	↑	1017	3.8	↑	417	1434
Darmkanker[4]	60.6	↑	5157	43.1	↑	4741	9898
Borstkanker[4]	0.8	↑	71	123.5	↑	11687	11758

[1]Hoofdstuk twee; [2]Hoofdstuk zes; [3]Hoofdstuk acht; [4]Nederlandse Kankerregistratie.
↑Incidentie neemt toe, ↓Incidentie neemt af, ↔ Incidentie blijft gelijk (tussen 1989 en 2003).

Wanneer er bij een patiënt de verdenking op longkanker is, volgt een verwijzing naar de longarts. Deze probeert de mate van uitbreiding van de longkanker vast te stellen en een stukje weefsel te verkrijgen voor microscopisch onderzoek. De uitbreiding wordt vastgesteld met beeldvorming, met name met CT en PET scan. Bij een PET scan wordt patiënten geïnjecteerd met radioactief gelabeld suiker. Wanneer mensen vervolgens onder de PET scan gaan liggen kleuren de plaatsen waar veel suiker wordt omgezet (zoals in tumoren) aan. Op deze manier kan het hele lichaam gescreend worden op uitzaaiingen (metastasen). Tumorweefsel wordt in het algemeen verkregen met een bronchoscopie. Tijdens dit kijkonderzoek in de binnenkant van de luchtwegen kunnen van afwijkende plekken hapjes (biopten) worden genomen of met borsteltjes (brushes) cellen van de binnenkant van de luchtwegen worden verzameld. Dit materiaal wordt vervolgens onder de microscoop beoordeeld door de patholoog.

Longkanker kent twee grote ondersoorten, namelijk kleincellig en niet-kleincellig longkanker. Deze beide soorten worden op verschillende manieren behandeld.

Kleincellig longkanker is de agressiefste vorm van longkanker, en komt voor bij ongeveer 15% van alle patiënten met longkanker. Kleincellig longkanker wordt gekenmerkt door een snelle groei en meestal grote tumoren op het moment van diagnose. Wanneer er geen uitzaaiingen worden aangetroffen (1/3 van de patiënten) spreekt men van beperkte ziekte (limited disease). Wanneer er wel metastasen op afstand zijn spreekt men van uitgebreide ziekte (extensive disease). De behandeling van limited disease bestaat in principe uit een combinatie van chemotherapie en radiotherapie op de tumor in de borstkas. Dit resulteert in een 5-jaars overleving van circa 15%. De prognose van extensive disease (wordt alleen met chemotherapie behandeld) is veel slechter: na 5 jaar is nog slechts 1% van de patiënten in leven. Verder worden alle patiënten die reageren op chemotherapie behandeld met profylactische hersenbestraling om de kans op het ontstaan van hersenmetastasen te verkleinen. Het grootste probleem bij kleincellig longcarcinoom is niet dat de tumor niet goed reageert op de therapie (in de meeste patiënten vermindert het tumorvolume snel nadat gestart is met chemotherapie) maar dat de tumor snel weer terug komt in nagenoeg alle patiënten. Geselecteerde patiënten kunnen dan opnieuw met chemotherapie behandeld worden.

Niet-kleincellig longkanker wordt nog weer verder onderverdeeld in adenocarcinoom, plaveiselcelcarcinoom en grootcellig carcinoom. Ongeveer 80% van alle patiënten met longkanker hebben een niet-kleincellig longkanker. Momenteel zijn de behandelingsmogelijkheden en de prognose niet verschillend voor deze ondersoorten. Voor patiënten in de zeer vroege stadia (zonder uitzaaiingen naar de lymfeklieren achter het borstbeen of naar andere plekken in het lichaam; in totaal ongeveer 15% van alle patiënten) is een operatie de beste behandelmethode, mits de resterende longfunctie het toelaat. Sinds kort is het standaard om na een complete resectie van de tumor 3 tot 4 kuren chemotherapie te geven om de kans op terugkeer van de kanker te verminderen. Desondanks overlijdt nog steeds een substantieel deel van de patiënten met een compleet verwijderde tumor aan longkanker: de 5-jaars overleving loopt van 65 tot 30%, afhankelijk van het stadium. Voor patiënten met longkanker die uitgezaaid is naar de lymfeklieren achter het borstbeen maar zonder uitzaaiingen elders in het lichaam (ongeveer 20% van alle patiënten) is gecombineerde radio- en chemotherapie de behandeling van keuze, resulterend in een 5-jaars overleving van ongeveer 25%. De overgrote meerderheid van patiënten met niet-kleincellig longcarcinoom heeft echter reeds uitzaaiingen op het moment van diagnose. Zij kunnen slechts met palliatieve chemotherapie behandeld worden. Deze therapie is niet meer gericht op genezing, maar op verlenging van het leven en verbetering van de kwaliteit van leven.

Chemotherapie voor zowel kleincellig als niet-kleincellig longkanker bestaat meestal uit een combinatie van middelen toegediend via een infuus. Deze middelen (ook wel cytostatica genaamd) grijpen meestal aan op de celdeling. Chemotherapie kent een groot scala aan mogelijke bijwerkingen, zoals misselijkheid, haaruitval, malaise en een verminderde bloedaanmaak met daardoor een vergrote kans op infecties.

De laatste jaren zijn verschillende nieuwe medicijnen op de markt gekomen voor de behandeling van longkanker. De nieuwe zogenaamde "biologicals" hebben een ander werkingsmechanisme dan cytostatica. Zij grijpen niet primair aan op de celdeling, maar beïnvloeden de (uit)groei van kankercellen doordat ze specifieke groeifactoren te remmen. Deze middelen kennen dan ook een ander, meestal gunstiger, bijwerkingenprofiel dan de klassieke cytostatica. Enkele biologicals worden al voorgeschreven in Nederland; de verwachting is dat de komende jaren meer zullen volgen en dat ook de plaats in de behandeling beter gedefinieerd wordt. Bovendien komen ook steeds meer technieken beschikbaar om het effect van de behandeling en de prognose beter te voorspellen. Dit zal mogelijk leiden tot het ontstaan van geïndividualiseerde therapie, in tegenstelling tot de huidige praktijk, waarbij elke patiënt in eenzelfde stadium dezelfde behandeling aangeboden krijgt.

Thymustumoren

Thymustumoren ontstaan uit epitheliale cellen in de thymus (zwezerik). De thymus is een orgaan van zo'n 60 gram bij volwassenen, en is gelegen in het voorste mediastinum, de ruimte tussen het borstbeen en het hart. De thymus heeft bij kinderen en jongvolwassenen een belangrijke rol in het afweersysteem; de rol van de thymus bij volwassenen is niet precies bekend. Waarschijnlijk is het een niet-werkzame rest van het werkzame orgaan op de kinderleeftijd.

Thymustumoren zijn, in tegenstelling tot longkanker, erg zeldzaam. Jaarlijks worden er slechts ongeveer 50 gevallen gediagnosticeerd in Nederland (Tabel 1), meestal tussen het 50-ste en 60-ste levensjaar. Eén derde wordt aangetroffen bij patiënten zonder klachten, de overige patiënten hebben meestal klachten vanwege de grootte van de tumor en druk op omliggende weefsels, wat kan resulteren in bijvoorbeeld benauwdheid, hoesten of pijn. Interessant is de relatie tussen thymustumoren en de spier-zenuwziekte Myasthenia Gravis. Eiwitten uit de tumor beïnvloeden de spier-zenuwovergang, zodat de klassieke symptomen van Myasthenia Gravis ontstaan. Dit gebeurt in ongeveer de helft van alle patiënten met een thymustumor.

De diagnose wordt in het algemeen gesteld op een stukje tumorweefsel, verkregen door een punctie of tijdens de operatie. Thymustumoren worden ingedeeld aan de hand van hun microscopische kwaadaardigheid (de WHO classificatie) en op basis van de ingroei in omliggende weefsels (de Masaoka classificatie).

Chirurgie is de eerste behandeloptie. Wanneer een thymustumor niet of niet helemaal verwijderd kan worden kan ook chemotherapie en/of radiotherapie gegeven worden. De overleving is afhankelijk van de microscopische agressiviteit, de mate van ingroei in de omliggende weefsels en natuurlijk van de behandeling. Voor de meeste patiënten is de prognose echter heel gunstig, de 10-jaars overleving voor alle patiënten loopt van 100% voor de gunstige stadia tot 40% voor de meest ongunstigste stadia.

Doel van dit proefschrift

Het doel van dit proefschrift was om te onderzoeken of de prognose van patiënten met een thoracale tumor beter voorspeld kan worden en of de behandeling van deze patiënten verbeterd kan worden.

Samenvatting van de hoofdstukken

In **hoofdstuk twee** werd de database van de Nederlandse Kankerregistratie geraadpleegd om het voorkomen, de histologische onderverdeling en de stadiumverdeling van longtumoren in Nederland te onderzoeken. In de periode 1989-2003 werd bij ruim 130.000 mensen een longtumor gediagnosticeerd. De overgrote meerderheid bestond uit niet-kleincellig en kleincellig longkanker. In de onderzochte periode nam de incidentie van longkanker bij de Nederlandse mannen af (met name plaveiselcelcarcinoom en kleincellig longkanker), terwijl bij de vrouwen alle ondersoorten in aantal toenamen (Tabel 1). Deze veranderingen worden niet alleen in ons land maar wereldwijd waargenomen, en hebben waarschijnlijk veel te maken met de verandering in rookgewoonten. Mannen zijn de laatste decennia minder gaan roken, terwijl het aantal rokende vrouwen eerst toenam, en pas de laatste jaren vermindert. Aangezien er een lange tijd tussen de blootstelling aan rook en het ontstaan van longkanker zit worden de effecten van het veranderende rookgedrag nu pas zichtbaar. Inmiddels komt longkanker zelfs meer voor onder ex-rokers dan onder huidige rokers.

Een tweede bevinding was dat bij het stellen van de diagnose meer en meer patiënten zich al in vergevorderde stadia bevinden. Dit wordt waarschijnlijk niet verklaard doordat patiënten eerder uitzaaiingen ontwikkelen maar doordat deze uitzaaiingen eerder opgespoord worden door betere beeldvormende technieken. Met name de introductie van de PET scan heeft hierin een belangrijke rol gespeeld. Een laatste opvallende bevinding was dat het voorkomen van zeldzame, niet aan roken gerelateerde longtumoren niet veranderd is de afgelopen jaren.

In **hoofdstuk drie** wordt het inschatten van de prognose van patiënten met kleincellig longkanker besproken. In de kliniek wordt veel gebruik gemaakt van radiologische technieken (zoals bijvoorbeeld een CT scan) om de prognose te bepalen door patiënten in te delen in limited of extensive disease. Sommige onderzoeksgroepen claimen echter dat een combinatie van de conditie van een patiënt en enkele bloedwaarden de prognose ook adequaat voorspelt. In een groep van 156 patiënten stelden wij vast dat zowel modellen gebaseerd op radiologische technieken als

modellen gebaseerd op een combinatie van conditie en bloedtesten de prognose goed kunnen inschatten. Dit betekent dat beeldvorming (duurder dan bloedtesten) niet per se nodig is om de prognose te bepalen. Bovendien onderstrepen onze bevindingen de waarde van bloedtesten en de conditie van patiënten met kleincellig longkanker.

Hoofdstuk vier behandelt de prognostische waarde van de PET scan bij patiënten met een operabel niet-kleincellig longkanker. Zoals eerder beschreven, is de PET scan een manier om de metabole activiteit van een tumor te meten. Hoe meer suiker een tumor verbruikt, hoe sneller de tumor deelt, en hoe agressiever deze is. De metabole activiteit wordt uitgedrukt in de standard uptake value (SUV), en wordt voor elke pixel op een scan uitgerekend. Er bestaan verschillende SUVs: de hoogste SUV in een tumor (SUVmax) of een gemiddelde SUV in een tumorgebied.

In 66 patiënten uit twee ziekenhuizen hebben we vastgesteld dat SUVmax en gemiddelde SUVs dezelfde prognostische waarden met betrekking tot de overleving hebben, waarbij SUVmax het makkelijkst uit te rekenen is. Om patiënten in te delen in twee groepen van gelijke grootte werd gebruikt gemaakt van de mediane SUVmax (de mediane SUVmax is die SUVmax waarbij de helft van de patiënten een lagere SUVmax heeft, en de andere helft een hogere SUVmax). Patiënten met een lagere SUVmax bleken een betere prognose te hebben dan patiënten met een hogere SUVmax. In dit licht kan men zich voorstellen dat patiënten met een hogere SUVmax wel baat hebben bij chemotherapie na een complete resectie, terwijl patiënten met een lage SUVmax alleen maar bijwerkingen maar geen voordeel hebben van deze chemotherapie. Binnenkort start in Nederland een grote landelijke studie naar de waarde van chemotherapie na een complete resectie, waarbij de SUVmax waarde wordt gebruikt om patiënten in te delen in een groep die wel en in een groep die niet deze chemotherapie zal ontvangen (NVALT-8 studie).

De afgelopen jaren is steeds meer duidelijk geworden dat niet alleen genetische processen (zoals bijvoorbeeld genmutaties) maar ook epigenetische processen een rol spelen bij het ontstaan van (long-)kanker. Bij epigenetische veranderingen blijft, in tegenstelling tot mutaties, de DNA basen volgorde intact, maar wordt door koppeling van bijvoorbeeld methylgroepen aan het DNA het aflezen van een gen onmogelijk gemaakt. Dit proces heet promoter methylatie. Het effect van mutaties en promoter methylatie is feitelijk hetzelfde, namelijk dat een gen zijn functie (zorgen voor de productie van eiwitten) niet meer kan uitoefenen. Door het ontbreken van specifieke eiwitten gaan processen in de cel fout waardoor kanker kan ontstaan. De aanwezigheid van promoter methylatie in een gen kan aangetoond worden met een specifieke methode (methylatie-specifieke PCR, MSP). Er is niet veel bekend over de aanwezigheid en spreiding door de longen van promoter methylatie in patiënten met longkanker.

In **hoofdstuk vijf** hebben we op drie locaties in de luchtwegen van patiënten met longkanker de aanwezigheid van promoter methylatie bepaald. Promoter methylatie bleek –gemeten met een specifieke MSP-test- voornamelijk voor te komen in tumorcellen, en niet of nauwelijks in luchtwegepitheelcellen in de andere locaties buiten de tumor. Bovendien bevatten luchtwegepitheelcellen van controlepersonen ook geen promoter methylatie. Dit betekent dat tabaksrook niet door de hele long schade in de vorm van promoter methylatie achterlaat, maar dat het aantonen van promoter methylatie een goede voorspellende waarde heeft voor de aanwezigheid van longkanker. Deze methylatie test kan dus gebruikt worden in sputum of brushes als screeningstechniek voor de aanwezigheid van longkanker.

In **hoofdstuk zes** wordt een grote studie naar de incidentie, de diagnose en de behandeling en overleving van thymustumoren in Nederland gepresenteerd. Over een periode van 10 jaar werden 537 gevallen van een thymoom of thymuscarcinoom vastgesteld. We stelden vast dat jaarlijks bij gemiddeld 3,3 op de miljoen Nederlanders een thymustumor ontstaat, en dat de definitieve diagnose meestal pas gesteld wordt nadat de tumor chirurgisch is verwijderd. Verder bleek dat de meerderheid van de mensen met een thymustumor, afhankelijk van de pathologische classificatie en het klinische stadium, een kans loopt om aan de tumor te overlijden. Dit betekent dat de meeste thymustumoren niet goedaardig genoemd mogen worden. Als laatste bleek dat de overleving van patiënten waarbij de tumor niet volledig was verwijderd gelijk was aan de overleving van patiënten waarbij de tumor wel volledig was verwijderd. Deze opvallende bevinding kan mogelijk verklaard worden doordat patiënten met een onvolledig verwijderde tumor vaker aanvullende behandelingen als chemotherapie en/of radiotherapie ontvingen.

De gegevens uit hoofdstuk zes kwamen tot stand door een koppeling tussen de het landelijke pathologie register (PALGA) en de Nederlandse Kankerregistratie (NKR). PALGA verzamelt alle pathologieverslagen. De NKR registreert gegevens over alle nieuw gediagnosticeerde maligniteiten in Nederland. In **hoofdstuk zeven** wordt dieper op de problemen en voordelen van zo'n koppeling ingegaan. Het bleek dat minder dan de helft van alle thymustumoren voorkwam in de NKR. Dit heeft veel te maken met de werkwijze van beide registraties; de inclusiecriteria van de NKR zijn anders dan die van PALGA. Patiënten met een meer kwaadaardige histologie of een verder uitgebreide tumor hadden een grote kans om in de NKR opgenomen te worden. Wij pleiten ervoor dat voortaan alle thymustumoren worden geregistreerd in de NKR, zodat niet alleen de pathologische maar ook de klinische gegevens van deze zeldzame tumoren met een groot klinisch spectrum algemeen beschikbaar zullen zijn. De oprichting van een nationaal thymomen panel kan hieraan bijdragen door de diagnose en de aanmelding bij de NKR te standaardiseren. Bovendien kan zo'n panel een goed uitgangspunt zijn voor landelijke trials bij deze tumoren.

In **hoofdstuk acht** wordt een jongeman met een in de borstkas gelegen desmoid tumor gepresenteerd. Dit is een heel zeldzame, langzaam groeiende tumor die uitgaat van fibroblastcellen gelegen in de borstkas. Andere zeldzame tumoren die differentiaal diagnostisch nog in aanmerking kwamen werden uitgesloten met behulp van microscopische technieken. Ook wordt de behandeling van deze desmoid tumor beschreven. De chirurgische resectie bleek niet helemaal volledig te zijn. Wij kozen niet voor aanvullende radiotherapie maar voor een beleid van intensieve controle met MRI's om de groei van de tumor in een vroeg stadium op het spoor te komen en dan pas te starten met radiotherapie. Tot op heden, 2 jaar na de diagnose, is er echter geen sprake geweest van een terugkeer van de tumor. Deze casus demonstreert de dilemma's bij het kiezen voor een bepaalde behandeling bij zeldzame tumoren.

In **hoofdstuk negen** werd de optimale dosering van het nieuwe middel irinotecan, in combinatie met cisplatinum en radiotherapie, bestudeerd in patiënten met limited disease kleincellig longkanker. In deze zogenaamde fase I studie wordt de dosering van de chemotherapie langzaam opgehoogd om te bepalen van de maximale dosis is die patiënten kunnen verdragen wat bijwerkingen betreft. We kwamen ernstige bijwerkingen (diarree, ontstoken slokdarm, en verstoorde bloedaanmaak) tegen, ook bij relatief lage doseringen. Helaas moesten we daarom concluderen dat ons driewekelijkse schema met irinotecan en cisplatinum niet aanbevolen kan worden voor verder onderzoek.

In **hoofdstuk tien** wordt beschreven dat de combinatie van carboplatin en paclitaxel geen betere progressie-vrije overleving gaf in patiënten met extensive disease kleincellig longkanker dan de vroegere standaard combinatie cyclofosfamide, doxorubicine en etoposide (CDE). Hoewel de werking van carboplatin en paclitaxel niet beter was dan de werking van CDE, waren de bijwerkingen van carboplatin en paclitaxel wel minder ernstig. Doordat de belangrijkste bijwerking (verstoorde bloedaanmaak) minder vaak voorkwam waren er 37% minder ziekenhuisopnames nodig voor patiënten behandeld met carboplatin en paclitaxel dan voor patiënten behandeld met CDE. Dit grote landelijke onderzoek met meer dan 200 patiënten toonde aan dat CDE niet meer toegepast dient te worden als eerstelijns chemotherapie voor patiënten met extensive disease kleincellig longkanker in verband met de ernstige bijwerkingen, en dat carboplatin en paclitaxel een goed alternatief is.

Het grootste probleem voor patiënten met kleincellig longkanker is echter niet dat de tumor niet goed reageert op therapie, maar dat bijna bij alle patiënten de tumor na korte tijd weer uitgroeit. Dit recidief kan dan opnieuw behandeld worden met chemotherapie. In **hoofdstuk elf** wordt beschreven dat in ons ziekenhuis ruim de helft van alle patiënten met kleincellig longkanker met tweedelijns chemotherapie behandeld wordt, en bijna 20% zelfs met derdelijnschemotherapie. Het bleek dat

een kwart van de patiënten goed reageerden op derdelijnschemotherapie, en dat de bijwerkingen goed te verdragen waren. Deze resultaten suggereren dat derdelijnschemotherapie, voor geselecteerde patiënten, een goede behandelingsmogelijkheid is.

Conclusie

Samenvattend worden in dit proefschrift nieuwe ontwikkelingen voor het inschatten van de prognose en de behandeling van patiënten met longkanker besproken. Bovendien worden het voorkomen en de behandeling van zeldzame thoracale tumoren zoals thymustumoren en desmoidtumoren besproken. De in dit proefschrift besproken onderzoeken dienen als uitgangspunt voor verder onderzoek in de toekomst om patiënten met een veel voorkomende of een zeldzame thoracale tumor betere vooruitzichten te kunnen bieden.

Dankwoord

Gedeelde smart is halve smart, maar gedeelde vreugde is dubbele vreugde; het schrijven van een proefschrift met alles wat daar bij komt kijken doe je niet alleen. Mijn hartelijke dank aan allen die hieraan in meer of mindere mate hebben bijgedragen.

Dit dankwoord is een goede plek om enkelen van degenen die de afgelopen vier jaar bij dit proefschrift betrokken zijn specifiek te noemen.

Als eerste mijn promotor, Prof. dr. H.J.M. Groen. Beste Harry, ik ben erg blij dat jij mijn promotor was. Je enthousiasme, je grote wetenschappelijke kennis, je klinische vaardigheden en je inzet vòòr en achter de schermen; ik heb er veel van geleerd en heb het allemaal zeer gewaardeerd. Ondanks je soms wat "flexibele" agenda vond ik ook de persoonlijke omgang met je erg plezierig, hoewel ik me soms wel eens afvroeg wat je nu van mijn humor vindt.

Alle mede-auteurs van de artikelen in dit proefschrift wil ik ook danken voor hun hulp bij de totstandkoming van deze stukken.

Dr. M. Schaapveld van het Integraal Kankercentrum Noord-Nederland, beste Michael, veel dank voor de frequente analyses en je kritische aanvullingen.

Drs. J.H.G. Blaauwgeers, patholoog in het OLVG te Amsterdam, beste Hans, hartelijk dank voor de plezierige samenwerking de afgelopen jaren.

Dr. J. Louwagie en Dr. G.F. Verpooten van Oncomethylome Sciences S.A., beste Joost en Gonda, mijn oprechte dank voor de goede samenwerking.

Alie Smidt van het endoscopiecentrum wil ik bedanken voor haar hulp bij het verkrijgen van de brushes voor hoofdstuk vijf.

De leden van de beoordelingscommissie, Prof. dr. P.E. Postmus, Prof. dr. E.G.E. de Vries en Prof. dr. R.P. Stolk, wil ik hartelijk danken voor hun bereidheid om mijn proefschrift te beoordelen.

Alle (oud-) stafleden en (oud-) assistenten van de afdeling Longziekten van het UMCG wil ik hartelijk bedanken voor hun interesse, hulp en gezelligheid bij de totstandkoming van dit proefschrift en op andere momenten tijdens de afgelopen vier jaren. Ik verheug me erop om weer terug te komen op deze afdeling.

Trudy en Evelyn van het secretariaat Longziekten en de dames van het "Longoncologie Team" Thea, Ria en Christa: bedankt voor alle hulp en gezelligheid.

De medewerkers van de polikliniek Longoncologie wil ik hartelijk danken voor hun behulpzaamheid en vriendelijkheid.

Het gedeelde smart en gedeelde vreugde uit de eerste zin is zeker ook van toepassing voor mijn (oud-) onderzoekscollega's Judith, Renske, Janwillem, Franke, Naomi, Sandra, Fransien, Linda, Marieke, Erik, Margot, Hester, Toby, Floris en Henk. Een goede sfeer is heel belangrijk. Ik denk met veel plezier terug aan de vele leuke momenten binnen en buiten werktijd, zoals bijvoorbeeld "de muur" (voor nadere details zie "Fate of editors who rejected major scientific breakthroughs", WK de Jong et al, *Bulg. J. Low Impact*, 2007;45:234-9.), de TIO studie, en de uitjes en borrels. Ik ben erg blij dat jullie inmiddels altijd allemaal al mijn grappen en humor waarderen.

Beste Vrienden, weglopend voor het gevaar mensen voor het hoofd te stoten ga ik jullie niet stuk voor stuk noemen. Ik kan me, de eerste zin van dit dankwoord indachtig, slechts aansluiten bij Thomas Jefferson: "Friendship is precious, not only in the shade but also in the sunshine of life, and thanks to a benevolent arrangement the greater part of life is sunshine".

De paranimfen van dienst wil ik van ook danken voor hun inspanningen. Beste Judith, hiermee is onze tweemaandelijkse gezamenlijke ganzenpas voorlopig ten einde. Ik zie ernaar uit om ook gezamenlijk de opleiding te volgen. Beste Vincent, inhoudelijk liggen onze expertises mijlenver uit elkaar. Desondanks waardeer ik het zeer dat je mij vandaag bij de verdediging bijstaat. Had ik je trouwens al verteld dat je volgens de reglementen de verdediging in voorkomende gevallen over moet nemen?

Mijn schoonfamilie, Kees, Miek, Marieke en Kees-Jan, wil ik ook danken voor hun belangstelling voor mijn proefschrift. Ik heb het goed met jullie getroffen.

Mijn ouders, zus en zwager wil ik hartelijk danken voor hun gezelligheid, interesse en steun, zowel voor dit proefschrift als op andere momenten. Pa, Ma, Caroline en Daan: ik heb geluk gehad met jullie als familie.

Lieve Esther, jij en sinds kort Pieter zijn voor mij het allerbelangrijkste!

Beste lezers, that's it, want als het klaar is, is het af.

Wouter

Curriculum Vitae

Wouter Karst de Jong werd op 10 februari 1977 geboren in Noordlaren. Hij deed in 1995 eindexamen Gymnasium aan het Lingecollege te Tiel. Na twee jaar Biologie te hebben gestudeerd kon hij uiteindelijk in 1997 starten met de studie Geneeskunde in Groningen. De doctoraalopleiding werd afgerond met een afstudeeronderzoek in Toronto (Canada). Na de co-schappen werd in december 2003 met succes het arts-examen afgelegd.
Daarna begon hij als arts-onderzoeker op de afdeling longziekten van het UMCG, onder leiding van Prof. dr. Harry Groen. Hier verrichtte hij het onderzoek beschreven in dit proefschrift.
Op 1 januari 2008 begint Wouter met zijn opleiding tot longarts op de afdeling Longziekten en Tuberculose van het UMCG. Het eerste deel hiervan, de vooropleiding Interne Geneeskunde, volgt hij in het Martini Ziekenhuis te Groningen. De geplande einddatum van de opleiding tot longarts is 1 juli 2013.
Wouter is getrouwd met Esther Gieteling. Sinds de zomer van 2007 hebben zij een zoon Pieter.