THANOS SIORIS

Non-small Cell Lung Cancer

with Special Reference to Treatment Decisions and Staging by Molecular Methods, Immunohistochemistry and Computed Tomography

University of Tampere
Tampere 2000

Non-small Cell Lung Cancer

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University of Tampere, Medical School,
Department of Surgery
Finland

Tampere University Hospital,
Department of Surgery

Supervised by
Docent Matti Tarkka
University of Tampere
Docent Jarmo Salo
University of Helsinki

Reviewed by
Professor Ari Harjula
University of Helsinki
Docent Karin Mattson
University of Helsinki

Distribution

University of Tampere
Sales Office
P.O. Box 617
33101 Tampere
Finland

Tel. +358 3 215 6055
Fax +358 3 215 7150
taju@uta.fi
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ACADEMIC DISSERTATION

To be presented, with the permission of
the Faculty of Medicine of the University of Tampere,
for public discussion in the small auditorium of
Building K, Medical School of the University of Tampere,
Teiskontie 35, Tampere, on May 5th, 2000, at 13 o'clock.

University of Tampere
Tampere 2000

To Helena and our children

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LIST OF ABBREVIATIONS

AJC	American Joint Committee on Cancer
CT	Computed tomography
DGGE	Denaturing gel gradient electrophoresis
DNA	Deoxyribonucleic acid
FEV1	Forced expiratory volume in one second
kD	Kilodalton
MRI	Magnetic resonance imaging
NSCLC	Non-small cell lung cancer
PCR	Polymerase chain reaction
PET	Positron emission tomography
SSCP	Single strand conformation polymorphism
TNM	Tumor Node Metastasis classification
UICC	Union International Contre le Cancer
UV	Ultraviolet light
WHO	World Health Organisation

INTRODUCTION

Lung cancer is the leading cause of cancer deaths in the world (WHO 1997). Approximately 80-90% of cases are caused by cigarette smoking (Finnish Foundation for Cancer Research 1989, Levi et al . 1997). Never having smoked or giving up smoking are the only effective means of prevention (Risser 1996). Lung cancer is usually only detected at an advanced stage of development, and is almost invariably already disseminated at diagnosis. The prognosis has not been improved by chemoprevention or by sputum cytology or by chest radiography screening of patients at risk, mainly because of a lack of an effective systemic treatment (Fontana et al. 1991, Omenn et al. 1996). The number of elderly people, who are at the greatest risk of developing lung cancer, because of the cumulative effects of a lifetime of smoking, is increasing in the population. Treatment tailored to the needs of this group is needed. The incidence of lung cancer is slowly decreasing among men in developed countries, and rapidly increasing among women (Finnish Foundation for Cancer Research 1989, Levi et al. 1997). Of the three main histological types of non-small cell lung cancer (NSCLC), adenocarcinoma is now the most frequent type, followed by epidermoid and large cell tumors (Finnish Foundation for Cancer Research 1989, Levi et al. 1997,Wynder 1998).

A complete surgical resection offers the best chance of cure (Shields 1993). The five-year postoperative survival in the optimum stage, when the tumor is limited to the lung and no lymph node metastases are present, is 57-67%. Most patients die from a systemic relapse of their cancer (Mountain 1997). Only 25-30% of patients with NSCLC has operable disease at diagnosis, because of co-morbid disease or inoperability due to local invasion or extrathoracic spread.

The development of surgical treatment focuses on new techniques and strategies to increase the number of complete resections. The evolution of bronchoplastic techniques enables pneumonectomy to be circumvented in selected cases with or without limited respiratory reserve (Maggi et al. 1993). Elderly patients, even of 80 years of age or older, and often without other co-morbidies, have undergone curative resections without increased mortality or impairment of their quality of life (Pagni et al. 1997). Optimal treatment decisions and patient selection rely strongly on prognostic factors. The clinical stage, determined by imaging methods and endoscopic evaluation, is the most important guideline for surgical treatment decisions. Understanding the molecular basis of carcinogenesis has led to the development of ”molecular staging” techniques to predict cancer agression. One such technique is to detect mutations in p53, the principle gene controlling cell cycle arrest and the induction of apoptosis in

cells with DNA damage (Hussain and Harris 1998). Routine preoperative staging is carried out using computed tomography (CT), which can predict surgical resectability with an accuracy of over 80% (Lähde et al.1991, Daly et al. 1993). Lymph node staging remains a problem, and only some 50% agreement can be obtained between surgical and clinical TNM staging evaluations (Fernando and Golstraw 1990). A systematic perioperative lymph node dissection is essential for definitive staging (Goldstraw et al. 1994, Holmes 1994, Thomas 1994, Shields 1993). Serial sectioning of all lymph nodes and immunohistochemistry can detect metastases in lymph nodes that were judged as normal in routine histological examination (Kubuschok et al. 1999). Since modern combined modality treatments using preoperative chemotherapy may improve the resectability of locally advanced disease and improve the cure rate (Bunn and Soriano 1998), precise staging has become essential for final treatment decisions.

In this study of patients with non-small cell lung cancer, we wanted to determine if bronchoplasty is an adequate surgical procedure for achieving a complete resection of cancer, if surgery with curative intent can be justified in elderly NSCLC patients, if p53 mutations have prognostic value, if the addition of immunostaining to normal routine histology reveals metastases in histologically normal lymph nodes, and if preoperative computed tomography is a reliable method for staging lymph nodes.

REVIEW OF THE LITERATURE

GENERAL FEATURES OF LUNG CANCER

Epidemiology

In Finland in 1995 there were 1552 new cases of lung cancer among men and 421 new cases among women. The mean age of men at diagnosis is 68 years, and of women 71 years. Only 4.5% of men and 9.8% of women are under 50 years of age at diagnosis (Finnish Cancer Registry 1997). Due to changes in smoking habits, the risk of lung cancer has consistently increased among women since the 1950s, and the number of new cases per year is expected to increase to about 650 in the years 2004-2008. The incidence among men has been decreasing since the 1970s, but the number of new cases is not falling so quickly, because the number of old men at high risk of developing lung cancer is increasing (Finnish Foundation for Cancer Research 1989).

Based on histology, lung cancer is divided into small cell cancer, and non-small cell cancers (adenocarcinoma, epidermoid carcinoma and large cell anaplastic carcinoma). In a large Swiss study, the commonest cell type was squamous cell carcinoma which accounted for 37% of cases, with adenocarcinoma and small cell carcinoma accounting for approximately 18% of cases each, and other carcinomas accounting for 16% of cases (Levi et al. 1997). However, the incidence of adenocarcinoma is rising in both genders, probably because smoking low tar and low nicotine filter cigarettes allows the peripheral parts of the lungs to be exposed to various carcinogens (Levi et al. 1997, Wynder 1998)

Etiological risk factors

The most important risk factor for lung cancer is smoking, which accounts for 80-90% of all cases (Finnish Foundation for Cancer Research 1989). The majority of these lung cancer patients are over 70 years of age, because of the cumulative effects of a lifetime of cigarrette smoking (DeMaria and Cohen 1987, Shepherd et al. 1994).

In lifetime non-smokers, adenocarcinoma accounts for approximately 70% of lung cancers (Koo and Ho 1990). Occupational exposure to asbestos and other agents accounts for approximately 3-17% of lung cancers (Mossman et al. 1996).

Prevention and screening

Never having smoked or giving up smoking are the only effective means of prevention (Risser NL 1996). While dietary factors may have some protective effect (Wynder 1998), active chemoprevention trials have been completely unsuccessful (Omenn et al. 1996).

Regular screening of populations at risk using sputum cytology and chest radiography has had no significant impact on the survival of lung cancer patients, in spite of some increase in the detection of early stage tumours (Fontana et al. 1991, Tockman 1986, Melamed et al. 1984)

Symptoms

Symptoms are present in almost all patients at diagnosis, but none of them are specific to lung cancer, while many are associated with smoking related co-morbidities. Nearly half of the patients will have a cough, while the incidence of weight loss, dyspnea, chest pain and hemoptysis varies. The only presenting symptom in an invasive tumor of the lung apex (Pancoast tumor) can be chronic shoulder and upper extremity pain. Paraneoplastic symptoms, if present, are often due to lung cancer (Chute et al. 1985, Hyde and Hyde 1974). A summary of the commonest symptoms at presentation in a total of 5140 patients is presented in Table 1 (Auvinen 1980, Chute et al. 1985, Huhti et al 1980, Hyde and Hyde 1974).

Table 1. Commonest presenting symptoms of lung cancer, and their prevalence

Cough	60%
Loss of weight	56%
Dyspnea	46%
Chest pain	34%
Hemoptysis	27%

Pretreatment evaluation

The following recommendations were outlined by consensus reports from the International Association for the Study of Lung Cancer, and the Lung Cancer Study Group (Eagan 1994, Goldstraw et al. 1994, Holmes 1994, Thomas 1994): all staging protocols should be TNM based; all patients should have their clinical history taken; and should undergo a clinical examination, chest radiography, and blood tests to include a blood count, transaminases and alkaline phosphatase levels, and possibly lactate dehydrogenase levels. Bronchoscopy is a routine examination, and is especially indicated for patients with a central tumor or if a central extension is suspected. Computed tomography of the chest and upper abdomen to renal level, with intravenous contrast enchancement of the mediastinal vessels, should be carried out for all patients. An isotope bone scan, brain CT, abdominal ultrasound or whole abdomen CT should be performed only if clinical symptoms of metastatic spread are present (Goldstraw et al. 1994, Hatter et al. 1994). Direct mediastinal evaluation is recommended if CT shows nodes larger than 10mm in diameter, or for central tumors. Thoracoscopy is recommended if pleural effusion is present, even if the cytology is negative but a clinical suspicion remains (Goldstraw et al.1994).

If curative treatment appears possible, spirometry should be carried out and supplemented with further investigations if the findings are more than slightly abnormal (Reilly 1997)

Staging

The staging classification presently recommended by the Union International Contre le Cancer (UICC) and the American Joint Committee on Cancer (AJC) is based on the TNM classification (Sobin and Wittekind 1997), and has been updated recently as the Revised International System for Staging Lung Cancer (Mountain 1997). The aforementioned TNM and staging categories are presented in Table 2.

Table 2. A summary of the TNM and stage grouping according to the UICC 1997 classification.

TX	Positive cytology
T1	Diameter of 3 cm or smaller
T2	Diameter of over 3 cm, tumor in main bronchus but 2 cm or more from carina, invades visceral pleura, partial atelectasis
T3	Invades chest wall, diaphragm, pericardium, mediastinal pleura, tumor in main bronchus to less than 2 cm from carina, total atelectasis
T4	Invades mediastinum, heart, great vessels, carina, trachea, esophagus, vertebra; separate nodules in same lobe, malignant effusion
N1	Ipsilateral peribronchial, ipsilateral hilar
N2	Ipsilateral mediastinal, subcarinal
N3	Contralateral mediastinal or hilar, scalene or supraclavicular
M1	Distant metastases, includes separate nodule in different lobe

Occult carcinoma	TX	N0	M0
Stage 0	T in situ	N0	M0
Stage IA	T1	N0	M0
Stage IB	T2	N0	M0
Stage IIA	T1	N1	M0
Stage IIB	T2	N1	M0
	T3	N0	M0
Stage IIIA	T1-T2	N2	M0
	T3	N1-N2	M0
Stage IIIB	Any T	N3	M0
	T4	Any N	M0
Stage IV	Any T	Any N	M1

The perioperative staging should include a systematic mediastinal lymph node dissection, with node labeling according to an approved lymph node map (Naruke et al. 1978 , Mountain and Dressler 1997), for the staging to be accurate in resectable disease (Goldstraw et al. 1994, Shields 1993, Thomas 1994, Holmes 1994, Mountain 1994). Briefly, stages IA to IIB represent resectable disease where the tumor can usually be removed, and no mediastinal metastases are present. The specific categories are determined by the extent of the tumor, and the presence or absence of pulmonary or hilar lymph node metastases. Stage IIIA mainly represents disease that is usually non-resectable because of mediastinal lymph node involvement. Stage IIIB represents disease which is usually non-resectable because the tumor has invaded vital structures, or because of pleural spread. Stage IV represents disease with systemic metastases. Tumors with a satellite lesion in the same lobe, or tumors which are potentially resectable but locally invasive with pulmonary or hilar node metastases have been included in stage IIIB. Those patients with lesions in more than one lobe are included in stage IV. Even if these patients can be technically resected, their prognosis tends to be statistically closer to the general prognosis for patients in the categories to which they have been assigned.

Surgical treatment

The best chance for a cure is presently offered by complete surgical resection, while an incomplete resection will not improve survival at all (Shields 1993). A predicted post-resection FEV1 of more than 0.8 l is usually a pre-requisite for resection in the average-sized patient (Reilly 1997). In relation to the extent of disease, patients with a completely resectable primary tumor who do not have preoperatively verifiable mediastinal metastases are candidates for surgery (Ginsberg 1997, Shields 1993). Lobectomy is the most commonly performed pulmonary resection. When intralobar lymph node metastases are present in patients with upper lobe tumors, especially on the right side, lobectomy is usually as effective as pneumonectomy. In cases with nodal metastases in other lobes, a bilobectomy or pneumonectomy is recommended (Shields 1993). When the tumor extends towards or involves the lobar orifice and precludes an adequate clearance, ideally 2cm from macroscopic tumor, a sleeve resection of the main bronchus may be used to circumvent pneumonectomy (Shields 1993). A standard pneumonectomy is necessary when a lobectomy or its sleeve modification cannot remove the local tumor and its lymph node metastases (Shields 1993). A segmentectomy can be recommended for elderly patients or for those with limited respiratory reserve in order to preserve more functional lung

tissue (Shields 1993). The recurrence rate after segmentectomy was greater than after lobectomy, but the 3-year survival rates were essentially the same (Lung Cancer Study Group 1995).

In cases of mediastinal metastases or a borderline resectable tumor, preoperative chemotherapy has shown promising preliminary results, after which the patients can be re-evaluated for surgery or radical radiotherapy (Evans et al. 1997, Vansteenkiste et al.1998).

Conservative treatment

Radiotherapy, combined with chemotherapy when feasible, should be considered for the patients who are definitely not suitable for a curative resection (Bunn and Soriano 1998, Evans et al. 1997). With the best available combinations of chemotherapy and radiotherapy, the five-year survival in locally advanced non-resectable disease has increased from 5% to as much as 20%. For patients with systemic metastases, chemotherapy has improved the quality of life, and produced a small improvement in survival (Bunn and Soriano 1998).

Palliative treatment

Palliative radiotherapy and/or chemotherapy, endoscopic resection with diathermy or laser, application of intrabronchial stents, brachytherapy, chemical pleurodesis, and pleuroperitoneal shunts are presently available to relieve symptoms in inoperable disease (Petrou and Goldstraw 1993, Monnier et al. 1996). Palliative surgery is hardly ever justified, as it offers no advantage of survival but exposes the patient to all the morbidities associated with thoracotomy (Shields 1993).

Prognosis

Survival probabilities for each stage category have been published together with an introduction to the present revised staging system devised by C.F. Mountain. The calculations are based on a study of over 5000 patients treated in registered hospitals (Mountain 1997). The survival of clinically staged patients is worse than that of surgical-pathological staged patients, because clinical staging tends to underestimate the extent of the disease.

Briefly, the 5 year survial of patients with resected disease without lymph node metastases (Stage IA-IB) is 57-67%. In similar cases with intrapulmonary or hilar lymph node metastases, or more extensive tumor without nodal metastases (stage IIA-IIB), the 5-year survival is 39-55%. If mediastinal lymph

node metastases are present, even though they may be completely resectable, or if only pulmonary nodes are present but the tumor is locally invasive, even if resectable (Stage IIIA), the 5-year survival is 13%. In cases of mainly inoperable disease due to extensive tumor invasion or systemic metastases (Stage IIIB-IV), the 5-year survival is 5% and 1%, respectively. If no anticancer treatment is given whatsoever, the only determinant for the length of the survival is the presence or absence of lymph node metastases, and no patient will survive for five years (Vrdoljak et al. 1994).

BRONCHOPLASTIC PROCEDURES

The bronchoplastic procedure

A bronchoplastic procedure is a lung tissue saving operation in which a portion of the main bronchus is resected in continuity with the involved lobe to preserve distal parenchyma, with subsequent end-to-end anastomosis. The first brochoplastic resection, to remove a carcinoid tumor from the right main bronchus, was performed in 1947 by Sir Clement Price Thomas. In 1952, Allison used a sleeve resection of the right main bronchus to remove a bronchial carcinoma from the right upper lobe orifice (Firmin et al. 1982). For tumors that involve the trachea, carina or proximal main bronchus, the same principles can be applied (Shields 1993). Bronchoplastic surgical techniques are the ideal form of excisional therapy for benign and low-grade malignant endobronchial tumors in the proximal airways. The same techniques can also be applied to lung cancers (Lowe et al.1982, Lowe and Sabiston 1992). It is estimated that the procedure is suitable for 5-8% of lung cancer patients with resectable tumors (Lowe et al. 1982, Tedder et al. 1992). It has been most often used in elderly patients, in those with a limited respiratory reserve, as a palliative procedure, and in upper lobe tumors which extend to the main bronchus (Vogt-Moykopf et al. 1982).

Bronchoplasty in elderly patients

Resections beyond lobectomy have not been encouraged in elderly patients (Pagni et al. 1997, Morandi et al. 1997), even though succesful pneumonectomies have been reported (Mizushima et al. 1997). An overall pneumonectomy-related morbidity of 72.7% has been reported in patients over 70 years of age, compared to 30% in patients of less than 70 years of age (Morandi et al. 1997). The operative mortality of patients over 70 years of age has been 13-19%, with pneumonia and ischemic heart disease

as the main causes of death (Borelly et al. 1992, Shields 1993). In a contemporary review, the mortality after a sleeve lobectomy was 5.5% (Tedder et al.1992), and patients of over 70 years of age were included in the series. General condition and co-morbid disease must be taken into account when planning any resection in an elderly patient.

Bronchoplasty in patients with impaired respiratory reserve

Patients with a predicted postoperative (PPO) FEV1 (PPO FEV1) of less than 0.8L are considered to be at a high risk of postoperative complications (Reilly 1997), and any resection resulting in a PPO FEV1 of less than 30% of baseline is generally contraindicated (Shields 1993). The risk can be further analysed in this group by assessing exercise capacity. A diminished respiratory reserve is often due to chronic obstructive lung disease caused by cigarette smoking. An intensive rehabilitation and cessation of smoking, for a month prior to surgery, is recommended for high risk patients, and may improve lung function (Reilly 1997).

Bronchoplasty as a palliative procedure

The five-year survival rate after an incomplete resection for lung cancer is practically zero (Shields 1993). The cause of death is usually a systemic, as well as local, relapse (Kumar et al. 1996). Median survival with the best available therapies is 6-10 months, with most people dying within two years of diagnosis (ASCO 1997). Bronchoplastic procedures, as well as other surgical procedures, are not generally justified in these patients. Other palliative therapies for airway obstruction, infection, hemoptysis and pain can provide relief in practically all cases. These include endoscopic laser or diathermy resection with intraluminal stent placement (Petrou and Goldstraw 1993, Monnier et al. 1996), and brachytherapy for the control of obstruction and bleeding (Cortese and Edell 1993).

Bronchoplasty for the upper lobe orifice tumor

An upper lobe orifice squamous cell carcinoma provides the optimum situation for a bronchoplastic procedure. Bronchoplasty is recommended for this condition even if respiratory function is normal (Deslauriers et al. 1986 , Firmin et al. 1983, Tedder et al. 1992). The upper lobe lymphatic drainage is fairly constant to the ipsilateral hilar and paratracheal locations especially on the right side (Deslauriers

et al. 1986), and in cases of squamous cell carcinoma (Belli et al. 1986). Systematic lymph node dissection does not prevent anastomotic healing (Rendina et al. 1993). The aortico-pulmonary nodes can contain metastases of left upper lobe tumors, and should be dissected even if the hilar nodes are normal (Libshitz et al. 1986). Five-year survival does not appear to depend on which side an upper lobe tumor is situated (Deslauriers et al. 1986). The lymphatic drainage from the other lobes is more variable (Naruke 1993) and the justification for a lower lobectomy with bronchoplasty has been questioned (Firmin et al. 1983). Successful results have, however, been reported for median and lower lobe tumors (Maggi et al. 1993, VogtMoykopf et al. 1987).

Surgical technique

Familiarity with exposing the main stem bronchus and carina is essential for a succesful procedure. An intubation tube that permits one lung ventilation, and a standard posterolateral or muscle saving incision is used (Deslauriers et al. 1986). Systematic sampling of the lymph nodes, or dissection of the ipsilateral mediastinal, subcarinal and hilar, as well as the interlobar, nodes is recommended (Goldstraw et al. 1994; Lowe et al. 1982, Lung Cancer Study Group 1988, Naruke 1993, Shields 1993). If the dissection is carried out first, and resectable hilar or interlobar nodal metastases (N1) are found , the procedure can still be completed (Belli et al. 1985, Deslauriers et al. 1986, Maggi et al. 1993). The same is true if totally resectable minimal intracapsular metastatic disease is found in the mediastinal nodes (Maggi et al. 1993). Extensive nodal or bronchial wall involvement, or extension of the tumor across fissure lines, should be considered a contra-indication (Lowe et al. 1982). The macroscopic free bronchial margin to the tumor must be at least 1cm, preferably 1.5-2cm, and it must be confirmed from frozen section of both the proximal and distal edges (Belli et al. 1985, Tedder et al. 1992). It is essential for the anastomotic healing, that pale bronchial edges are resected until healthy-looking bleeding tissue is encountered (Vogt-Moykopf et al. 1987). A sleeve of the main bronchus should be resected, with subsequent end-to-end anastomosis. Any difference in caliber can be overcome by a correct placement of the interrupted sutures (Firmin et al. 1983, Rendina et al. 1993). A wedge-like partial excision of the lobar bronchus offers no advantage, but predisposes to kinking and should probably be avoided (Rendina et al. 1993). Absorbable, preferably monofilament, suturing is recommended for the bronchial anastomosis (Maggi et al. 1993, Rendina et al. 1993, Vogt-Moykopf et al. 1987), but monofilament non-absorbable suturing has also been used succesfully (Firmin et al. 1983). Braided non-absorbable sutures have a tendency to form granuloma, and should not be used (Maggi

et al. 1993, Rendina et al. 1993, Vogt-Moykopf et al. 1987). A complete wrapping of the anastomosis with a pleural (Vogt-Moykopf et al. 1987), pericardial (Maggi et al. 1993) or intercostal muscle flap (Rendina et al. 1993) is recommended (Maggi et al. 1993) as well as the use of fibrin glue (Tissucol; Immuno, Vienna, Austria) (Maggi et al. 1993), but has also all been successfully omitted (Deslauriers et al. 1986). A pleural flap does not add to the perfusion of the bronchial anastomosis (Tedder et al. 1992), so vitality of the edges is still essential. If the pulmonary artery is involved with the tumor or with lymph node metatastases, it can be angioplastically resected using a low dose systemic heparinisation. It can be either primarily sutured end to end using standard vascular technique, or augmented after resection by using a venous (Maggi et al. 1993) or pericardial patch (Rendina et al. 1993). The risk of complications will increase if a combined angioplastic procedure is performed, and only small series have been published, but the procedure is recommended by several authors for patients who cannot tolerate pneumonectomy but are otherwise deemed suitable for the procedure. Interposition of a pleural or pericardial flap to prevent bronchovascular fistula formation has been recommended by the same authors (Maggi et al. 1993, Rendina et al. 1993,Vogt-Moykopf et al. 1987).

Postoperative care

Postoperative care is the same as for a standard resection, with special attention to physiotherapy, breathing excersises and clearing of secretions by coughing (Lowe et al. 1982). The use of routine bronchoscopic suctioning may stimulate the production of secretions and is not recommended (Tedder et al. 1992 ,Vogt-Moykopf et al. 1987). However, it should be carried out immediately if there is any sign of retention (Tedder et al. 1992). Bronchoscopic follow up at 6 and 12 month intervals is recommended, and anastomotic strictures and granulomas have been succesfully treated using endobronchial Nd-YAG laser resection, and covered stent placement if necessary (Rendina et al. 1993). A removable silastic stent has also been used, until the scar has matured, after which a non -covered Wallstent (Schneider AG, Bulach, Switzerland) can be succesfully applied (Petrou and Goldtraw 1994).

Surgical complications

The 30-day mortality of 0% (Belli et al. 1985, Deslauriers et al. 1986) to 3.4% (Baros and Djuric 1990) after sleeve lobectomy is less than the 5.7% for pneumonectomy (Damhuis and Schutte 1996), and is comparable the 2.9% mortality rate for standard lobectomy (Ginsberg et al. 1983). Complications of

sleeve lobectomy are pneumonia (6.7%), atelectasis (5.4%), benign stricture or stenosis (5.0%), bronchopleural fistula (3.5%), and empyema (2.8%) (Tedder et al. 1992). Local recurrence has been documented in 10.3%, and may require a completion pneumonectomy, assuming that extensive nodal or metastatic disease is absent and that the patient can tolerate it (Tedder et al 1992). Palliative endoscopic procedures are otherwise to be considered (Monnier et al.1996, Petrou and Goldstraw 1994).

Preservation of lung function, and long term survival

Preservation of homogenous respiratory function in the conserved part of the lung, without gas trapping, has been documented using spirometry and ventilation-perfusion studies; the reimplanted lobe/lobes contribute significantly to the remaining lung function (Deslauriers et al. 1986, Roder et al. 1986). This is never the case after pneumonectomy.

The postoperative survival rates after bronchoplastic resections are comparable to those after pneumonectomy, with five year survival rates for stage I, II and III carcinoma of 63%, 37% and 21%, respectively (Tedder et al. 1992). The presence of lymph node metastases will adversely affect the prognosis (Belli et al. 1985). Five-year survival rates of 50%-71% for N0 patients (Belli et al. 1985 , Deslauriers et al. 1986, Firmin et al. 1983), versus 60% -17% for those with N1 involvement (Deslauriers et al. 1986, Firmin et al. 1983), and 9.7% for those with N1 and/or N2 disease (Belli et al. 1985) have been reported. In completely resectable N2 disease, a five year survival of 23.4% has been reported (Maggi et al. 1993).

Sleeve pneumonectomy

A sleeve pneumonectomy should probably be limited to patients with large tumors limited to the carina or tracheobronchial angle, with adequate cardiopulmonary reserve, and who are otherwise in good health (Tedder et al. 1992). A high frequency jet ventilator can be used for ventilating the other lung. The effect of cardiopulmonary bypass on long term cancer- related survival, if used to improve exposure, is not definitely known (Shields 1993). If the carina or proximal main bronchus on the right side is invaded, a sleeve pneumonectomy can be performed using a posterolateral right thoracotomy. The left lung can be removed via a left sided thoracotomy, and the carinal or tracheal resection and reconstruction is then carried out from a right-sided thoracotomy (Shields 1993). Due to the high morbidity, and a 30 -day mortality of over 20% (Shields 1993, Tedder 1992), this procedure should not

be carried out if upper mediastinal metastases are present, as the prognosis is non-existent (Mountain 1990, Shields 1993). A three-year survival of 34% in patients with carinal extension of the tumor, and with isolated subcarinal metastases, has been reported (Dartevelle et al. 1989).

ELDERLY PATIENTS

Justification for lung cancer surgery in the elderly patients

The justification for lung cancer surgery in the elderly depends on four factors (Pagni et al. 1997). Firstly, the life expectancy of the patients must exceed their projected survival if the neoplasm is not treated, or if it is treated non-operatively. Secondly, the actual long-term survival of the resected patients must confirm the theoretical advantage of the operative approach. It is suggested that, in a cohort of elderly patients, death from all causes is considered, instead of only cancer-related death. Thirdly, the operative mortality rate should be sufficiently low that it does not abolish the long term benefit. Fourthly, the quality of life must not be sacrificed for limited added survival.

Life expectancy

The life-expectancy at 75 years in Finland in 1995 was 8.48 years for men, and 10.70 years for women; at 80 years, it was 6.30 and 7.72 years, respectively (Statistics Finland, 1997). The corresponding life expectancy of an 80 year old in Japan, in 1990, was 6.9 years and 8.7 years, respectively (Osaki et al. 1994). Octogenarians currently comprise approximately 3% of the population in the United States (Naunheim et al. 1994), and about 3.5% in Finland (Statistics Finland, 1997). Age is a major determinant for the risk of developing lung cancer. Almost half of all lung cancers occur in individuals older than 65 years of age (Shepherd et al. 1994). In the Nordic countries (Denmark, Finland, Iceland, Norway and Sweden), a 100 fold increase has occurred in the incidence of lung cancer in men and a more than 20 fold increase in women between the ages of 35 and 75 years (Olsen and Hirsch 1996). A survey of the annual cancer death rates in the United States and 73 other countries for which World Health Organisation data exist, showed nearly identical relationships to the respective percentages of elderly people in their populations. The same trend was present in lung cancer death rates. This supports the notion that biological age is intrinsically linked to cancer. A downward trend in the cardiovascular death rate has probably resulted in more elderly people falling prey to cancer

(Tallarida 1995). The increase in cancer mortality, between 1970 and 1990 in England and Wales, was only observed in men over 75 years of age and women over 55 years of age. One of the main causes was the increase in lung cancer mortality (Grulich 1995). In Finland in 1995, 23% of male and 6% of female cancer deaths in people over 75 years of age, were caused by lung cancer (Statistics Finland 1996). These findings are in accord with the observation that men will suffer the greatest cumulative exposure to cigarette smoke in their lifetime by their 7^{th} and 8^{th} decades (De Maria and Cohen 1987). If no anti-cancer therapy whatsoever is given for non-small cell lung cancer, survival depended only on whether lymph node metastases were present on diagnosis: in N0 patients, the mean survival was 17 months; in N1 or N2 patients it was less than 11 months. No-one lived for more than three years (Vrdoljak et al. 1994). Radiotherapy with curative intent has resulted in a 5-year survival of 21% in stage I (T1-2N0M0) disease (Haffty et al. 1988), and of 17% in stage I-II (T1-2N1-2M0) disease in patients more than 75 years old (Katano et al. 1997). A 5-year survival of 5-10% is most common, and median survival times are less than one year (O´Rourke and Crawford 1988). At present, only a complete surgical resection offers the best hope of a cure (Shields 1993). Otherwise, the life expectancy will be significantly shortened, even for patients over 80 years old.

Long term survival

In curatively resected lung cancer patients over 70 years old, the 5-year overall survival can be 32% (Berggren et al. 1984). A 20% survival was reported in an unstaged group of patients of the same age, in which the 30-day mortality was rather high (20%), and 31% underwent pneumonectomy (Borrelly et al. 1991). In stages I, II, and IIIA, the 5-year survivals were 67%, 40% and 32%, respectively (Ishida et al. 1990c). Corresponding overall survivals of 51% and 17% for stages I and II , and a 4-year survival of 8% in stage IIIA have been reported (Morandi et al. 1997). In a series comprising only pneumonectomied patients, the 5-year survival was 30% for stage I and II combined, and 16.4% for IIIA (Mizushima et al. 1997).

In patients over 80 years old, an overall 3-year survival of 40% for those who survived more than 30 days is reported (Naunheim et al. 1994). The median overall survival was 19.1 months for stage I and II combined (Nugent et al. 1997). The 5-year survival was 57% in stage I, but zero for stage II and III combined (Pagni et al. 1997). A 5-year overall survival of 32% in completely resected patients, and a relative survival rate (survival rate of the subjects/survival rate of age- and gender matched population) of 61% has been presented (Osaki et al. 1994). Even though the long term survival after a complete

resection was reduced compared with the expected survival of an unselected matched population, it is high enough to justify surgical treatment in elderly patients, even in those over 80 years of age (Berggren et al. 1984, Osaki et al. 1994). However, if the disease extends beyond the lung parenchyma, or lymph node metastases are present, the prognosis is poor and extended procedures should generally be avoided (Pagni et al. 1997).

Operative morbidity and mortality

The hospital mortality (within six weeks) after lobectomy in patients over 70 years of age is 15.9% (Berggren et al. 1984). The mortality, within 30 days of surgery, is 38% after pneumonectomy and 11% after lobectomies and lesser resection combined (Borrelly et al. 1990). Figures of 5.7% after pneumonectomy, 4.4% after bilobectomy, and 1.4% after lesser resections have been reported (Damhuis and Schutte 1996). When co-morbid disease and other risk factors were controlled in a multivariate analysis, age of more than 70 years did not reach statistical significance in a prospective study, even though the morbidity and mortality was higher than for the younger patients (Duque et al. 1977).

In a study by the Lung Cancer Study Group, where systematic perioperative lymph node staging was routinely performed, the mortality was 5.9% after pneumonectomy and 7.3% after lobectomy. These results reflect the safety of complete staging and a careful selection of patients for pneumonectomy (Ginsberg et al. 1983). In a series that included pneumonectomies (all of whom survived), the overall incidence of complicatons was 21%, with 6% major complications and 3% operative mortality (Ishida et al. 1990c). In another study, the mortality of patients more than 70 years old undergoing pneumonectomy was 22.2%; significantly higher than the 3.2% in younger patients. The same difference applied to complication rates: 33.3% versus 8.4% (Mizushima et al. 1997). There was, however, no significant difference in overall morbidity: 43.8% versus 43.8%. However, major and minor cardiovascular complications were significantly more frequent (29.4% vs. 13.8%) in the older patients, even though it was the minor ones, such as supraventricular arrhytmias, which mostly determined the difference (Morandi et al. 1997).

In patients over 80 years old, the operative mortality (in hospital or within 30 days) was 16% for resected patients. Complications occurred in 45% of these patients, and were major in 30% (Naunheim et al. 1994). The reported figures depend on the definition of operative mortality, whether it is within 30 days of surgery or within the same stay in hospital. Osaki reported a 30 day mortality of 3% in

octogenarians but this rose to 18% if deaths during the same stay in hospital were included. Overall, complications occurred in 67% of the patients (Osaki et al. 1994). If pneumonectomies were generally avoided, the operative mortality was 3.7%, with 42% suffering complications (11% major and 31% minor) (Pagni et al. 1997). A limited resection, defined as a less than lobectomy, carries a 75% increased risk of recurrence in T1NOMO disease, and should not be used routinely. There was no significant difference in the 5-year survival, however (Lung Cancer Study Group 1995). If the tumor has crossed an intersegmental plane, the risk of local recurrence is especially high, approximately 20% (Miller 1993). If the tumor is a T1 lesion, the location is peripheral, and the margins are easily encompassed by resection, a limited resection offers a good alternative (Miller 1993), especially if co-morbid disease is present and the patient is over 80 years old. McKenna reported on nine 80 year old patients who underwent thoracoscopic lobectomy (8 patients) and penumonectomy (1 patient), with lymph node sampling. In this series the mortality rate was zero (McKenna 1994). Since extremely high risk patients can be resected using rapid conventional open techniques, in conjunction with the postoperative epidural anesthesia, the advantage offered by video assisted techniques has been questioned (Miller 1993). What level of surgical mortality is acceptable in a disease that has a 100% mortality rate? (Berggren et al. 1984). The risks associated with lobectomy are acceptable, even in octogenarians; whereas pneumonectomy should be reserved for the most exceptional of cases (Yellin 1994, Naunheim et al. 1994, Pagni et al. 1997), although sleeve lobectomies as an alternative have been used as a succesfully alternative in some cases (Pagni et al. 1997).

Quality of life

In a study of all Finnish centenarians, 36% were completely independent. Of the rest, 19% were in hospital, and 18% depended completely on other people´s assistance. Only 20% of the men were clearly demented, in comparison to 50% of the women (Louhija 1994). This indicates the importance of quality of life, even at an extreme age. Since 50% of octogenarians would be expected to die within 5 years, even if they did not have lung cancer, quality of life is as important as survival (Yellin 1994). Patients who are in nursing homes, are non-ambulatory, or are mentally impaired are not candidates for surgery (Naunheim et al. 1994). In octogenarians, surgery should probably be limited to patients who are capable of caring for themselves, and carrying out normal everyday activities independently. In these patients, there is every expectation that a major lung resection can be tolerated, and long term survival enjoyed with an excellent funtional status (Naunheim et al. 1994).

Octogenarians often had a poor perfomance status after discharge from the hospital if major cardio-respiratory complications arose after surgery. This was probably due to a reduction in the patients major organ reserve, including mental function (Osaki et al. 1994). Of the octogenarians who survived the operation, 94% were discharged directly home after the operation, and the mean hospital stay was 8,1+/- 3,5 days. No chronic morbidity associated with the procedure was observed in these patients (Pagni et al. 1997).

The recommendations of Yellin (Yellin 1994), for ensuring a good quality of life for elderly lung cancer patients following surgical treatment are definitely worth citing: " These patients should be managed carefully and expertly… with complete staging, careful and conservative operations, and a well-tempered optimism".

MOLECULAR STAGING OF LUNG CANCER

Biological prognostic factors in lung cancer

During malignant transformation, cancer cells acquire genetic mutations which override the normal mechanisms controlling cellular proliferation (Hahn et al. 1999). The essential steps are a capability for unlimited division (immortality) due to ectopic telomerase activity, stimulation of growth by oncogenes, and loss of the growth-regulating function of the p53 gene in the mutated cells. Tumorigenic conversion of normal epithelial and fibroblastic human cells by providing the afore-mentioned conditions has recently been demonstrated (Hahn et al. 1999).

Growth-stimulating oncogenes associated with lung cancer are the *ras, myc, neu, myb, raf* and *bcl*-1 genes, epidermal growth factor and platelet-derived growth factor. Mutations in growth-regulating anti-oncogenes such as the rb (retinoblastoma gene), p53, and in chromosome 3 have been found in familial cancer disorders such as the Li-Fraumeni syndrome and von Hippel-Lindau disease, and also in lung cancer (Gazdar 1992). Oncoproteins, proliferation activity, neovascularisation, blood-group antigens and neuroendocrine differentiation have also been studied (Apolinario et al. 1997, Casson et al. 1994, Costa et al. 1996, Fontanini et al. 1997, Rainio et al. 1996, Smit et al. 1996). Several of the aforementioned factors have been associated with impaired prognosis, but none has emerged as a single consistent and independent indicator of poor prognosis (Pastorino et al 1997, Scagliotti et al. 1995).

The p53 gene is of special interest because it functions in many pathways associated with growth

regulation and DNA repair, and because of its capability to arrest the division of cells, even those with multiple genetic defects (Hussain and Harris 1998).

The staging system currently used (Mountain 1997) is based on the anatomical extension of the primary tumor and the presence of lymph node metastases, and of distant metastases. It groups the various tumor, node, and metastasis (TNM) categories (Sobin and Wittekind 1997) into stages, which predict survival and offer guidelines for treatment. However, even in the optimal post-surgical stage IA (T1N0M0), 33% of patients will relapse and die of cancer within five years of surgery (Mountain 1997). In stage IA and IB disease (T1-2N0M0), recurrence has been regional in 7%, and systemic in 20% (Martini et al. 1995). One possible explanation here is that microscopic metastases are present but are missed in the preoperative evaluation and the routine histopathological examination of the surgically removed lymph nodes.

Since new preoperative (neoadjuvant) chemotherapeutic protocols are showing promising preliminary results in lung cancer (Vansteenkiste et al. 1998), methods are needed to select the individuals with a poor prognosis in spite of curative resection (Mountain 1995). Neoadjuvant therapy should not be given to all, since the known side-effects would result in poor compliance by patients who have undergone a potentially curative resection (Niiranen et al 1992). The expense of neoajuvant therapy would also limit administration, since lung cancer is one of the commonest malignancies in the world.

The p53 gene

The human p53 gene is located in 20kb of chromosome band 17p13.1 (Pich 1998). It is composed of 11 exons, the first of which is non-coding. The p53 gene product is a 53kD nuclear phosphoprotein , which acts as a transcriptional factor able to activate several other genes (Pich 1998, Wang and Harris 1997).

The p53 product protein was discovered by several groups in 1979. It was associated with the large T antigen in SV40, the simian tumor-producing virus found as a contaminant of rhesus monkey kidney cells being used to cultivate polio viruses for vaccines. The p53 protein was first thought to be a tumor antigen in view of its nuclear accumulation in tumor cells (Lane and Crawford 1979). In the 1980s, p53 was thought to be an oncogene. These are usually normal cellular genes (proto-oncogenes) which, when activated, result in enchanced cellular growth (Gazdar 1992). This conception was formed because the first p53 DNA to be isolated, which was cloned from tumor cells, was able to induce neoplastic transformation in rodent cells when cotransfected with other oncogenes such as *ras* (Wang

and Harris 1997, El Deiry 1998). A mutated p53 gene can regulate the tumor suppression function in a negative fashion because the mutant p53 protein can bind and inactivate the wild-type protein, (Vogelstein and Kinzler 1992). In the late 1980s, however, it was discovered that the p53 gene isolated earlier was mutated; the wild type isolated from normal cells did not induce neoplasms but inhibited tumor growth. These findings reclassified p53 as a tumor-supressor gene (Wang and Harris 1997). In 1990 it was discovered that p53 regulates a cell-cycle checkpoint in response to DNA damage, and in early 1990s that p53 determines the chemo- and radiosensitivity of tumors (El-Deiry 1998).

Mechanisms of p53 action

1. Cell cycle arrest and apoptosis

The growth of human cells, including those bearing multiple genetic lesions, can be suppressed by wild-type but not mutant p53 (Takahashi et al. 1992). Following exposure to DNA-damaging agents, wild-type p53-containing cells are arrested primarily in the G1 phase (Smit et al. 1996, El-Deiry 1998). This phase is the period before DNA synthesis and mitosis, during which repair of DNA damage can proceed, thus reducing the probability of mutations (Harris and Holstein 1993). One of the mechanisms involved p53 transcriptionally induces the production of p21WAF1, a universal cell cycle inhibitor which induces the arrest of cells in G1 (El Deiry 1997, Pich 1998).

If the DNA damage is irreparable during the G1 phase arrest, p53 can trigger apoptosis. Apoptosis can be defined as a physiological form of programmed cell death whereby a cell is triggered to die by external stimuli, but provokes its own demise through the implementation of an endogenous suicide mechanism (Manning and Patierno 1996). The precise mechanisms whereby p53 induces apoptosis are as yet unclear (Wang and Harris 1997). Loss of wild-type p53 function reduces cellular apoptosis in response to environmental and cancer-therapeutic agents which cause DNA damage (Wang and Harris 1997). P53 can also trigger apoptosis by upregulating effector signals such as BAX and Fas/APO1, and downregulating inhibitor signals such as bcl-2 (El Deiry 1998, Wang and Harris 1997). Other mechanisms involved in the induction of apoptosis have also been detected, for example signaling with caspases (El Deiry 1998) or the poisoning of cellular helicases (Wang and Harris (1997),.

2. Modulation of DNA repair

It has been suggested that transcription is repressed when p53 protein binds to transcription factors, and that the binding of p53 protein to replication protein A (among others) controls the nucleotide excision and repair (NER) pathway. Accumulation of high levels of p53 protein in DNA damaged cells can also act as a sensor of damaged regions and may possibly trap a major component of the NER machinery (transcription factor TFIIH) at sites where it is needed (Wang and Harris 1997). Inactivation of the p53 gene or its protein can lead to an increase in mutation frequency as a result of inefficient NER and genomic instability, manifested as the gene amplification, aneuploidy and chromosomal aberrations which are associated with malignant progression (Wang and Harris 1997).

3. Other regulatory functions of p53

In the absence of oncogene stimulation, the protein mdm2 interacts with the p53 protein, inhibits its ability to activate gene expression, and also targets it for degradation. Positive growth regulatory signals from oncogenes can trigger the cellular protein p14ARF, which binds with mdm2 and stops it binding with p53. The activation of p53 then causes cell senescence or triggers apoptosis. This ”oncogene checkpoint” allows cells to prevent the deregulated growth which would otherwise result from oncogene activation (El-Deiry 1998).

The wild type p53 protein may also regulate neoangiogenesis through the downregulation of vascular endothelial growth factor (Fontanini et al. 1997).

Detection of p53 alterations

Incidence and types of p53 mutation

P53 gene mutations can be detected in tumor samples obtained by non-surgical methods such as fiberoptic bronchoscopy, thoracocentesis and percutaneous needle aspiration (Murakami et al. 1996). Mutated p53 is present in about 80% of small cell and 50% of non-small cell lung cancers (Gazdar et al. 1994) Mutations in exons 5 - 8 comprise 98% of the p53 mutations in human tumours (Mitsudomi et al. 1993). Missense mutations make up 82% of these, and result in the production of abnormal p53 protein (Wang and Harris 1997). A frame-shift mutation inserts or deletes a single base so that the reading frame is changed for the entire subsequent sequence. In a nonsense mutation there is a base change which results in a stop codon and subsequent premature termination of protein synthesis. In

frame-shift or nonsense mutations, the levels of p53 protein are usually undetectable, because the protein is absent, truncated or unstable. These categories constitute less than 20% of the p53 mutations in human tumors (Hussain and Harris 1998). In squamous cell carcinoma, p53 mutations are more common (59%-61%) than in adenocarcinoma (33-38%) (Fukuyama et al. 1997, Husgafvel-Pursiainen et al. 1999, Ridanpää et al. 1994). Mutations that result in the production of a truncated or unchanged protein may be more common in adenocarcinoma than in squamous cell carcinoma (Ridanpää et al. 1994, Wild et al. 1995).

In this latter cancer, mutations in p53 are related to the number of pack years smoked, whereas in adenocarcinoma only k-*ras* mutations are associated with cigarette consumption (Ridanpää et al. 1994), and the presence of p53 and k-*ras* mutations appears to be inversely correlated (Husgafvel-Pursiainen et al. 1999). Both mutations have been detected in lung tumors from smokers who had not smoked for decades, and metastases harbored indentical mutations. These findings suggest that k-*ras* and p53 mutations may be early events in the development of lung cancer (Husgafvel-Pursiainen et al. 1995, Pich 1998). A possible precursor lesion of adenocarcinoma has not been identified because of adenocarcinoma's peripheral origin, in contrast to centrally arising cancers such as squamous and small cell carcinoma (Gazdar 1994). However, based on a topographical analysis of the distribution of p53 mutations in tumor tissue, these mutations are homogenously distributed in lung adenocarcinomas and its metastases. This suggests that these mutations occur during the earliest phases of tumor formation, and in small precursor lesions (Li et al. 1994). P53 or k-*ras* mutations were found more than a year prior to clinical diagnosis in sputum samples from patients who developed lung adenocarcinoma (Mao et al. 1994). This is in line with the afore-mentioned finding.

Detection of the p53 protein

The wild-type p53 protein has a half life of 20 to 30 minutes, compared to several hours for the mutant or otherwise stabilised protein. Only the latter can therefore be detected by immunohistochemistry (Carbone et al. 1994, Fontanini et al. 1993). The commonest antibodies currently used are CM1, Pab1801, Pab421, Pab240, DO1 and DO7. They recognise both wild-type and mutant p53 protein (Pich 1998). The advantages of immunohistochemical analysis are that it can be performed during routine diagnosis and can identify p53 protein which has been stabilised and inactivated, e.g by binding with oncoproteins such as mdm2, even without the presence of a p53 mutation (Soussi et al. 1994). Immunohistochemistry cannot identify mutations which do not induce p53 protein over-expression,

but these are present in less than 20% of cases (Soussi et al. 1994). On the other hand, most of the available antibodies react with both mutant and wild type p53 protein, and the latter can be stabilized but functional under certain circumstances (El Deiry 1998).

Detection of p53 gene mutations

The whole p53 gene can be sequenced, to give detailed information about each mutational event, but the technique is laborious and time-consuming. Direct sequencing can identify p53 mutations only when wild-type alleles do not exceed two thirds of the material analysed. False-negative results may also be obtained (Pich 1998).

The most common molecular analysis methods used to screen for unidentified sequence alterations are polymerase chain reaction-denaturing gradient gel electrophoresis (PCR-DGGE) and polymerase chain reaction-single strand conformation polymorphism (PCR-SSCP). These methods amplify the desired exon fragments using a polymerisation chain reaction (PCR) and observe the melting pattern using denaturing gradient gel electrophoresis (DGGE)(Ridanpää et al. 1994), or by radioactively labeling the amplified fragments and observing the electrophoretic shift of the separated DNA strands using the single-strand conformation polymorphism (SSCP) technique (Marchetti et al. 1993). The latter techniques are rapid, and automated sequencing systems are available. The accuracy of molecular analysis is, in general, between 90% and 100%, because firstly the whole gene is not sequenced, secondly some mutations may be present outside the exon 4 - 8 area usually examined, or thirdly mutations may only be present in a small proportion of cells within a tumor sample (Soussi et al. 1994, Marchetti et al. 1993).

Correlation between p53 mutations and detectable p53 protein

The concordance between p53 detection and the presence of a mutation has been variable. Rates of 73% (Isobe et al. 1994), 67% (Carbone et al. 1994), 59.5% (Mitsudomi et al. 1995) and 54% (Marchetti et al. 1993) have been reported. One group (Nishio et al. 1996) obtained 90% concordance, and attributed this to microwave pre-treatment for antigen retrieval. Microwaving and using the same cut-off value for a positive result (10% of positively staining cells) resulted in only 54% concordance in another study (Mitsudomi et al. 1995), therefore other factors must be involved. Alterations in p53 were discovered in 69% of NSCLC cases by combining both methods (immunohistochemistry and mutation analysis), compared to 50-52% when either method was used alone, and the probability of a

false-negative result by either individual method was estimated to be 20% (Top et al. 1995). In squamous cell carcinoma, the combined method resulted in the detection of p53 alterations in 86% of cases, and it was impossible to distinguish a subgroup of any useful size , while in adenocarcinoma the frequency was 56% (Top et al. 1995). In a review of 84 studies, the sensitivity of immunohistochemistry in detecting mutations was 75%, but the positive predictive value was only 63%, with considerable variation depending on the type of the tumor (Pich 1998). Immunoreactivity can still be considered an approximate indicator of tumors with altered p53 function (Harris and Holstein 1993).

Explanations for the poor concordance

The possibility that the mutation is of a type which results in the production of an undetectable protein has already been discussed. Conversely, it is possible that immunostaining detects p53 protein, but no mutation is found. The mutation can sometimes lie outside the exon 5-8 area usually examined (Top et al. 1995), but this is happens in less than 5% of cases (Wild et al. 1995). The wild-type protein can be sequestered and stabilised, and cause staining in the cytoplasm, by mechanisms which interfere with its transport from the site of synthesis to the nucleus. This has been demonstrated in some cases of breast cancer and small cell lung cancer, as well as in NSCLC (Top et al. 1995). Wild-type p53 can also be stabilised and inactivated by binding to over-expressed oncoproteins such as mdm2, or to viral oncoproteins such as those of the human papilloma, Epstein-Barr or hepatitis B viruses (Pich 1998)
A wild-type functional p53 protein can be over-expressed , and its half life increased by several mechanisms involved in oncogene expression, such as hypoxia, hypoglycemia or exposure to gamma rays or other agents that cause double-strand breaks. The mechanisms associated with stabilisation due to UV exposure and nucleotide depletion are not known (El-Deiry 1998). Most available antibodies recognise both wild-type and mutated p53 proteins, while only some are specific for the latter. Neither may p53 immunoreactivity be restricted to neoplastic or dysplastic cells (Pich 1998).

The prognostic significance of p53 alterations

Over-expression of p53 protein, and prognosis in lung cancer

No association with prognosis was found in one study of radically resected NSCLC patients, when an immunohistochemical assessment of p53 protein, *bcl*-2, bax and neovascularisation was undertaken.

The antibodies Pab1801 and DO7 were used, but positive immunoreactivity only to the first suggested shorter survival. Both antibodies detect wild-type and mutated p53, but not the same epitopes. The type of antibody used was thought to affect the results (Apolinario et al. 1997) . In other studies, immunopositivity to Pab1801 had no correlation with prognosis (Costa et al. 1996, Fontanini et al. 1995).

Immunopositivity to Pab1801 has been found to be associated with hilar and mediastinal lymph node involvement, and post-surgical tumor stage (Fontanini et al. 1993). If a p53 alteration was detected (as a mutation in exons 4 - 9 using PCR-SSCP, or immunopositivity to Pab1801), the hilar or mediastinal lymph nodes were significantly more frequently involved. Neither abnormality alone was associated with lymph node involvemenrt, and survival was not studied (Marchetti et al. 1993). Immunopositivity to CM1 was also significantly associated with the presence of lymph node metastases and an unfavourable prognosis in node-negative patients (Dalquen et al. 1996). Conversely, immunopositivity to the DO7 antibody correlated with higher tumor stage, as well as with a worse prognosis, but not with nodal metastases (Fujino et al. 1995). Immunopositivity to DO1 was associated at borderline significance with the detection of micrometastatic tumor cells in the bone marrow of curatively resected patients. The micrometastases were associated with a significantly impaired prognosis in comparison to patients without such findings (Ohgami et al. 1997).

Immunopositivity to Pab1801 was significantly associated with regional lymph node metastases, distant metastases and pathological stage, but only in adenocarcinoma, not in squamous cell carcinoma. A difference in the stage of time when the p53 alteration occurred between these histological types was suggested as an explanation (Hiyoshi et al. 1992). Another study found immunopositivity to DO7 to be a significant marker for impaired prognosis, but only in adenocarcinoma patients who had undergone potentially curative resection. No significant difference was found in squamous cell carcinoma or if both histologies were analysed as a single cohort (Nishio et al. 1996). In curatively resected patients with stage I adenocarcinoma, p53 alterations (detected either as a mutation in exons 5 - 8 using PCR-DGGE or as immunopositivity to the DO7 antibody), were a significant indicator of poor prognosis. The immunopositivity alone was also significant, while the presence of a mutation alone only approached significance (Isobe et al. 1994).

Immunopositivity to DO7 correlated with earlier local recurrence in NSCLC patients treated with radiotherapy. Fewer p53-positive patients responded to radiotherapy, although the difference was not

statistically significant. The function of p53 as an inductor of G1 phase arrest and of apoptosis following irradiation, was suggested as an explanation (Langendijk et al. 1995).

When more than 50% of cells showed positive staining with DO7, a significantly better prognosis was observed, especially in patients with non-squamous histology or in those whose lymph nodes were involved. The prognosis was better than for patients with low expression (less than 50% staining cells) or negative staining. In patients with no nodal involvement or squamous cell carcinoma, immunopositivity had no association with survival. However, it has been suggested that some patients with no positive staining may nonetheless have a genetic mutation. Moreover, a high expression of functional p53 protein has been documented as a normal response associated with DNA repair after UV irradiation of human and mouse cells, and it is speculated that the presence of such p53 protein in a high percentage of cells might have rendered the tumor less aggressive (Lee et al. 1995). Similar results have been obtained using Pab1801, when patients who were extremely high expressors of p53 protein, and those with no expression at all were observed to have a better prognosis, than those demonstrating moderate or slight levels of expression. Others have speculated that some mutant proteins may not form tetramers and so be unable to interfere with the wild-type protein, or that the antibody detects different types of protein depending on the type of mutation, and the oncogenic potential may vary accordingly (Morkve et al. 1993). Yet another study found immunopostitivity to Pab1801 to be associated with a better prognosis in completely resected stage I-II patients only. The ability of highly expressed and detectable wild-type p53 protein to arrest the cell cycle for DNA repair was proposed as a possible explanation (Passlick et al. 1995).

The creation of a multivariate model to quantify the risk of recurrence and cancer death in stage I patients has been suggested. Immunopositivity of p53 to Pab1801 antibodies has been one of the independent predictors of early recurrence and cancer death (Harpole 1995). A similar approach was applied to stage I patients, but p53 immunopositivity to DO7 or CM1 was not associated with survival (Pastorino et al. 1997)

P53 mutations and prognosis

In a study of 19 curatively resected NSCLC patients, two out of three with a p53 mutation detected using SSCP had a second primary malignancy (Casson et al. 1994). Significantly impaired prognosis was found in resected stage I-II patients with mutations exon 5 - 8 of p53 using the SSCP technique (Fukuyama et al. 1997). Impaired survival in both early stage (I-II) and late stage NSCLC was

associated with a p53 mutation in exon 5 - 8 as detected by SSCP (Horio et al. 1993). In another study, a mutation detected by PCR-SSCP in exons 5 - 8 was associated with a significantly worse prognosis in a univariate analysis of stage III-IV patients, but not stage I-II patients. This may be due to the effect of p53 mutations on the sensitivity of the tumor to chemotherapy and radiotherapy. (Mitsudomi et al. 1993).

The frequency of hilar and mediastinal lymph node metastases was observed to be significantly higher in patients with p53 mutations detected by PCR, with direct sequencing of exons 5 - 8. The association was strongest with exon 8 mutations, which were also the most common mutation in the series . Survival was not investigated (Lee et al. 1994). In another study, exons 5 - 9 were examined using PCR-SSCP. The presence of an exon 5 mutation in squamous cell carcinoma patients was associated with impaired prognosis. It was concluded that since this exon codes for those amino acids which constitute part of the central core domain of the p53 protein essential for sequence-specific DNA binding, a missense mutation within this domain would abolish the supressor function (Vega et al. 1997).

In adenocarcinoma, the presence of a p53 mutation in exons 5 - 8 detected by SSCP, or immunopositivity to DO1, were significant indicators of poor prognosis in univariate analysis. In multivariate analysis, neither was significant (Mitsudomi et al. 1995). The Lung Cancer Study Group observed that neither mutations in exons 4 - 8 of p53, nor *ras* mutations detected using SSCP analysis, had any impact on prognosis. However , immunopositivity to the BP53-12 antibody was associated with a signficant impairment of prognosis. The dominant transforming function of the mutant p53 protein was suggested as an explanation (Carbone et al. 1994). Elsewhere, again, the presence of a p53 alteration (detected as either immunopositivity to DO7 or BP53-12, or as an exon 5 - 8 mutation in PCR-DGGE analysis) was inititially associated with improved prognosis. The difference was only of borderline significance, and the material rather small. The presence of K-*ras* mutations was also studied and found to be inversely correlated with the presence of a p53 alteration. When all K-*ras*-positive patients were excluded, all of them with adenocarcinoma, the p53 alteration completely lost its significance as a prognostic marker. This would appear to indicate that p53 status alone does not influence postoperative survival (Top et al. 1995). A K-*ras* mutation has been identified by several investigators as a negative prognostic factor (Smit et al. 1996, Van Zandwijk et al. 1995), although not significant by others (Carbone et al. 1995).

Factors causing variation in the impact of p53 alterations on survival

Recents studies indicate that some p53 mutations can induce apoptosis without being able to inhibit growth via G1 phase cell cycle arrest, and vice versa. These two pathways appear to be separate, yet interactive in p53 mediated tumor suppression (Wang and Harris 1997). However, there are other cellular factors which can bind to and inactivate the wild-type p53 protein, for example mdm-2 (Pich 1998). Furthermore, in interpreting the results of survival studies, several factors must be considered. Firstly, the techniques used to define p53 alterations range from the various genetic analysis methods to immunohistochemistry. Secondly, the choice and concentration of the antibody used, and the staining methods used in immunohistochemistry vary. The different antibodies also recognise different epitopes of the p53 protein, leading to variations in immunohistochemical detection. Thirdly, the definition of p53 positivity depends on an arbitrary cut-off for the percentage of cells stained, and may therefore be subjective. Fourthly, the size of the studies varies. It appears that studies with more patients demonstrate prognostic significance more frequently. Fifthly, the independence of p53 as a prognostic marker from other well established factors such as TNM and stage should be assessed using multivariate analysis (Pich 1998).

MICROMETASTASES IN LUNG CANCER

The effect of lyph node metastases on prognosis

The presence of lymph node metastases is the most important prognostic factor in operable non-small cell lung cancer (Mountain 1997, Tarkka et al. 1988). If the tumor category is not considered, 5-year survival among completely resected patients without nodal metastases will be 63%, but if pulmonary or hilar nodes are present (N1), the survival will be 41.8%, and if complely resectable mediastinal metastases are present, survival will be 17.2% (Naruke 1993). Relapses are systemic in over half the patients, irrespective of stage or histological type (Mountain 1995). The lymphatic route is assumed to be the primary pathway for dissemination. Lymphatic vessels are thin-walled and more susceptible to invasion than veins or arteries. A poor prognosis has been demonstrated in patients with lymph vessel invasion, even when the primary tumor was T1 or T2 and no lymph node metastases were present (Ichinose et al. 1994).

Preoperative evaluation of lymph node status

The currently available imaging methods, computed tomography (CT) and magnetic resonance imaging (MRI), are not reliable for detecting lymph node metastases (Shields 1993, Gdeedo et al. 1997, Izbicki et al. 1992). Sensitivity and specificity are 48.3% and 53.3%, respectively (Gdeedo et al. 1997) Nodal status is correctly predicted in 51-58% (Gdeedo et al. 1997, Izbicki et al. 1992). Positron emission tomography, when correlated with CT, has a sensitivity of 93% and a specificity of 96% for mediastinal (N2) disease. However, metastases of a few millimeters in diameter, especially if close to the tumor, are not identifiable (Vansteenkiste et al. 1997). If cervical mediastinoscopy is routinely performed, 10% of N2 node metastases will still only be found at surgery, and N1 nodes cannot be reached. In such patients, the resectability was 95% but the 5-year survial only 30% (DeLeyn et al.1997).

Perioperative evaluation of lymph node status

A complete perioperative systematic lymph node dissection is essential for accurate lymph node staging, and all accessible sites in the ipsilateral mediastinum, including the subcarinal nodes, should be included according to the Lung Cancer Study Group (Thomas 1994), and the IASLC (Goldstraw 1994). The procedure does not involve increased mortality or morbidity (Izbicki 1994), and will double the rate of detection of mediastinal metastases, compared to the removal of suspicious nodes only (Bollen et al. 1993).

Histopathological examination and serial sectioning of lymph nodes

Examination of a single hematoxylin-eosin stained section from each node removed remains the present standard practice (Pelkey et al. 1996). In a mathematical model, the probability of detecting a 2 mm peripheral metastasis located in a 10 mm lymph node using this technique was calculated to be only 30.2%, but was increased to 81.9% if the node was sectioned into four equal parts properly orientated (Wilkinson and Hanse 1974). The Ludwig breast cancer study group re-examined nodes from 921 patients who were node negative according to routine histopathological examination. They cut six sections from each node, and found metastases in 9%. The technique was considered laborious and time-consuming (International Breast Cancer Study Group 1990). Analysing more than three sections per node is not considered feasible as a routine technique (Izbicki et al. 1997). However,

cutting the nodes into 2 mm sections along the longitudinal axis perioperatively, and macroscopic inspection has been recommended (Nicholson et al. 1997).

Immunohistochemical methods for detecting micrometastases

A lymph node micrometastasis is defined as a nodus of tumor cells less than 2 mm in diameter (Nicholson et al. 1997). Another definition is a metastasis of less than 2 mm that has been missed by routine hematoxylin-eosin staining (Passlick et al. 1996). Some investigators exclude clusters of more than three immunostainable cells, since these could be detected by standard hematoxylin-eosin staining (Kubuchok et al. 1999). The need for a consensus on the definition has been urged (Kubuschok et al. 1999).

Immunohistochemistry is able to detect microscopic metastases of only one to three cells in lymph nodes, and in bone marrow. The techniques in question are described as simple, and the reagents are readily available (Chen et al. 1993). The antibodies commonly used are anti-cytokeratins and BerEP4. Molecular analysis techniques for the detection of mRNA coding for specific tumor markers such as the carcinoembryonic antigen, or the detection of tumor-associated oncogene mutations such as *ras* or p53, have been successfully used, but are technically more demanding (Ahrendt et al. 1997, Liefers et al. 1998, Luketich et al. 1998, Pelkey et al. 1996, Salerno et al. 1998). The specificity of these techniques has also been questioned, since normal hematopoietic cells have been found to express low amounts of these markers (Kubuschok et al. 1999).

The cytokeratins

A large proportion of the cytoplasm of vertebrate cells is composed of filaments which are components of the cytoskeleton. Filaments cotaining keratin-like proteins (”cytokeratins”) are characteristic of epithelial cells. During tumor development, this cell type specificity is conserved. The cytokeratins expressed in the different epithelia vary in polypeptide composition, molecular weight and isoelectric pH value. A given epithelium or epithelial cell can therefore be characterised by its cytokeratin components (Moll et al. 1982). The intermediate-sized cytokeratin polypeptides 7 and 8, and the small acidic ones 17, 18 and 19 are found in lung carcinomas (Chen et al. 1993, Moll et al. 1982). Broad-spectrum anti-cytokeratin reagents are highly sensitive, since cytokeratin filaments are a feature of all lung carcinomas regardless of their degree of differentiation, and can be used on paraffin- embedded tissue (Chen et al. 1993). These reagents are not specific for lung cancer cells: polymorphonuclear

leukocytes and macrophages in lymph nodes show cytoplasmic and nuclear staining, but tumor cell nuclei do not stain and the cells are morphologically different (Chen et al. 1993). Mesothelial cells which have migrated into the lymph nodes of lung cancer patients can given false-positive results (Nicholson et al. 1997). Anti-cytokeratin staining has been used to detect tumor cells in the bone marrow and lymph nodes of patients with various types of cancer, including small cell and non-small cell lung cancer (Beiske et al. 1992, Chen et al. 1993, Funke et al. 1998, Pelkey et al. 1996, Nicholson et al. 1997)

The Ber-EP4 antibody

BerEP4 is a monoclonal antibody directed against two glycopolypeptides of 34 and 49kd found in the cytoplasm and on the surface of all epithelial cells except hepatocytes and parietal cells, and in the apical cell layers in squamous epithelia (Latza et al. 1990). BerEP4 does not bind to non-epithelial cells, so it can be used to differentiate between carcinoma cells and mesothelial cells (Daniel et al. 1991, Latza et al. 1990), or reactive mesothelial cells in smears of serous effusions (Latza et al. 990). Benign epithelial cells have not been found in thoracic lymph nodes (Kubuschok et al. 1999). The amount of BerEP4 antigen varies in different tissues. Furthermore, formalin fixation for more than 48 hours can destroy the epitope recognised by the monoclonal antibody, and lead, on occasion, to the complete removal of reactivity with BerEP4. For these reasons, the BerEP4 antibody may not always work satisfactorily in paraffin wax sections of tissues expressing small amounts of the antigen, for example lung carcinoma and hepatocellular carcinoma. This has been noted specifically in lung adenocarcinoma. However, the antibody works reliably on frozen sections fixed in acetone, cytospins and cell smears of the afore-mentioned tissue (Latza et al. 1990). Authors who have used BerEP4 to detect lymph node metastases of lung carcinoma (Izbicki et al. 1996, Kubuschok et al. 1999, Passlick et al. 1994, Passlick et al. 1996) and esophageal carcinoma (Izbicki et al. 1997) state that the antibody will work in paraffin-embedded material, but they all only used snap-frozen and acetone-fixed tissue for the immunohistochemistry. This latter decision was based on initial experiments on lymph node dissemination using different anti-epithelial antibodies, where specific mention was made of anti-cytokeratin antibodies, but not BerEP4, which did not all work on paraffin-embedded tissue (Passlick et al. 1996).

Presence of micrometastases in various cancers

Immunostaining has detected metastases in the lymph nodes of 33%-39% of histologically node negative colorectal carcinoma patients (Adell et al. 1996, Cutait et al. 1991, Sasaki et al. 1997), 23.5%-40% of gastric carcinoma patients (Ishida et al. 1997, Maehara et al. 1996), 15.2%-27.4% of non-small cell lung cancer patients (Izbicki et al. 1996, Kubuschok et al. 1999, Passlick et al. 1994, Passlick et al. 1996), 17% of esophageal cancer patients (Izbicki et al. 1997), and 27% of gall-bladder carcinoma patients (Yokoyama et al. 1998). Two reports on lung cancer are markedly contradictory , with one giving a 6% incidence of metastases in histologically node negative patients (Nicholson et al. 1997) and the other 63% (Chen et al. 1993). The possibility of false- positive results due to the inclusion of mesothelial cells, and a higher primary detection rate due to perioperative sectioning of the nodes at 3 mm intervals along the longitudinal axis before routine histology are offered as explanations (Nicholson et al. 1997).

Immunostaining has also demonstrated bone marrow micrometastases in 32% of small cell carcinoma patients (Beiske et al. 1992), 21.9%-54.3% of non-small cell carcinoma patients (Cote et al. 1995, Ohgami et al. 1997, Pantel et al. 1993, Pantel et al. 1996), 29% of colorectal patients, 25% of gastric cancer patients, and 58% of pancreatic cancer patients (Juhl 1994). Several studies have failed to find a correlation between lymph node involvement and bone marrow micrometastases, suggesting that different determinants exist for the dissemination of cancer cells via lymphatic and hematogenic routes (Izbicki et al. 1997, Pantel et al. 1993, Passlick et al. 1994, Passlick et al. 1996). Micrometastatic spread into lymph nodes is erratic in lung cancer (Izbicki et al. 1996, Passlick et al. 1994) and also in esophageal carcinoma (Izbicki et al. 1997), and does not follow established lymphatic routes.

The prognostic significance of micrometastases

In almost all of the afore-mentioned studies, the presence of lymph node or bone marrow micrometastases appeared to predict a shortened recurrence-free interval, and shorter cancer-related survival in comparison to cases without micrometastases. In a critical meta-analysis (involving 2,494 patients) on the significance of bone-marrow micrometastases, the results suggested that the prognostic impact of epithelial cells in the bone marrow remains to be substantiated by further studies using standardised methodic protocols prior to its suggested entry in the TNM classification. However, a statistically significant odds ratio for breast cancer patients could be calculated based on this material (Funke and Schraut 1998). One study applied rigorous perioperative macroscopic sectioning of the

lymph nodes, and detected cytokeratin positive cells in only 6% of lung cancer patients, none of whom relapsed during a three-year follow-up (Nicholson et al. 1997). Only one prospective study with a five-year follow-up applied multivariate analysis, but established the presence of BerEP4 positive lymph node micrometastases as an independent indicator of poor prognosis in non-small cell lung cancer (Kubuschok et al. 1999).

The presence of lymph node micrometastases does not only predict a local relapse, nor do bone micrometastases only predict skeletal metastases (Izbicki et al. 1996, Izbicki et al. 1997, Ohgami et al. 1997, Pantel et al. 1993) . Moreover, the relapse-free interval did not depend on the number of immunostainable cells detected (Izbicki et al. 1996) . Bone marrow micrometastases have been demonstrated months after curative resections for gastrointestinal cancers, and appear to indicate minimal residual disease (O´Sullivan et al. 1997). The cells may remain dormant for extended periods before exhibiting their metastatic potential, and long-term follow-up is needed (Kubuschok et al. 1999). Micrometastases are therefore thought to be an indicator of the potential of a tumor to spread aggressively. Host-related factors will also determine whether these micrometastases will develop into true metastases (Izbicki et al. 1996, Izbicki et al. 1997, Pantel et al. 1996, O´Sullivan et al. 1996) or be destroyed (Adell et al. 1996, Cutait et al. 991).

PREOPERATIVE IMAGING

Non-invasive preoperative staging

CT or MRI of the chest does not accurately stage primary lung carcinoma according to the TNM classification (Lewis et al. 1990, Shields 1993); there was only 45.4% agreement with the operative staging (Lewis et al. 1990). In a study that compared the staging results obtained by preoperative imaging and at surgery, the clinical and histopathological T categories concurred in 81.6% of cases, and the N category in 55.3% (Fernando and Goldstraw 1990). Even though 86.6% of the tumors that CT predicted would be resectable, indeed proved to be completely resectable, an insufficiency in defining lymph node metastases and infiltrative growth was evident (Lähde et al. 1991). A normal CT scan also predicted a better outcome. In patients whose mediastinal CT was abnornal and in whom resectable N2 metastases were found at surgery, the 5-year survival was 6.6% as opposed to 13.5% in those who had a normal CT scan, but in whom metastases were found at surgery (Cybulsky et al. 1992).

Neither tumor size nor the presence or absence of hilar lymph node enlargement is a reliable prediction of N2 disease (Arita et al. 1995). Mediastinal node metastases have been found in 12-25% of patients with a tumor of only 1.1-3 cm in diameter (Ishida et al. 1990b, Yano et al 1998), with an overall incidence of 21% in T1 tumors (Seely et al. 1993). Mediastinal node metastases without N1 disease (so-called "skip metastases") have been found in 7%-33% (Libshitz et al. 1986, Tateishi 1994), and were more common in adenocarcinoma than in squamous cell carcinoma, as were N2 metastases in general (Libshitz et al. 1986).

The normality criterion for lymph nodes is usually based on their size on CT images, and is not reliable. It has been observed that malignant mediastinal nodes are no larger than benign nodes, and small nodes are not infrequently malignant (Kerr et al .1992). In a study of squamous cell carcinoma, optimum sensitivity and specificity (both 76%) has been obtained by measuring the minor axis, with size limit of 9.3 mm for normality (Kobayashi and Kitamura 1995). Nevertheless, mediastinal lymph nodes which were less than 10 mm in diameter had metastases in 15-15.5% of cases (Arita et al. 1995, Kerr et al. 1992), but a lower incidence of 7.3%-9% has been reported (Daly et al. 1993, Kayser et al. 1990). Furthermore, agreement among radiologists as to whether there were mediastinal nodes larger than 10 mm ranged from fair to substantial, and the disagreement is sufficient to contribute to suboptimal sensitivity and specificity of CT in detecting tumor spread (Gyatt et al. 1995). Interobserver variability with only 58% agreement has been observed even among experienced radiologists (Webb et al. 1993). Benign adenopathy is common, especially in patients with acute pulmonary inflammation (Kerr et al. 1992). If mediastinal nodes with a minor axis of 15 mm or more on the CT scan were considered abnormal, the sensitivity would be 26%, specificity 81%, and accuracy 69% (Cole et al. 1993). Up to 35.7% of nodes with a diameter of more than 20 mm have not been infiltrated (Vogel et al. 1990).

Transesophageal echography appears to be more sensitive than CT for detecting nodes larger than 10 mm in the lower mediastinum, but is inferior in the upper mediastinum and hilum (Jakob et al. 1990), Used together, echography and CT appear to complement each other (Kondo et al. 1990).

Direct preoperative mediastinal staging

Direct mediastinal evaluation is suggested by the Lung Cancer Study Group for patients who have a tumor wholly or partly within the medial half of the chest cavity, and for peripheral T2 lesions (Eagan 1994). In a consensus report on minimal pretreatment staging, mediastinoscopy is recommended in all

cases involving nodes of over 10 mm on the CT scans, supplemented by additional evaluation of the subaortic fossa if the tumor is in the left upper lobe or main bronchus (Goldstraw et al. 1994). In view of the 92.9% negative predictive accuracy of CT for N2 disease, invasive staging is deferred for thoracotomy if no mediastinal nodes over 10 mm are seen (Lewis et al. 1990). Conversely, routine mediastinoscopy has also been recommended (Kristensen et al. 1995).

The 5-year survival for surgical patients according to their N2 status on mediastinoscopy is 47% for negative results, 14% for false negative results, and 6% for positive results (Funatsu et al. 1992). Patients with only intranodal growth fare better (Bollen et al. 1994, Ishida et al. 1990a), but mediastinoscopic lymph node involvement can only be classified as intranodal or extranodal in 25% of cases at the most (Bollen et al. 1994, Theunissen et al. 1994). For this reason, some authors recommend that patients should not be systematically excluded from surgery on the basis of positive mediastinoscopic findings (Levasseur et al. 1992). However, no patient deemed inoperable because of N2 disease on mediastinoscopy survived for 5-years (Van Klaveren et al. 1993), whereas if a resection was performed, approximately 2% would survive for 5 years (Shields 1990). Surgery for only those patients who have completely intranodal N2 disease on one ipsilateral level, which is not macroscopically suspected (”minimal N2 disease”), appears justifiable with an expected 5-year survival of 20.8%-31% (Mountain 1994, Shields 1990, Vansteenkiste et al. 1997).

Thoracoscopy has been specifically recommended in cases of cytologically normal pleural effusion, suspicion of mediastinal infiltration on the CT scans, or contralateral lymphadenopathy not accessible by mediastinoscopy (De Giacomo et al. 1997), as well as suspicion of intrapulmonary metastases (Naruke 1993). An ultrasound probe attached to the mediastinoscope has been reported to give a better view of mediastinal and subcarinal nodes (Nakano et al. 1994), and transesophageal ultrasound can be used to guide fine-needle aspiration of posterior subcarinal nodes (Wiersema et al. 1994)

Perioperative mediastinal staging

Perioperative mediastinal palpation is deemed insufficient, and careful extensive dissection is recommended with separate labeling of the nodes according to a known lymph node map (Goldstraw et al. 1994, Holmes 1994). Mobilisation of the aortic arch in left-side tumors has not been an obligatory step, but appears to result in more detailed staging of the N2 region (Izbicki et al. 1995). A complete ipsilateral mediastinal dissection is recommended, because metastases to the lower mediastinum from upper lobe tumors, and vice versa, have been described (Watanabe et al. 1990).

Patients found to have N2 disease at surgery and undergoing complete resection have a 5-year survival of 10% -34.5% (Mountain 1994, Nakahara et al. 1993, Nakanishi et al. 1997, Naruke 1993, Van Klaveren et al. 1993, Yano et al. 1998). A 3-year survival of 49% has been reported for patients with clinically evident disease who nonetheless undergo complete resection with radical lymph node dissection, (Martini et al. 1980). However, the completely resected group constituted only 18% of those patients who had clinically evident N2 disease (Martini et al. 1980), while several underwent an incomplete resection. Patients with N2 disease discovered only at surgery should still undergo complete resection, if feasible, and a 5-year survival of 15-30% can then be expected (Mountain 1994, Shields 1990). However, this group constitutes less than 20% of all patients with N2 disease, and at the most only 6% of all such patients can be expected to live more 5 years following surgery alone (Shields 1990).

PURPOSE OF THE STUDY

The aim of the present investigation was:

1) to evaluate the applicability of bronchoplastic resection for the curative treatment of non-small cell lung cancer;
2) to evaluate the justification of surgery with curative intent for patients over 75 years of age, with special reference to those over 80 years old;
3) to evaluate the prognostic significance of (tumor) p53 mutations on the prognosis of surgically treated non-small cell lung cancer patients;
4) to ascertain whether immunostaining for high- and low-molecular-weight cytokeratins and BerEP4 would reveal metastases in histologically normal lymph nodes in non-small cell lung cancer patients; and
5) to evaluate the reliability of preoperative computed tomography (CT) for assessing lymph node status in non-small cell lung cancer.

PATIENTS AND METHODS

Patients

There were five separate studies of patients altogether.

I. Bronchoplastic resections.

This study included all the 28 lung cancer patients who had undergone bronchoplastic resection at the Department of Thoracic Surgery at Helsinki University Central Hospital between1973 and1993.

II. Surgery for lung cancer in elderly patients

This study included all the 75 patients, aged 75 years or older, who underwent surgery for non-small cell lung cancer at the Department of Thoracic Surgery at Helsinki University Central Hospital, between 1976 and 1996. Thirteen of the 75 were 80 years or older, three undergoing surgery between 1985 and 1990 and 10 between 1991and 1997.

III. The effect of p53 mutation on prognosis.

The study population here consisted of 101 consecutive primary lung adenocarcinoma or epidermoid carcinoma patients, who underwent surgery with curative intent at the Department of Thoracic Surgery at the Helsinki University Central Hospital between 1988 and 1993.

IV. Immunohistochemistry for detecting lymph node metastases.

This study population comprised 19 patients who had undergone surgery and systematic mediastinal lymph node dissection for non-small cell lung cancer at the Department of Thoracic Surgery at Tampere University Hospital between March 1997 and September 1998, and who had no mediastinal metastases according to routine histology. Of the 36 patients operated on during this period, eight were excluded because a mediastinal node dissection had not been performed, and a further nine were excluded because of mediastinal node metastases.

V. Reliability of preoperative computed tomography for lymph node staging.

The study population consisted of 26 consecutive patients who had undergone surgery and systematic mediastinal lymph node dissection for primary non-small cell lung cancer between March 1997 and September 1998 at the Department of Thoracic Surgery at Tampere University Hospital. Of the 36 patients operated on during this period, 10 were excluded because a complete mediastinal node dissection had not been performed.

Preoperative investigations

All patients had their medical history taken, and underwent a physical examination. Laboratory tests included a blood count, and transaminases and alkaline phosphatase levels.

Other preoperative evaluations included standard chest radiography, fiberoptic bronchoscopy and spirometry. Computed tomography of the thorax and upper abdomen was performed routinely from the 1980s. Abdominal ultrasound, brain CT and isotope bone scans were not performed unless indicated by clinical suspicion of metastatic disease. Radiospirometry and/or diffusion capacity evaluation was carried out if spirometry was more than slightly abnormal.

Histological classification and staging

Histological classification was performed according to the World Health Organisation criteria (WHO, 1981). The assessment of TNM category according to the present classification (Sobin and Wittekind 1997) and staging according to the Revised International System for Staging Lung Cancer (Mountain 1997) were based on patient documents in studies I-III, and from clinical, perioperative and histopathological observations in studies IV-V. The previous staging classification (Mountain 1986) was used in the study of bronchoplastic surgery, and both classifications were used in the study of elderly patients. Systematic lymph node dissection was performed in patients in studies IV and V. Otherwise, mediastinal exploration was limited to lymph nodes which aroused suspicion on palpation.

Surgical techniques and the definition of a complete resection

Postero-lateral or muscle-sparing thoracotomy was performed in all cases. General anesthesia, with intubation providing single-lung ventilation, was used. All lung resections were carried out according to standard routine care procedures. The extent of the procedure was defined as a limited resection (less than lobectomy), lobectomy, bilobectomy or pneumonectomy. The bronchoplastic procedures were either sleeve or wedge resections of the main bronchus, or sleeve pneumonectomies.

Systematic lymph node dissection, when performed, was according to the recommendations of the Lung Cancer Study Group (Thomas 1994), and all nodes were labeled according to the Naruke map (Naruke et al. 1978). All nodes from the base of the lobar bronchus and interlobium in the case of lobectomy, and/or hilar extramediastinal nodes (Naruke map nos. 10, 11, and 12) in the case of pneumonectomy, were removed. In some cases these were dissected from the resected lung tissue by

the surgeon. All accessible nodes from the ipsilateral mediastinum were removed together with fatty tissue if present. On the right side, this included nodes under the azygos vein (Naruke map no. 4), nodes lateral and anterior to the trachea up to subclavian artery level (Naruke map nos 2 and 3) and nodes anterior to the superior vena cava (Naruke map N:o 3a). On the left side, nodes along the aorta (Naruke map no. 6), those in the subaortic fossa and also those medial to the Botallo ligament (Naruke map nos 5 and 4) were removed. The aortic arch was not mobilised. On both sides, subcarinal and pulmonary ligament nodes (Naruke map nos 7 and 9) were routinely removed. Paraesophageal (Naruke map no. 8) nodes below the carina level were also removed if present.

In all studies, the resection was regarded as complete only if all macroscopic tumor tissue was removed, no microscopic tumor tissue was found at the bronchial resection margin, no mediastinal lymph node metastases or extrathoracic metastases were present, and the operating surgeon felt that no residual tumor remained. A non-anatomical resection, defined as a wedge-excision of the tumor from the lobe in which it was, was regarded as an incomplete (non-radical) resection.

Results of surgery, and follow-up

In the studies on bronchoplastic resection (I) and elderly patients (II), the mortality and morbidity was obtained from patient records; in these as well as the study on p53 mutations (III), the completeness of resection was also noted.

Follow-up in respect of survival and cancer-related survival of all patients except those in the immunohistochemistry study (IV), was conducted by record linkage with the Central Population Register of Finland. A unique personal identification number assigned to every resident in Finland since 1967 was used in this linkage. Survival status, dates and causes of death were obtained from Statistics Finland.

Statistics

In the study on bronchoplastic resections, the actuarial method was used to estimate overall and cancer-related 5-year survival.

In the study of elderly patients, a comparison of their survival with that of an age-and sex-matched population was made by constructing an actuarial life table. The Kaplan-Meier method was used to compare the survival of stage I and IA patients to the other stage patients, stage I patients who underwent a limited resection to those who underwent a lobectomy, and the 75-79 year old group to the

80 years or older group. Fisher´s exact test and the contingency table method were used to compare operative mortality and complication rates between patients with limited and standard FEV1, between the 75-79 year-old group and the 80 or more year-old group, and between those who underwent a limited resection or lobectomy and those who underwent a more extensive procedure.
In the study on p53 mutations, the Kaplan Meier method was used to compare the survival of the p53-positive and -negative adenocarcinoma and epidermoid carcinoma patients, and specifically in low-stage (IA-IIB) and high-stage (IIIA-IV) patients with these tumor histologies. Cox´s multivariate analysis was employed to quantify the association between overall and cancer-related survival, and indicators of p53 mutation, stage, smoking, asbestos exposure, gender, age, histological type and completeness of resection.

Detection of p53 mutation, asbestos exposure and smoking history

A complete preoperative occupational history and smoking history was prospectively obtained in a personal interview during the hospital stay. A standardised questionnaire was used. The patients were classified into four exposure categories according to the probability of past occupational exposure to asbestos: definite; probable; possible; and unlikely (Karjalainen et al.1993). In the statistical analysis, all patients with definite or probable exposure were considered exposed, and those with possible or unlikely exposure as non-exposed.
Representative samples of fresh tumor tissue were frozen at -80°C for analysis. DNA was extracted from the tissue samples by phenol-chloroform extraction as described elsewhere (Ridanpää et al. 1994). All lung tumour samples were examined for p53 alterations using denaturant gradient gel electrophoresis (DGGE) of PCR amplified fragments of the p53 gene. PCR-DGGE and direct sequencing of exons 4-9 was performed as previously described (Husgafvel-Pursiainen et al. 1999).

Immunohistochemistry for detecting lymph node metastases

The thoracic cavity of 14 patients was lavated prior to any lung manipulation with 100ml of 0.9% NaCl solution, and the aspirate taken for cytopathological study. Systematic lymph node dissection was performed in 28 cases as previously described.
The pleural lavage sample was fixed in 50% ethanol and processed into a cytocentrifuge preparation. This was stained using the Papanicolaou method. The removed lymph nodes were fixed in buffered 10% formalin and sent for paraffin study. Nodes of 1 cm in diameter or less were cut in half and four

sections taken from one cut surface. Larger nodes were cut into three parts. One section was stained with hematoxylin and eosin, while the adjacent sections were stained for high- molecular-weight (MW) cytokeratin, low-molecular-weight cytokeratin, and BerEP4. For immunoperoxidase staining of the above antigens, 4 μm-thick paraffin sections were cut on ChemMate^{TM} capillary gap microscope slides (DAKO a/s, Glostrup, Denmark). Before immunostaining, rehydrated sections were heated in a microwave oven at 800 W for two 7-minute cycles using 0.01 mol/l citrate buffer (pH 6.0) as the antigen retrieval solution. Staining was carried out using an indirect streptavidin-biotin peroxidase sequence in TechMate^{TM}500 immunostainer (DAKO a/s). Mouse monoclonal antibodies to the epithelial antigen BerEP4 (DAKO, dilution 1:150), low-MW cytokeratins (clone C-51, Neomarkers, Fremont, California, USA, dilution 1:500) and high-MW cytokeratins (clone DE-SQ, Neomarkers, dilution 1:1000) were visualised using a ChemMate^{TM} detection kit (DAKO). Normal human skin served as control for high-MW cytokeratins, and normal large-bowel mucosa for BerEP4 and low MW-cytokeratins.

In the three patients who had metastatic disease on follow-up, the removed lymph nodes were re-studied: all removed lymphatic tissue had been preserved in paraffin blocks, and was re-examined for metastases using a serial sectioning technique. Histology and immunohistochemistry were performed as previously described, but the four sections were taken at 50-micron intervals in all the removed lymph nodes. These patients had also all participated in the pleural lavage study.

Preoperative imaging

All patients underwent a chest and upper abdomen CT, extended to suprarenal gland level. Native and contrast-enhanced sections of 8 and 10 mm were taken with a Siemens Somatom non-spiral device. All images were independently interpreted by on-duty radiologists, and a thoracic radiologist. The median interval between CT and surgery was 1 month (range 1 week to 3 months). All nodes of 10 mm or longer, as measured along the short axis, were considered abnormal. Their exact anatomic location according to the Naruke map (Naruke et al. 1978) was noted.

All lymph nodes were labeled according to the map, and the tumor category was noted by the surgeon according to measurements of the resecate immediately before fixation. Special attention was paid to any lymph node sites defined as abnormal by the radiologist.

Tumor histology and lymph nodes were studied from paraffin-embedded material at Tampere University Hospital by pathologists experienced in pulmonary pathology. The central section of nodes under 10 mm were studied, and the larger ones were cut into three parts**.**

Tumor and node status, as interpreted by both the on-duty radiologist and a thoracic radiologist, were compared to each other and to the postoperative surgical pathological stage.

RESULTS

Bronchoplastic resections

Bronchoplastic resection was performed on 28 patients, whose characteristics are given in Table 3.

Table 3. Patient characteristics in studies I-V.

STUDY	*TOTAL PATIENTS*	*AGE **(years)***		*SEX*	*N (%)*	*TUMOR HISTOLOGY*	*N (%)*
BRONCHO-PLASTY (study I)	28	Median Range	60 46-76	Male Female	27 (96) 1 (4)	Squamous Adeno Large cell Bronchiolo-alveolar Small cell	24 (86) 0 1 (4) 1 (4) 2 (6)
ELDERLY PATIENTS (study II)	75	Median Range	77 75-84	Male Female	56 (75) 19 (25)	Squamous Adeno Large cell Bronchiolo-alveolar	36 (48) 20 (27) 9 (12) 10 (13)
P53 MUTATION (study III)	101	Median Range	65 38-79	Male Female	84 (83) 17 (17)	Squamous Adeno Large cell Bronchiolo-alveolar	59 (58) 38 (38) 0 4 (4)
IMMUNO-HISTOCHE-MISTRY (study IV)	19	Median Range	68 52-83	Male Female	16 (84) 3 (16)	Squamous Adeno Large cell Bronchiolo-alveolar	11 (58) 6 (32) 0 2 (10)
PREOPERA-TIVE IMAGING (study V)	26	Median Range	68 57-83	Male Female	21 (81) 5 (19)	Squamous Adeno Large cell Bronchiolo-alveolar	14 (54) 10 (38) 0 2 (8)

The tumor was in the right upper lobe in 22 patients (79%), right median lobe in 3 (11%), left upper lobe in 2 (7%), and left lower lobe in one (4%). Six patients (21%) had a right upper lobe orifice tumor. The indication for bronchoplastic resection was a limited respiratory reserve (defined as a predicted postpneumonectomy FEV1 of less than 1.0 L) in 13 patients (46%), a perioperative decision to preserve functioning lung tissue in 12 (43%), inadvertent transsection of the carina in 1 (4%), and tumor involvement of the carina in 2 (7%). Bronchoplasty was performed in 25 patients (89%), and sleeve pneumonectomy in 3 (11%). The lung resections carried out were lobectomy in 21 patients (75%), bilobectomy in 4 (14%), and pneumonectomy in 3 (11%). Nine patients had stage I (Mountain 1986) tumors, three patients (11%)had stage II tumors, 15 patients (54%) had stage IIIA tumors and one patient (4%) had a stage IIIB tumor.

A complete resection was achieved in 13 patients (46%). Incomplete resections were due to mediastinal metastases found at surgery in 9 patients (32%) and microscopic tumor at the bronchial resection line in 6 patients (22%). Two patients in the latter group underwent a completion pneumonectomy.

Major complications (bronchopleural fistula, empyema, pneumonia and postoperative hemorrhage requiring thoracotomy) occurred in 25% of the bronchoplasty patients. No clinically significant kinking of the anastomosis was noticed, even though approximately the same number of bronchial wedges and sleeve resections were performed. In the majority of cases, anastomosis was performed using absorbable sutures, and none showed clinically significant suture granuloma formation. The operative mortality was 8% among the bronchoplastic resection patients. The deaths were caused by pneumonia and bronchopleural fistula with pneumonia. The mortality in the sleeve pneumonectomy group was 33% (1/3), due to pneumonia.

Follow-up was maintained until the end of May, 1995. The overall 5-year survival, excluding patients who underwent a completion pneumonectomy, was 30%, and the cancer-related 5-year survival was 40%. After a complete resection, the cancer-related 5-year survival was 54%. For stage I and stage II disease (Mountain 1986), the cancer-related 5-year survivals were 67% and 66%, respectively, compared to 18% for stage IIIA.

Surgery for lung cancer in the elderly

There were 75 patients in this study,of whom 13 were 80 years old or older. Their characteristics are given in Table 3. Lung cancer was the only illness in 44% of the whole group, and in 46% of the over-

80-year olds. The predicted post-pneumonectomy FEV1 was < 1.0 L in 18 patients and 3 patients, respectively.

There were 36 epidermoid carcinomas(48%), 20 adenocarcinomas(27%), 9 large cell carcinomas(12%), and 10 bronchiolo-alveolar carcinomas(13%). Surgical techniques were standard and routine for all patients. Lobectomy was performed in 47 patients (63%), bilobectomy in 10 patients (13%), and limited resection in 8 patients (11%). -According to the earlier classification (Mountain 1986), 43 patients (57%)had stage I disease, 10 (13%)had stage II disease, 15 (20%) had stage IIIA disease, 4 (5%) had stage IIIB disease, and 3 (4%) had stage IV disease. According to the revised classification (Mountain 1997), the figures were 13 (17%), 30 (40%), 2 (3%), 13 (17%), 10(13%), 4(5%) and 3(4%) for stages IA, IB, IIA, IIB, IIIA, IIIB and IV, respectively.

A complete resection was achieved in 59 (79%) of the 75 patients. In patients with a predicted post-pneumonectomy FEV1 of less than 1.0 l (limited FEV1 group), and over 1.0 l (standard FEV1 group), the resection was incomplete in 22% and 21%, respectively. Pneumonectomy would not have been curative in patients with a limited respiratory reserve.

The operative mortality in all patients, in those younger than 80 years (75-79 group), and those 80 years or older (80+ group) was 9%, 8%, and 15% respectively. The mortality rate was zero after a limited resection, 6% after lobectomy, and 20% after bilobectomy or pneumonectomy. In the limited FEV1 and standard FEV1 groups the mortality was 6% and 11%, respectively. There was no statistically significant difference in operative mortality between patients undergoing limited resection or lobectomy and those undergoing bilobectomy or pneumonectomy, between the 75-79 and the 80+ groups, or between the limited and standard FEV1 groups. The number of lobectomies or limited resections did not differ significantly between the two age groups (71% versus 85%).

The incidences of overall and major complications were 29% and 21%, respectively. In both the 75-79 and the 80+ group the incidence of complications was 29%: 13% after a limited resection, 21% after lobectomy, 50% after bilobectomy and 60% after pneumonectomy. The incidence of complications was significantly lower after a limited resection or lobectomy than after bilobectomy or pneumonectomy (Fisher´s exact p-value 0.008). The mean postoperative hospital stay for the 68 surviving patients was 14.9 days (SD=5.9, range 7-39 days), of whom 60 (88%) returned to their own homes, 2 (3%) to retirement homes, and 6 (9%) to local health care units.

Follow-up was maintained until the end of June 1997. The actuarial postoperative 5-year survival was 56% of the 5-year survival in a size-, age-, and gender-matched standard population (Fig. 1).

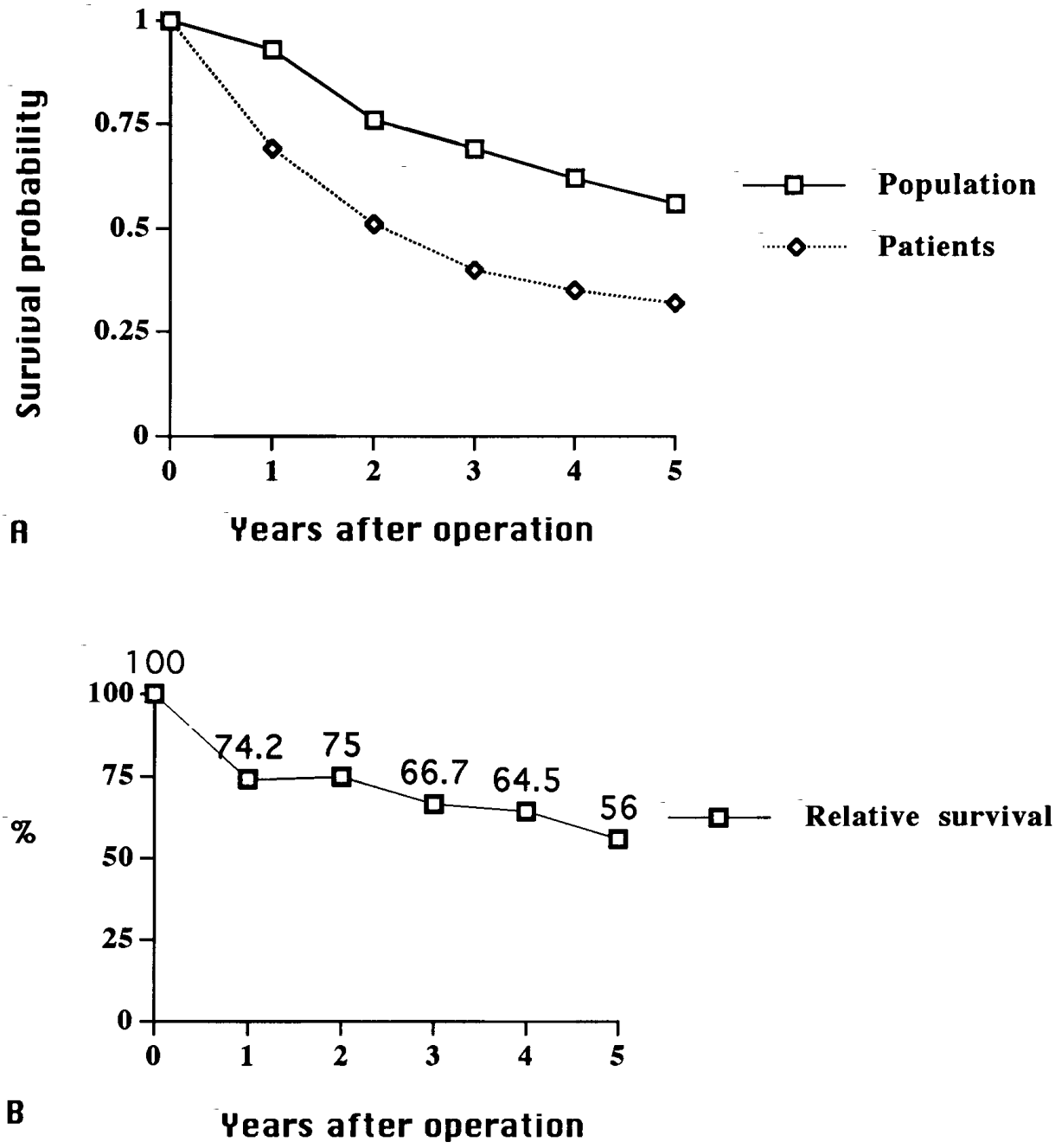

Fig 1. (A) Actuarial survival for patients versus matched population, and (B) relative survival rate in % (our patients/ that of matched population).

The cumulative overall and cancer-related 5-year survivals according to the previous (Mountain 1986) and present (Mountain 1997) classification are presented in Table 4.

Table 4. Cumulative overall cancer-related 5-year survival probabilities among elderly patients surgically staged according to the previous (Mountain 1986) and present (Mountain 1997) classifications.

STAGE 1986	OVERALL SURVIVAL	CANCER SURVIVAL
I	0,32	0,54
II	0,57	0,67
IIIA	0,14	0,35
IIIB	0,12	NOT CALCULATED
IV	0	0

STAGE 1997	OVERALL SURVIVAL	CANCER SURVIVAL
IA	0,31	0,77
IB	0,27	0,40
IIA	NOT CALCULATED	NOT CALCULATED
IIB	0,41	0,61
IIIA	0,22	0,44
IIIB	0,12	NOT CALCULATED
IV	0	0

The cumulative survival in stage IIA and IIIB are not presented as there were only 2 and 4 patients in these categories.

There was a statistically significant difference in survival (p=0.013) and in cancer-related survival (p=0.038) between patient with stage IA disease and those with other stages of disease (Fig. 2), but not between stage I and the other stages according to the earlier classification (Mountain 1986) (Fig. 3.).

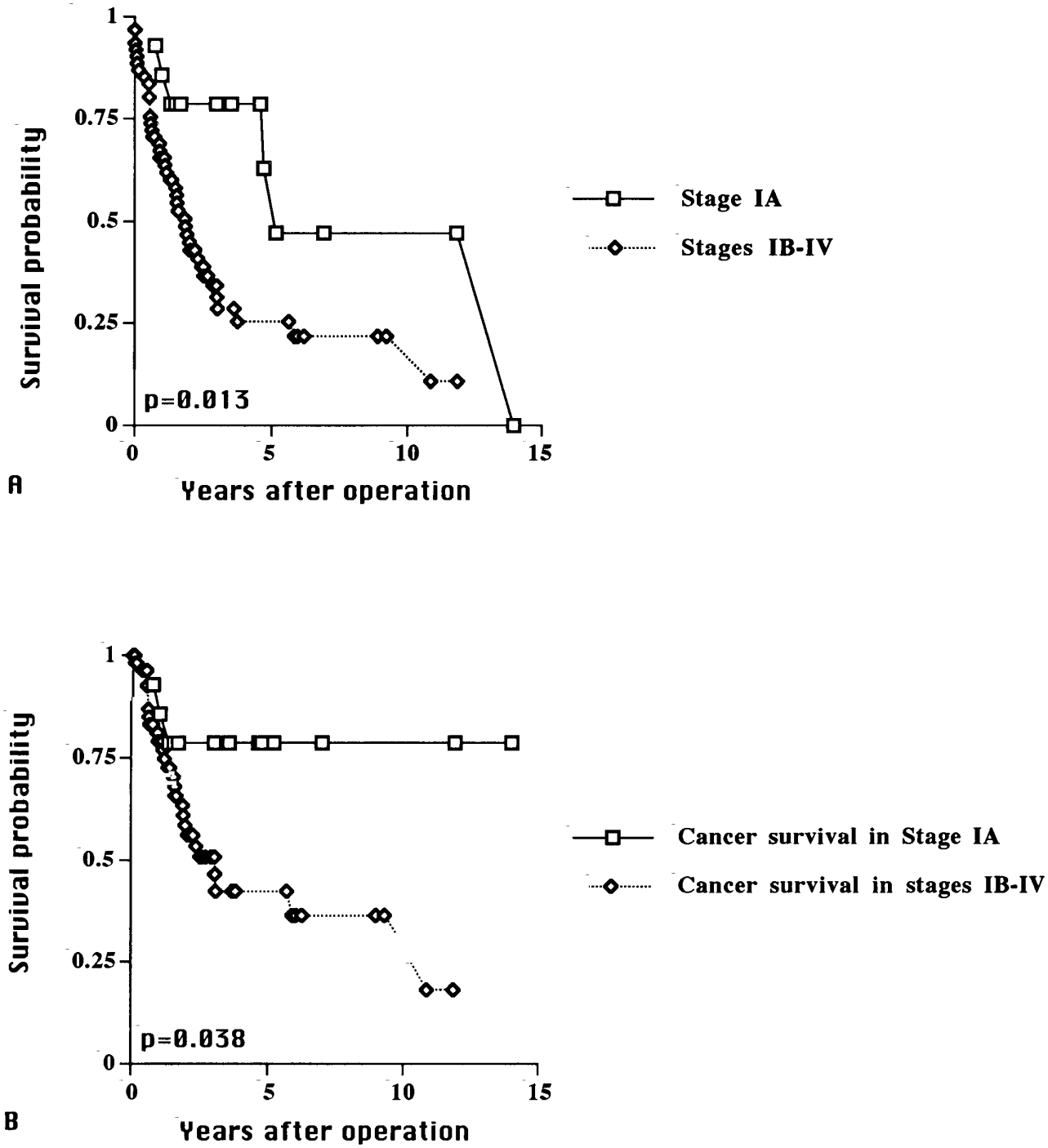

Fig 2. (A) Overall cumulative survival, and (B) cancer-related survival for stage IA (n=14) versus the other stages combined (n=61).

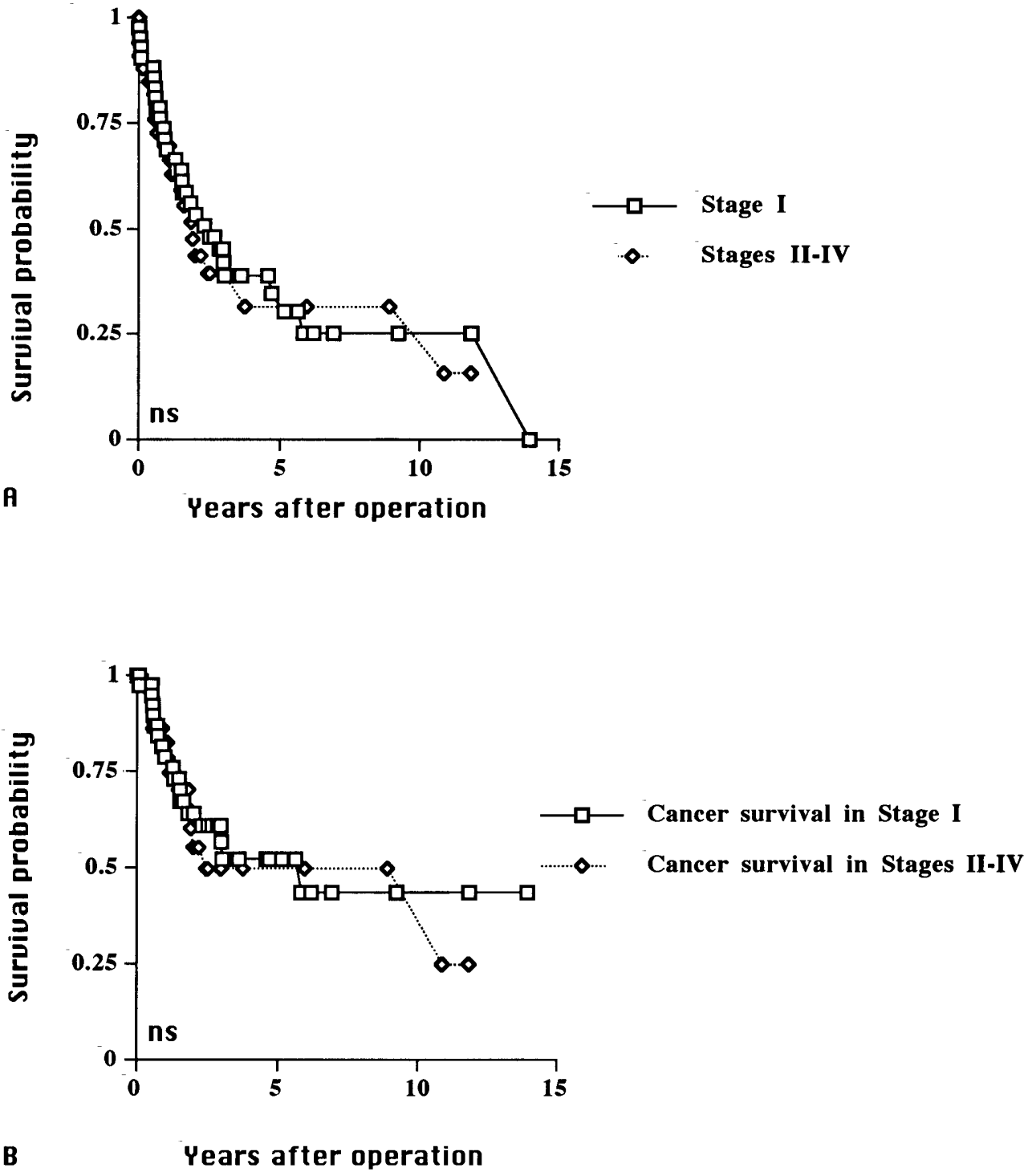

Fig 3. Cumulative overall survival, and (B) cancer-related survival for stage I (n=43) versus the other stages combined (n=32). ns=no statistical significance.

There was no significant difference in the cumulative 5-year survival) of stage I patients who underwent a limited resection or a lobectomy (cancer survival: 63% vs 51%; overall survival: 50% vs 57%). Likewise, there was no statistically significant difference in overall survival (30% vs 25%) or cancer-related survival (45% vs 78%; p=0.09) between the 75-79 group and the 80+ group.

The effect of p53 mutation on prognosis

A total of 101 patients were included in this study; their characteristics are given in Table 3. Only patients with epidermoid and adenocarcinoma tumors were studied. Four patients with bronchiolo-alveolar carcinomas were included in the adenocarcinoma group. Lobectomy was performed on 53 patients (52%), bilobectomy on 13 (13%), pneumonectomy on 32 (32%), and non-anatomical resection on 3 (3%). The resection was incomplete in 33 (33%) of the cases. Seventeen patients (17%) had stage IA disease (Mountain 1997), 32(32%) had stage IB disease, 3 (3%) had stage IIA disease, 19 (19%) had stage IIB disease, 21 (21%) had stage IIIA disease, 4 (4%) had stage IIIB disease, and 5 (5%) had stage IV disease.

Of the 101 patients participating in this study, 48 (47%) were shown to have tumors with a p53 mutation. The mutations occurred more frequently in male patients, and in tumors with squamous cell histology. In both squamous cell carcinoma and adenocarcinoma, the mutations were evenly distributed across the various stage groups. In the contingency table analysis, the allocation of patients into a low-stage group (stage IA-IIB) and a high-stage group (stage IIIA-IV) did not correlate with the presence of p53 mutations (adenocarcinoma, p=0.86; squamous cell carcinoma, p=0.59).

Follow-up was maintained until the end of December 1997 . The overall four-year survival of the patients was 40% (40/101), mean survival 3.8 years (standard error (SE) 0.3 years). For those with squamous cell carcinoma, the four-year survival was 37%, and the mean survival 3.8 years (SE 0.4 years). For those with adenocarcinoma, four-year survival was 43%, mean survival 3.8 years (SE 0.5). The deaths were cancer-related in 75% of all the patients, in 73% of patients with squamous cell carcinoma, and 79% of patients with adenocarcinoma.

For patients with a wild type p53 gene, the overall four-year survival was 43% (23/53), with a mean survival of 4.0 years (SE 0.4 years). For those with a mutated p53 gene, the four-year survival was

35% (17/48), and the mean 3.45 years (SE 0.4 years). The deaths were cancer-related in 77% of the patients without a p53 mutation, and in 74% of those with a mutation.

Univariate analysis did not indicate a significant difference in the cancer-related survival of the p53 mutated cases in either histological group, but the p-value was suggestive (p=0.10) in adenocarcinoma. The analysis also suggested an association between p53 mutation and poor cancer-related survival in low-stage (IA-IIB) adenocarcinoma (p=0.08), but not in low-stage squamous cell carcinoma.

In the Cox multivariate analysis of overall survival, a high stage was significantly associated with poor survival in both histologies . In squamous cell carcinoma, heavy smoking (OR 1.9, 95% CI 0.9-3.8), defined as above the median of pack-years smoked, had a suggestive prognostic significance.

In the case of cancer-related survival the stage was of prognostic significance in both histological groups . In adenocarcinoma, p53 mutation was significantly associated with a poor prognosis (OR 3.5, 95% CI 1.1-12). In the stepwise model, p53 mutation assumed only suggestive significance (OR 2.2, 95% CI 0.9-5.6). Asbestos exposure was not associated with the overall survival or cancer-related survival of patients with squamous cell carcinoma or adenocarcinoma .

Immunohistochemistry for detecting lymph node metastases

Out of 36 patients operated upon with curative intent, 19 qualified for the study group. Their characteristics are given in Table 3. The tumor was on the right side in 10 patients(53%) and the left in 9 (47%). It was in the upper lobe in 13 patients (68%), and in the median or lower lobes in 6 (32%). Lobectomy was performed on 10 patients (53%), bilobectomy in 3 (16%), and pneumonectomy in 6 (32%). Four patients (21%) had stage IA disease (Mountain 1997), 6 patients (32%) had stage IB disease, 6 patients (32%) had stage IIB disease, none had stage IIIA diseasse, 1 patient (5%) had stage IIIB disease and 2 patients (11%) had stage IV disease. The TNM category of the stage IV patients was T1N0M1 in both cases. One had an epidermoid carcinoma and the other a bronchiolo-alveolar carcinoma. The metastases were in the contralateral ribs and in the contralateral lung (lingula), respectively, and were verified as present at the time of the primary resection.

Immunostaining with high-MW and low-MW cytokeratin antibodies and BerEP4 did not detect metastases in lymph nodes which were negative to routine hematoxylin and eosin staining.

The survival analysis was cut off in November 1998, when all the available follow-up data had been obtained from the Department of Oncology at Tampere University Hospital. The median follow-up time was 12 months (range 3-16 months).

Aggressive disease was found in three patients despite a T1-2N0 disease. The first of these patients was a 67 year old man who underwent left upper lobectomy for T2N0M0 squamous cell carcinoma. He had undergone a curative resection for carcinoma of the penis 21 years earlier, and had had no recurrences. An 8 cm metastasis in the remaining left lower lobe, as well as mediastinal and liver metastases, were seen on a CT scan 10 months after the lobectomy. Squamous cell carcinoma was verified from a fine needle biopsy of the liver. The second patient was a 67-year old man who underwent upper and median lobectomy for T1N0 adenocarcinoma of the right median lobe. There was a solitary 8 mm tumor in the lingula of the contralateral lung, seen on the preoperative CT. It was removed six weeks after the primary operation, and was histologically identical to the primary tumor. Both had some features of a clara cell bronchioloalveolar carcinoma. He also had a poorly differentiated (atypical) 17 mm carcinoid tumor in the left upper lobe. The third patient was a 64-year-old man who underwent left upper lobectomy for a T1N0 squamous cell carcinoma. He had a diagnosis of chronic alcoholism, and the tumor showed signs of lymph vessel invasion. There were several deposits in the ribs on the contralateral side on an isotope bone scan taken one month before surgery. In a control scan taken 6 months after surgery, multiple new bone deposits were seen at the same site, and also elsewhere in the skeleton. Ultrasonography demonstrated liver metastases. In these three patients with agressive disease, serial lymph node sectioning combined with immunostaining did not reveal further undetected metastases.

Reliability of computed tomography in lymph node staging.

A total of 26 patients underwent mediastinal lymph node dissection and qualified for surgical staging. Their characteristics are given in Table 3. The surgical procedures were exploratory thoracotomy and biopsy only in 1 patient (4%), wedge excision of tumor in 1 patient (4%), lobectomy in 13 patients (50%), bilobectomy in 3 patients (12%), and pneumonectomy in 8 patients (31%). Systematic mediastinal lymph node dissection was carried out in all except the seven cases where inoperable N2 disease was found. The metastatic sites were confirmed from frozen sections, and mediastinal dissection was not carried further. The surgical-pathological (pT and pN) categories of the patients were T1 in 23% (6/26), T2 in 62% (16/26), T3 in 4% (1/26) and T4 in 12% (3/26) (two cases with satellite tumors, one of pleural carcinosis at surgery); the N category was N0 in 54% (14/26), N1 in 19% (5/26) and N2 in 27% (7/26).

The T and N categories assigned to the 26 patients by a thoracic radiologist, compared to the pT and pN categories are shown in Table 5.

Table 5. T categories (A) and N categories (B) assigned by a thoracic radiologist (THR), compared to surgical-histopathological results (pT and pN) of 26 non-small cell lung cancer patients

	T4	0	0	0	3
	T3	0	1	0	0
THR	**T2**	1	10	1	0
	T1	5	5	0	0
		T1	**T2**	**T3**	**T4**
			pT		

A**.**

	N2	1	1	3
	N1	1	3	0
THR	**N0**	12	1	4
		N0	**N1**	**N2**
			pN	

B.

The concordance in T category between radiologists and the thoracic radiologist was 85%, between the radiologists' results and pT 62%, and between the thoracic radiologist's results and pT 69%. The T-category was underestimated by the radiologists in 35% (9/26), and overestimated in 4% (1/26). The T-category was underestimated by the thoracic radiologist in 23% (6/26), and overestimated in 8%

(2/26). The commonest error was to underestimate the tumor's size, placing too many tumors in the T1 category. This error constituted 67% (6/9) of the underestimations by the radiologists, and 83% (5/6) of the underestimations by the thoracic radiologist..

The concordance in the N category between radiologists and a thoracic radiologist was 65%, between the radiologists and the surgical-pathological results (pN) 54%, and between the thoracic radiologist and pN 69%. The N-category was underestimated by the radiologists in 31% (8/26) of cases, and overestimated in 15% (4/26). The N-category was underestimated by the thoracic radiologist in 19% (5/26) of cases, and overestimated in 12% (3/26). The commonest error was a failure to detect N2 disease, which constituted 75% (6/8) of the underestimations by the radiologists, and 80% (4/5) of the underestimations by the thoracic radiologist. N2 disease unsuspected by the radiologists was found at surgery in 23% (6/26) of the patients, and they failed to detect 86% (6/7) of the total N2 disease on the CT scans. Mediastinal metastases were not found at surgery in 75% (4/5) of patients classified as N2 by the radiologists. N2 disease undetected by the thoracic radiologist was found in 15% (4/26) of patients at surgery. The thoracic radiologist failed to detect N2 disease on the CT scans in 57% (4/7) of the patients with N2 disease. Mediastinal metastases were not found at sugery in 40% (2/5) of patients classified as N2 by the thoracic radiologist. In no case did the radiologists detect N2 disease that had been missed by the thoracic radiologist.

In 60% (3/5) of the N2 cases missed by the thoracic radiologist, the clinical T category was interpreted as T1 by both the radiologists and the thoracic radiologist. The tumor was on the left side in 80% (4/5), on the right side in 20% (1/5). It was in the upper lobe in 80% (4/5) and lower lobe in 20% (1/5). Two patients with left upper lobe tumors had metastases in the sub-aortic fossa, and the patients with right upper lobe tumor had a metastasis in the pretracheal nodes. Two patients with left lower lobe tumors had metastases in the lower mediastinum, but also in the subaortic fossa. One patient, with a left upper lobe T2 squamous cell carcinoma, had metastases only in the pulmonary ligament node.

None of the patients had a complication that could be directly attributed to the lymph node dissection, for example recurrent nerve paralysis, or adult respiratory distress syndrome-type changes in the remaining lobes. One patient developed a bronchopleural fistula after right-side pneumonectomy. He had purulent pneumonia in the removed lung at the time of operation, and had been treated with up to 30mg/day prednisone for several months before and also after surgery, because of obstructive pulmonary disease. For three to four days after the operation, several patients had a copious serous discharge from their pleural drains.

DISCUSSION

Reliability of the study

The advantage of the study were that all the patients were treated at two university hospitals, which followed broadly similar guidelines on eligibility for surgery, preoperative studies, surgical techniques and postoperative care. The follow-up data were based on accurate medical records and reliable mortality data from the Central Population Registry of Finland. The study involved patients from a Scandinavian country with a relatively homogeneous population, where modern health care is uniformly available to all citizens. This in itself further improves the reliability of the follow-up.

One significant limitation of the study was the small number of patients. There are approximately 2000 new cases of lung cancer in Finland each year, of whom some 500-600 are eligible for surgery. These patients are distributed among the five university hospitals, and occasionally patients are resected at central hospitals. The number of patients resected each year is therefore less than 100 per hospital, with most university clinics apart from Helsinki resecting only 30 to 50 patients a year. For this reason, the numbers of patients in all our studies were small. The study of bronchoplasty patients was only descriptive, as no statistical comparisons could be made using this small material. In the elderly patient group there was no reference group of mixed-age or younger patients for comparison with respect to surgical morbidity and mortality. Data on patients with different stages of disease had to be combined to form groups sufficiently large for analysis, and univariate analyses could not be applied between individual stage categories. This entailed a possible dilution of the effect of stage on prognosis in our studies.

Another limitation on reliability was that systematic lymph node dissection was not routinely performed until 1997, when it was introduced as a routine procedure at Tampere University Hospital. This, as well as the fact that general thoracic surgery is not a sub-speciality in Scandinavia, resulted in variations in the perioperative staging of lung cancer patients. Systematic lymph node dissection has doubled the detection rate of mediastinal node metastases, since the time when only nodes which appear suspicious on palpation are removed (Bollen et al. 1994). The lymph node dissections performed in these studies did not include mobilisation of the aortic arch on the left side. Mobilisation

of the aortic arch somewhat improves the detection of metastases (Izbicki et al. 1995), although fortunately metastases to the high paratracheal nodes are very rare if all other nodes are negative (Tateishi et al. 1994)

Bronchoplastic resections

Approximately 100 lung cancer resections were performed per year at Helsinki University Hospital, and a yearly bronchoplastic procedure rate of 1.4% was relatively constant during the 20 years studied. This is rather low, considering that a bronchoplastic resection can be performed in approximately 5-8% of patients with resectable lung cancer (Bennett and Smith 1978, Mountain 1986). The rarity of suitable patients with a central tumor but no mediastinal metastases, as well as unfamiliarity with the technique, are possible explanations. In 79% of our cases, the tumor was in the upper lobe. The lymphatic drainage of the right and left upper lobes is relatively constant so that upper lobectomy with bronchoplasty is therefore an adequate oncological-surgical procedure. In the case of the other lobes, or in the presence of nodal metastases, this adequacy has been questioned (Firmin et al. 1983, Shields 1989, Vogt-Moykopf et al. 1987).

The frequency of incomplete resections affects the number of patients likely to benefit in the long-term. The resection was incomplete in 21% of patients because of tumour infiltration discovered post-operatively. The minimum healthy margin that should be verified by frozen section is 1 cm at both bronchial resection lines (Belli et al. 1985), and 1.5 to 2cm is recommended (Paulson et al. 1970). Bronchoplasty was performed to palliate obstruction in 32% of cases, in spite of mediastinal metastases. Other modalities such as laser or diathermy resection and /or stenting are now available for palliation, so that an incomplete resection can no longer be recommended (Shields 1993).

The operative mortality of 8% associated with our bronchoplasty procedures is comparable to the 7.5% reported elsewhere (Tedder et al. 1992), and comparable to a post-pneumonectomy mortality of approximately 6%. However, the level is twice as high as that associated with standard lobectomy (Ginsberg et al. 1983, Shields 1989). Sleeve lobectomy mortality rates of only 0%-3.4% have been reported (Belli et al. 1985, Deslauriers et al. 1986, Baros and Djuric 1990). We only performed three sleeve pneumonectomies so no comparisons could be made, but a 20.9% operative mortality for this procedure has been reported (Tedder et al. 1992).

The complication rate was similar to that in previous reports: pneumonia in 6-7%, empyema in 2.8% and bronchopleural fistula in 3.5% (Tedder et al. 1992). We observed no clinically significant strictures

or anastomotic kinks in spite of several wedge resections. A sleeve resection is recommended to avoid such complications, whereas leaving a narrow segment of intact bronchus apparently offers no real benefit (Toomes and Vogt-Moykopf 1985). Suture granulomas were not encountered in spite of the use of non-absorbable sutures in some early patients. Interrupted absorbable sutures were mainly used, and are recommended for avoiding granulomas (Maggi et al. 1993, Vogt-Moykopf et al. 1987).

The overall 5-year survival of 30% is comparable to the 25-34% rates reported elsewhere (Roder et al 1987, Tedder et al. 1992, Vogt-Moykopf et al. 1982, Vogt-Moykopf et al. 1987). The cancer-related survival rates are comparable to a reported mean survival of 28.1-40.8% overall (Belli et al. 1985, Maggi et al.1993), and of 66% for stage I (5), 42-43.5% for stage I and II combined (Vogt-Moykopf et al. 1982, Vogt-Moykopf et al. 1987), 12-25% for stage III (Belli et al. 1985, Vogt-Moykopf et al. 1987), 37-67% for node-negative patients (Belli et al. 1985, Deslauriers et al 1986, Vogt-Moykopf et al. 1987), 10% for node-positive patients (Hambraeus 1993), and 42% after a complete resection (Vogt-Moykopf et al.1987). In our series, 15 incompletely and two completely resected patients received postoperative radiotherapy, but its effect on survival remains undetermined (Slater et al. 1991). There were no fatal complications or late bronchial fistulas associated with the radiotherapy. Other complications were not studied.

Bronchoplasty is an adequate cancer-surgical procedure in upper lobe orifice tumors, and can be used to obviate pneumonectomy. A better quality of life was experienced than after pneumonectomy (Vogt-Moykopf et al. 1987). Lung resection permanently reduces ventilation and oxygen uptake because of the loss of lung tissue, which is worsened by distension and an increase in the residual volume of the remaining tissue (Hambraeus 1993). If pneumonectomy is performed in elderly patients with a limited biological reserve, they will suffer additional co-morbidity from late pulmonary hypertension and subsequent ventilatory disability. The increasing availability of follow-up data on bronchoplasty, and technical advances such as perioperative videobronchoscopy and jet ventilation, as well as advances in postoperative care, are likely to encourage the contemporary surgeon to consider this procedure.

Surgery for lung cancer in the elderly

The contemporary life expectancies of 8.48 years in men and 10.7 years in women over 75 years of age (Statistics Finland 1997) support a surgical approach to lung cancer in the elderly Untreated lung cancer patients will live a median of 6 months, and very rarely over one year (Lee-Chiong Jr and Matthey 1993, Vrdoljak et al. 1994). The five-year survival after radiotherapy is usually 5-10%, with

reports of 17-21% in stage I disease (Haffty et al. 1988, Katano et al. 1997, O´Rourke and Crawford 1988).

The 9% overall operative mortality among our elderly patients, and the 15% mortality in our 80 years+ group were higher than the 3.7-6.6% reported for mixed-age patients (Deslauriers et al. 1994, Duque et al. 1997, Ginsberg et al. 1983), which suggests an age-related increase in risk. Advocates of lung cancer surgery in the elderly have reported similar mortality rates of 3-19% (Berggren et al. 1984, Borrelly et al. 1992, Damhuis and Schutte 1996, Naunheim et al. 1994, Osaki et al. 1994, Pagni et al. 1997, Riquet 1994, Sherman and Guidot 1987). Our complication incidence resembled reported rates in mixed-age series, viz. 17-27% major (Deslauriers et al. 1994, Kearney et al. 1994) and 32.4% total complications (Duque et al. 1997), and those for elderly patients, viz. 11-30% major and 42-67% total complications (Naunheim et al. 1994, Osaki et al. 1994, Pagni et al. 1997). Resection beyond lobectomy has been discouraged in elderly patients (Morandi et al. 1997, Pagni et al. 1997, Yellin 1994), yet successful pneumonectomies have been reported (Mizushima et al 1997). We observed that a resection beyond lobectomy is not contraidicated but carries an increased risk. A bronchoplasty can circumvent pneumonectomy in selected cases (Sioris et al. 1997). Safe, radical surgery therefore seems feasible in elderly patients, even with below-normal FEV1, which is probably an age-related phenomenon rather than an indicator of severe pulmonary disease.

The postoperative quality of life appears to be almost unimpaired. The mean hospital stay of 14.9 days accords with the 8.1-14 days reported for other elderly patients (Naunheim et al. 1994, Pagni et al. 1997). Postoperative performance status comparable with preoperative status is indicated by the 88% of patients discharged to their own homes. A rate as high as 94% has been reported (Pagni et al 1997).

The lower overall 5-year survival in our stage I patients, compared to the 51-67% previously reported (Ishida et al. 1990, Morandi et al. 1997), and of our stage IA patients compared to our stage IIB patients, is explained by a difference in non-cancer-related deaths between the patient groups studied. Surprisingly, the cancer-related 5-year survival rate was higher in stage II than in stage I, though the differences were not statistically significant. This may imply that the prognosis in various stages of limited disease is similar if the tumour is more advanced than T1 or has metastasised to regional, but not beyond hilar lymph nodes. The fact that lung cancer in the elderly presents at diagnosis with less metastatic and less extensive disease than in younger patients (De Maria Jr and Cohen 1987, Lee-Chiong Jr and Matthay 1993, O´Rourke et al. 1987, Sarma 1987,) may be attributable to biologically less aggressive tumors (O´Rourke and Crawford 1988), senescent host factors or obliterative changes in

the lymphatic system (Sarma 1987). Studies which have used the previous staging classification have not found a stage-related difference in long-term survival in patients older than 80 years (9). The revised (Mountain 1997) classification appears to be a better predictor of survival, as it separates stage I according to the extent of the tumor.

Long-term survival is indicated by a 56% relative 5-year survival in relation to an age- and sex-matched general population and comparable to a previously reported figure of 60.5% (Osaki et al. 1994). The survival rates declined as the stage progressed to or beyond IIIA in the revised classification (Mountain 1997), and curative removal of tumors seems warranted only in stages IA-IIB.

Limited resection has been recommended instead of lobectomy for octogenarians or patients with cardiopulmonary or major organ dysfunction (Takao et al. 1997). The Lung Cancer Study Group found a 75% increase in recurrence rate and a threefold increase in locoregional relapse, excluding second primaries, in patients undergoing limited resection rather than lobectomy for T1N0 lung cancer. However, there was no difference in 5-year survival (Lung Cancer Study Group 1995). There were no 5-year survivors in our 80+ group, and pulmonary resection in octogenarians has been recommended only for exceptional cases (Pagni et al. 1997). Limited resection is an adequate alternative to lobectomy if comorbidity favors that choice.

Our findings support investment of surgical resources in the treatment of elderly lung cancer patients. Individual planning of resection, taking into account both cancer extent and co-morbidity, is essential to ensure that the acute risk does not outweigh the potential long-term benefit. However, even octogenarians who are otherwise healthy can return to their homes after successful surgery for lung cancer.

The effect of p53 mutations on prognosis

P53 mutation is more commonly observed in squamous cell carcinoma (59-61%) than in adenocarcinoma (33-38%) (Fukuyama et al. 1997 Husgafvel-Pursiainen et al. 1999, Ridanpää et al. 1994), which is in line with our findings. The association with higher stage may be due to a specific type of mutation (Lee et al. 1994), and was not observed in our study.

The multivariate analysis indicated that a p53 mutation significantly reduced the cancer-related survival of adenocarcinoma patients. In squamous cell carcinoma patients, the presence or absence of p53 mutation was not related to survival.

Our findings are in keeping with those in other studies, which suggest that p53 alterations - either mutations or protein accumulation - predict poor survival in lung cancer patients (Quinlan et al. 1992, Mitsudomi et al. 1993, Kwiatkowski et al. 1998, Levesque et al. 1998,). Certain investigators have associated p53 alterations with a poor prognosis specifically in adenocarcinoma (Horio et al. 1993; Mitsudomi et al. 1995; Nishio et al. 1996; Fukuyama et al. 1997; Huang et al. 1998) and others only in squamous cell carcinoma (Levesque et al. 1998). These partially contradictory findings may be due to differences in the timing of p53 mutation with respect to tumor development and clonal expansion in adenocarcinomas and squamous cell carcinomas (Fukuyama et al. 1997, Li et al. 1994). Other independent prognostic factors, as yet unaccounted for, may also be involved.

Disease stage and completeness of resection are well-established prognostic factors. A late stage was, as expected, significantly associated with an unfavorable prognosis in both squamous cell carcinoma and adenocarcinoma. The same was observed for both cancer-related and overall survival. The completeness of resection was mainly associated with stage and did not emerge as an individual prognostic marker.

The Kaplan-Meier analysis suggested that p53 mutation is associated with poor prognosis in stage IA-IIB adenocarcinoma, but not in squamous cell carcinoma. This is in line with the results of our multivariate analysis. The number of late-stage cases was small because we were only looking at potentially operable patients, and meaningful analyses could not be undertaken. To observe the effect of p53 alteration in such a heterogeneous group is also extremely difficult (Fukuyama et al. 1997).

Two well-known etiological factors underlying lung cancer, tobacco smoking and occupational exposure to asbestos fibres, did not affect cancer-related survival in the multivariate model. However, poor overall survival was associated with heavy smoking (defined by more than median pack-years) in squamous cell carcinoma patients. A comparison with non-smokers could not be made, because they were too few. This would indicate the importance of other smoking-related diseases as causes of death, even among lung cancer patients.

The published work on p53 alterations and cancer prognosis in NSCLC is still controversial. In part this may be due to the variety of methods used to examine p53 mutation status. Poor concordance between the presence of a p53 mutation and immunohistochemical p53 detection (Hussain and Harris 1998), often the method of choice in prognostic studies, may explain some of the discrepancies. Different types of the p53 protein are known to have specific biological functions (Hussain and Harris 1998), and it is possible that alterations in certain regions may have distinct prognostic consequences.

Studies on breast cancer have indicated that mutations in the core domain of the gene, resulting in conformationally altered non-functional protein, are related to the poorest survival (Borresen et al. 1995).

In theory, molecular staging can predict cancer cell aggression and be used to select patients for adjuvant treatment trials for whom a poor prognosis is to be expected in spite of a curative resection (Mountain 1995). However, no single molecular marker has sufficed to replace lymph node staging in predicting prognosis (Pastorino et al. 1997, Scagliotti et al. 1995). Based on the present findings, and given the increasing incidence of adenocarcinoma observed world-wide, further investigation of the significance of the p53 mutation as a prognostic factor would seem justified especially in lung cancer of this histology.

Immunohistochemistry for detecting lymph node metastases

We found no immunohistochemically detectable micrometastases in our 19 patients who had histologically normal regional lymph nodes. This is somewhat unexpected in the light of a 15.7% incidence of nodal micrometastases in lung cancer found using BerEP4 (Passlick et al. 1996, Kubuschok et al. 1999), and 6% when using anti-cytokeratins in one study (Nicholson et al. 1995), and 63% in another (Chen et al. 1993). We believe this is due to our small material. Serial sectioning of the lymph nodes of all patients would have minimised any sampling error. It increases the detection rate, but has not gained widespread acceptance because of a poor cost-benefit ratio (Nicholson et al. 1997) . More than three sections per node has been claimed to be impractical for routine use (Passlick et al. 1996). Moreover, in 80% of the patients studied using BerEP4, micrometastases were always found in more than one of the three lymph node sections (Kubuschok et al. 1999), and the frequency of detection of micrometastases did not reflect the extent of nodal sectioning, as further sectioning of immunohistochemically negative nodes did not detect tumor cells (Chen et al. 1993).

The effect of micrometastases on survival could not be determined, as we had no such patients. Impaired prognosis has been demonstrated in node-negative lung cancer patients with nodal micrometasases detected by cytokeratin immunostaining (Chen et al. 1993), and the same technique has predicted early recurrence in colorectal carcinoma patients (Sasaki et al. 1997). Lung cancer patients with nodal micrometastases detected by BerEP4 had impaired survival (Izbicki et al. 1996, Passlick et al. 1994, Passlick et al. 1996), and the presence of such micrometastasees was an independent marker for poor prognosis for five-year survival in a prospective study (Kubuschok et al.

1999). The capability of a few immunohistochemically demonstrable cancer cells to develop into a true metastasis has also been questioned, as no effect was observed on the survival of lung cancer patients with micrometastases detected with cytokeratin antibodies (Nicholson et al. 1997). However, the presence of cytokeratin-detected cancer cells in the bone marrow of lung cancer patients has been linked to a higher rate of recurrence (Cote et al. 1995, Pantel et al.1996) and impaired prognosis (Ohgami et al. 1997).

We had two stage I patients with systemic metastases at the time of surgery, and one who suffered regional and systemic recurrences within a year. This is in line with a 14% incidence of M1 disease on presentation in T1N0 patients (Quint et al. 1996), and with a 20% incidence of systemic relapse in completely resected stage I (T1-2N0M0) disease (Martini et al. 1995). The regional relapse was probably due to an intrapulmonary metastasis (Yoshino et al. 1997) in the left lower lobe, and subsequent metastases into the mediastinum and liver. The histologically identical tumor in the lingula was either a metastasis or a synchronous primary lung carcinoma, which occur in 0.8% to 1% of cases (Pommier et al. 1996). The patient in question had a poorly differentiated carcinoid tumor in the right upper lobe, which might indicate a tendency to develop multiple tumors. The patient with bone metastases in the contralateral ribs at the time of surgery had a long history of heavy alcohol abuse, which may have weakened his immune system both directly and via malnutrition. He also had lymph vessel invasion of the tumor, which has been associated with a poor prognosis (Ichinose et al. 1994).

A histological as well as immunohistochemical study of all lymph nodes was made at 50 micron intervals to exclude insufficient sampling in patients with aggressive disease, considering that 1-3 tumors cells will have a thickness of approximately 10-60 microns (Nicholson et al. 1997). The epitope detected by BeEP4 can be inactivated by formaldehyde fixation for more than 48 hours, and lung cancer has a weaker expression of the Ber-EP4 antigen than other cancers, for example breast cancer (Latza et al. 1990). However, adequate staining of paraffin-embedded material has been noted by other authors (Passlick et al. 1996, Kubuschok et al. 1999) even though they used frozen section material. The detection rate and sensitivity of cytokeratin antibodies in paraffin-embedded material has been so high (Chen et al. 1993) that one might suspect an error towards false positives (Nicholson et al. 1997). Furthermore, it has been shown that the presence of immunohistochemically demonstrable bone marrow micrometastases and lymph node metastases are independent of each other, supporting the view that different determinants must exist for the lymphatic and systemic spread of tumor cells (Passlick et al. 1996); this is in accord with our findings.

Preoperative studies such as CT, magnetic resonance imaging (MRI), positron emission tomography (PET) or routine mediastinoscopy cannot detect lymph node metastases with complete accuracy (DeLeyn et al. 1997, Gdeedo et al. 1997, Izbicki et al. 1992 Vansteenkiste et al. 1997). Systematic perioperative lymph node dissection is essential for accurate staging (Bollen et al. 1993), but a routine histopathological examination of one to three sections per node will not detect minimal metastases (Chen et al. 1993, Passlick et al. 1996, Kubuschok et al. 1999). According to the literature, immunohistochemistry appears to be a tool worth further study for staging lung cancer patients. Cytokeratins are expressed by all non-small cell lung cancers (Chen et al. 1993), but can react with benign mesothelial inclusions in the lymph nodes, thus not being completely specific (Passlick et al. 1996). BerEP4 is more specific than the cytokeratins, as it does not react with mesothelial cells or lymphatic tissue. It can be used on either frozen or paraffin-embedded tissue (Kubuschok et al. 1999, Passlick et al. 1996, Nicholson et al. 1997).

Immunohistochemistry yielded no more information than standard histology in our series, but we had so few patients that no conclusions could be drawn. It was an easy technique to apply, especially using automated immunostaining. The absence of micrometastases in lymph nodes, even in stage I patients, will not assure a good prognosis, and other predictors of cancer cell aggression must be found to complement nodal staging.

Reliability of computed tomography in lymph node staging

Our interobserver rates of accord of 85% for the T category and 65% for the N category are similar to those found in the literature: 73-90% for the T category, and 58- 61% for the N category (Gyatt et al. 1995, Webb et al. 1993). Our 69% rate of accord for the T category and 69% for the N category between the CT interpretation of a thoracic radiologist and the surgical pathological results, are comparable to the reported 81.6% concurrence for the T category and 55.3%-84.2% concurrence for the N category (Fernando and Goldstraw 1990, Lewis et al. 1990, Lähde et al. 1991, Gyatt et al. 1995, Webb et al. 1993). Even though thoracic radiologists appeared to obtain more accurate results than on-duty radiologists, statistical comparisons could not be made because there were so few patients.

We used a 10-mm size limit for the normality of lymph nodes, as recommended by several authors (Daly et al. 1993, Kayser et al. 1990, Goldstraw et al. 1994). In a lymph node size criteria study on squamous cell carcinoma, optimum sensitivity and specificity, both of 76%, were obtained using a size criterion of 9.3-mm (Kobayashi and Kitamura 1995).

Unsuspected N2 disease was found in 15 % of our cases, of whom 60% had clinical T1 disease. Similar percentages of 12-25% for unsuspected N2 found at surgery are reported elsewhere (Fernando and Goldstraw 1990, Ishida et al. 1990, Seely et al. 1993). Some metastatic nodes appeared normal until cut open, and some sites such as the subcarinal space could not be evaluated by palpation of the unopened mediastinal pleura. The detection rate for N2 disease doubles if systematic nodal dissection is performed, compared to only removing nodes suspicious on mediastinal palpation (Bollen et al. 1993). This supports the need for complete nodal dissection (Mountain 1994, Naruke 1993). Our patients were too few for any comparison to be made with studies which have observed more N2 metastases and undetected N2 disease in adenocarcinoma patients (Libshitz et al. 1986). We observed no increased morbidity related to lymph node dissection, aside from the increased fluid secretion which has been observed by others (Bollen et al. 1993, Izbicki et al. 1995). Even though resectability can be fairly accurately predicted by CT (Daly et al. 1993, Lähde et al. 1991), the accuracy of staging in terms of lymph node status is poor and varies from 50% to 81% (Arita et al. 1995, Cole et al. 1995, Fernando and Goldstraw 1990).

New chemotherapeutic agents alone or in combination with either radiotherapy or surgery can improve survival and the quality of life for patients with locally advanced stage disease, and may increase the cure rate for earlier stage patients (Bunn and Soriano 1998, Einhorn 1998, Evans 1997). Accurate preoperative staging, possibly complemented by molecular staging in the future, is becoming essential for treatment decisions, and non-invasive preoperative staging alone is likely to become insufficient.

CONCLUSIONS

1. Bronchoplastic resection can be applied in selected cases where lung cancer extends to or near the main bronchus. It is an adequate cancer-surgical procedure, resulting in five-year survival.

2. Resection with curative intent is justifiable in elderly patients, even those of 80 years or more. Resection beyond lobectomy appears to increase the rate of complications. A 50-60% survival rate, relative to a standard population, indicates long-term survival. Only stage IA patients had a 5-year survival rate significantly different from that in other stages.

3. A p53 mutation appears to be a significant adverse prognostic factor in adenocarcinoma of the lung, but not in squamous cell carcinoma.

4. Immunostaining of lymph nodes with cytokeratin and Ber-EP4 antibodies did not reveal undetected metastases, even in patients with aggressive metastatic disease.

5. Preoperative computed tomography cannot reliably predict the lymph node status of lung cancer patients; lymph node dissection is needed.

SUMMARY

Lung cancer is the commonest cause of cancer deaths world-wide. The elderly population is increasing, and consequently so are the numbers of those at risk of developing lung cancer due to cumulative life-time exposure to cigarette smoke. The majority of patients are beyond curative resection when diagnosed by reason of disseminated cancer or smoking-related co-morbidity. Until recently there has been no effective systemic treatment for lung cancer. Developments in multimodality therapy have now shown promising results in several small series of patients. This has underlined the importance of being able to detect those patients who have a poor prognosis even after a curative resection. The presence of lymph node metastases is the most important prognostic factor in lung cancer. However, even if the tumor is limited to the lung, and no nodal metastases are histologically detectable, approximately one third of patients will relapse with cancer within five years of a complete resection. Molecular methods which detect genetic mutations in the tumor may predict cancer cell aggression, while immunohistochemical methods can detect occult metastases of one to three cells in apparently histologically normal lymph nodes. Systematic perioperative lymph node dissection has become an essential part of accurate surgical staging.

Bronchoplastic resections undertaken to circumvent pneumonectomy in lung cancer patients, and considered radical, resulted in a 40% cancer-related 5-year survival.

In patients 75 years old or older who underwent surgery for lung cancer, a 56% relative 5-year survival compared to an age- and sex-matched standard population was achieved. Thirteen patients who were 80 years old or older were included in the study. While the mortality did not differ, the complication rate was significantly higher for procedures beyond lobectomy. Only stage IA patients had a cumulative 5-year survival which diverged from that of the other stages combined. After surgery, 88% of the patients were able to return to their homes.

The presence of a genetic mutation of the p53 tumor supressor gene was determined by molecular methods in resected epidermoid lung carcinoma and adenocarcinoma patients. In multivariate analysis, a p53 mutation proved to be a statistically significant adverse prognostic factor in adenocarcinoma patients, but not in epidermoid carcinoma.

The usefulness of immunostaining, using cytokeratin and Ber-EP4 antibodies, in detecting occult metastases in systematically removed intrapulmonary and mediastinal nodes which were normal upon

histological examination was studied. Immunohistochemistry did not detect occult metastases, even when systemic metastatic disease was present.

The lymph node stage, as determined by preoperative CT, was compared to the surgical-pathological stage in lung cancer patients who underwent systematic perioperative lymph node dissection. The lymph node status accorded with the surgical pathological stage in 69% of cases. Unsuspected mediastinal node metastases were found at surgery in 15% of patients.

Bronchoplastic resection is an adequate oncological surgical procedure which can be used to obviate pneumonectomy in selected cases. Radical lung cancer surgery is justified in elderly patients, and a good postoperative course of events can be expected, but the extent of the resection should be carefully and individually considered. Molecular staging and the prognostic effect of a genetic p53 mutation, especially in adenocarcinoma, should be further studied in order to identify resected patients with a poor prognosis. The absence of detectable regional lymph node metastases, even when immunohistochemically sought, does not ensure a good postoperative prognosis. CT is not reliable for the accurate preoperative staging of intrathoracic lymph nodes.

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Thanos Sioris

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