Table 1: Patients who underwent VATS anatomical resection during this study period (n = 179)

Variables	No. of patients (%)
Age, median (years, range)	68 (26–87)
Sex	
Men	88 (49)
Women	91 (51)
Diagnosis	
Lung cancer	165 (92)
Metastatic lung tumour	6 (3)
Benign lung disease	8 (5)
Type of surgery	
Lobectomy	172 (96)
Right upper lobectomy	54
Right middle lobectomy	17
Right lower lobectomy	41
Right middle and lower lobectomy	2
Left upper lobectomy	37
Left lower lobectomy	21
Segmentectomy	7 (4)
Left S1+2–3	3
Right S3	1
Right S6	1
Left S4 + 5	1
Left S8–10	1
Preoperative 3D imaging	
Present	124 (69)
Absent	55 (31)

Table 2: Patients who underwent VATS anatomical resection with the preoperative 3D imaging (n = 124)

Variables	No. of patients (%)
Age, median (years, range)	68 (35–87)
Sex	
Men	60 (48)
Women	64 (52)
Diagnosis	
Lung cancer	115 (93)
Metastatic lung tumour	5 (4)
Benign lung disease	4 (3)
Type of surgery	
Lobectomy	119 (96)
Right upper lobectomy	38
Right middle lobectomy	13
Right lower lobectomy	27
Right middle and lower lobectomy	1
Left upper lobectomy	26
Left lower lobectomy	14
Segmentectomy	5 (4)
Left S1 + 2–3	2
Right S6	1
Left S4 + 5	1
Left S8–10	1
Conversion from VATS to thoracotomy	5 (4)
Operative time, mean (min, range)	230 (132–444)
Bleeding, mean (ml, range)	110 (0–1406)
Postoperative complications (Grade ≥ 2)	
Present	10 (8)
Arrhythmia	3
Prolonged air leakage	2
Chylothorax	1
Bacterial pneumonia	1
Bleeding	1
Empyema	1
Recurrent nerve paralysis	1
Absent	114 (92)
30-day mortality	0
90-day mortality	0

(96%) underwent lobectomy. This study involved 124 patients (69%) in whom 3D imaging was performed preoperatively and 55 patients (31%) in whom 3D imaging was not available because of contraindication to the use of contrast radiography (e.g., allergies to contrast medium, severe diabetes or bronchial asthma) or patient refusal against repeated imaging studies.

The characteristics and surgical outcomes of 124 patients undergoing 3D imaging are given in Table 2. There were 5 (4%) conversions from VATS to thoracotomy because of vessel bleedings. The frequency of patients presenting with complications with Grade 2 or above was 8% (n = 10), and there were no 30-day or 90-day operative mortalities.

According to intraoperative findings, PA branches were precisely identified on the basis of preoperative 3D-CT imaging (Table 3) in 97.8% (309 of 316) of vessels and 94.4% (117 of 124) of patients. There were 7 patients with undetected PA branches including 5 right upper lobectomies (RULs) and 2 left upper lobectomies (LULs). Undetected PA branches were the truncus arteriosus superior in 3 patients, the ascending artery in 2 patients, and the apical artery and lingular artery in 1 patient each. A representative image of an undetected ascending artery is shown in Fig. 1. The actual sizes of the 7 missed branches in the 7 patients were all less than 2 mm. The 3D image findings in PA branches were identical to operative findings in cases other than upper lobectomy. The number of patients with anomalous or uncommon PA branching patterns was 15 (12%). A summary of the 3D image findings in patients with anatomical variants of the PA is given in Supplementary material, Table S1. All anomalous or uncommon PA branches were accurately confirmed by 3D imaging. Of the 26 patients receiving LUL, 5 patients (19%) had anatomical variants of mediastinal lingular branches of the PA, which included the A4 + A5 type and either the A4 or A5 type. In addition, there were 5 patients with lingular PA arising from the basilar artery, 2 patients with 2 superior segmental arteries coming directly from the main PA, 2 patients with double ascending arteries, 1 patient without an ascending artery and 1 patient with triple middle lobe branches (Fig. 2). The 3D imaging showed 5 patients with anomalous PVs (Supplementary material, Table S2).

We evaluated the relationship between various clinical factors and the occurrence of postoperative complications (Table 4) or overall operative time (Table 5) in 165 patients with primary lung cancer. Sex (P = 0.002), pulmonary function test of forced expiratory volume in 1 s % (FEV1.0%; P = 0.011), and the presence or absence of respiratory comorbidities including chronic obstructive pulmonary disease, interstitial pneumonia, bronchial asthma etc. (P = 0.018) were found to be associated with the occurrence of complications. Conducting the preoperative 3D imaging tended to have associations, but the difference was not statistically significant (P = 0.054). On multivariate logistic regression analysis for these statistically or marginally significant factors, male gender was shown to be the only statistically significant independent predictor (risk ratio: 5.432, P = 0.013, Hosmer-Lemeshow χ^2 test = 0.89, P = 0.641), and 3D imaging also tended to be associated with the occurrence of complications (risk ratio: 2.852, P = 0.074).

There were significant associations between total operative time (dichotomized at mean operative time, 237 min) and conducting

Table 3: Identification rate of the 3D imaging in pulmonary artery branches according to type of surgery

Variables	No. of patients (%)	No. of PABs involved in resection		Identification rate (%)		Undetected PABs (no. of patients)
		3D images	Surgical findings	A per-vessel basis	A per-patient basis	
Overall	124 (100)	309	316	97.8	94.4	1 mm (1)/2 mm (6)
Type of surgery						
Right upper lobectomy	38 (31)	84	89	94.4	86.8	1 mm (1)/2 mm (4)
Right middle lobectomy	13 (10)	24	24	100	100	-
Right lower lobectomy	27 (22)	55	55	100	100	-
Right middle and lower lobectomy	1 (1)	2	2	100	100	-
Left upper lobectomy	26 (21)	99	101	98.0	92.3	2 mm (2)
Left lower lobectomy	14 (11)	33	33	100	100	-
Segmentectomy	5 (4)	12	12	100	100	-
Uncommon PAB pattern	15 (12)	50	50	100	100	-

PABs: pulmonary artery branches.

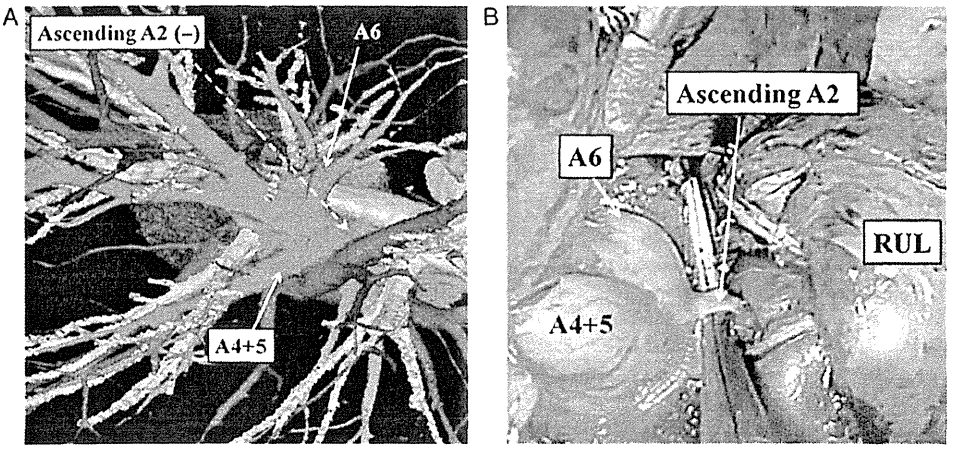

Figure 1: (A) A 3-dimensional computed tomographic image of the right pulmonary vessels. In this patient, a right upper lobectomy was performed. An ascending artery was not detected. (B) The intraoperative findings in this patient demonstrated the 2-mm ascending artery branches from the pulmonary artery.

the 3D imaging (risk ratio: 2.282, $P = 0.021$) and intraoperative blood loss (risk ratio: 1.005, $P = 0.005$) on univariate and multivariate analysis (Hosmer–Lemeshow χ^2 test = 5.92, $P = 0.656$).

DISCUSSION

We set out to identify the effectiveness of 3D-CT imaging for preoperative assessment of the branching patterns of pulmonary vessels and short-term surgical outcomes. A total of 97.8% of PA branches were precisely identified and all anomalous or uncommon PA and PV branching patterns were accurately confirmed by 3D imaging. In addition, patients undergoing preoperative 3D imaging tended to have lower incidences of postoperative complications and have significantly shorter operative time than those without the 3D simulations.

In reports in the literature concerning patients undergoing thoracoscopic and open surgery, 95–98% of PA branches were preoperatively identified using 3D-CT angiography, similar to our results [10–12]. Several authors also studied anomalous PA, PV or bronchial variations for surgery using 3D reconstruction [9, 12–15]. 3D simulation is considered to be useful in performing anatomical segmentectomy for small lung tumours for identifying the intersegmental veins as boundary lines of the pulmonary segments in order to determine the surgical margins using lateral 3D images and to identify the target segmental bronchi using vertical 3D images before segmentectomy [8, 16, 17]. These reports show that intraoperative visual guidance of the target pulmonary vessels and bronchi, and their relationship to one another as revealed by high-quality 3D images, could help thoracic surgeons perform safer anatomical lung resection and be prepared for more complicated operations.

In our clinical experience with using the Synapse Vincent software, there have been advantages of the 3D system that are based on volume-rendering techniques. First, a surgeon without expert knowledge concerning synthetic imaging can quickly and easily construct 3D images of each patient. The mean processing time required to construct a 3D angiographic image is approximately 5 min. The 3D imaging also allows us to freely rotate the objects and change the dimensions of images. The virtual 3D-CT can provide an overview of the 3D relationships of the pulmonary vessel pathways and the tracheobronchial tree. Thus, it is employed for preoperative simulations, which also help educate trainees about surgical anatomy. Secondly, unlike the currently

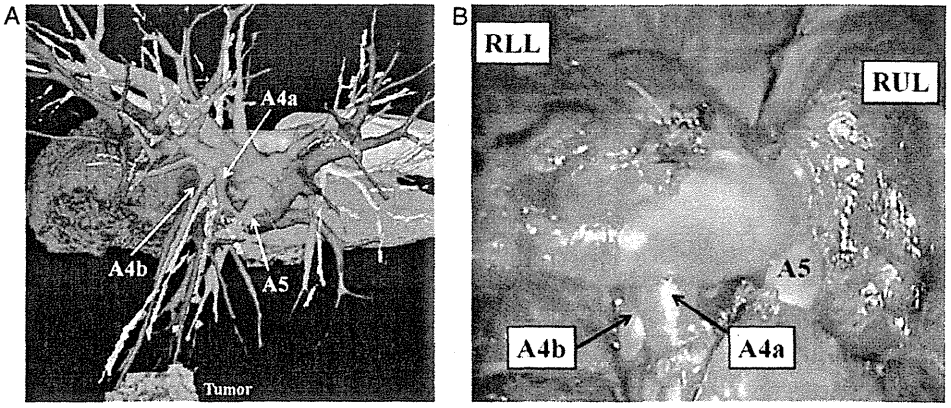

Figure 2: (A) The 3-dimensional computed tomographic image of the right pulmonary vessels showed that this patient had triple middle lobe pulmonary artery branches. (B) The intraoperative findings of this patient showed triple middle pulmonary artery branches corresponding to the 3-dimensional computed tomographic image.

Table 4: Association between clinical factors and the development of postoperative complications in patients who underwent VATS anatomical resection with primary lung cancer (n = 165)

Variables	Postoperative complications		Univariate analysis	Multivariate analysis		
	Present	Absent	*P*-value	RR	95% CI	*P*-value
Overall	18	147				
Sex						
Men	15	65		5.432		
Women	3	82	0.002	1	1.427–20.683	0.013
Age, median (years, range)	69 (27–87)		0.46			
Clinical staging						
IA	11	104				
IB	5	28				
IIA	1	7				
IIB	1	3				
IIIA	0	4				
IIIB	0	1	0.81			
Tumour location						
Right upper lobe	4	47				
Right middle lobe	2	14				
Right lower lobe	4	35				
Left upper lobe	6	34				
Left lower lobe	2	17	0.87			
Preoperative 3D imaging						
Present	9	106		1		
Absent	9	41	0.054	2.852	0.904–8.999	0.074
FEV1.0%, median (%, range)	74 (44–89)		0.011	0.962	0.891–1.039	0.861
Operative procedure						
Lobectomy	18	143				
Segmentectomy	0	4	0.48			
Conversion from VATS to thoracotomy						
Present	0	6				
Absent	18	141	0.38			
Intraoperative blood loss, mean (ml, range)	112 (0–1406)		0.89			
Operative time, mean (min, range)	237 (131–455)		0.65			
Respiratory comorbidity						
Present	8	29		1.153	0.233–5.716	
Absent	10	118	0.018	1		0.25
Cardiovascular comorbidity						
Present	1	10	0.51			
Absent	17	137				
Diabetes						
Present	0	7	0.34			
Absent	18	140				

RR: risk ratio; CI: confidence interval; FEV1.0: forced expiratory volume in 1 s.

Table 5: Association between clinical factors and operative time (<237 min vs ≥237 min) in patients who underwent VATS anatomical resection with primary lung cancer (n = 165)

Variables	Univariate analysis, *P*-value	Multivariate analysis		
		RR	95% CI	*P*-value
Operative time, mean (min, range)	237 (132–455)			
Sex	0.26			
Age	0.15			
Clinical staging: IA/IB/IIA/IIB/IIIA/IIIB	0.25			
Tumour laterality: right/left	0.77			
Preoperative 3D imaging: present/absent	0.019	2.282	1.131–4.604	0.021
Conversion from VATS to thoracotomy: present/absent	0.77			
FEV1.0%	0.31			
Intraoperative blood loss	0.001	1.005	1.001–1.009	0.005
Operative procedure: lobectomy/segmentectomy	0.21			
Respiratory comorbidity: present/absent	0.08			
Cardiovascular comorbidity: present/ absent	0.24			
Diabetes: present/absent	0.68			

RR: risk ratio; CI: confidence interval; FEV1.0: forced expiratory volume in 1 s.

available 3D-CT software programs, this system can show 3D images of the PA and the PV separately from the data of only one conventional CT scan. It can thereby reduce the radiation exposure dose. In contrast to the conventional method, we do not have to inject contrast media rapidly, and the infusion rate is sufficient at 1.5–2 ml/s. Consequently, we have not had any leakage accidents during contrast media infusion.

The disadvantages of 3D imaging include its potential deficiency in identifying bilateral upper lobe PA branches owing to the anatomically complex overlap of PA and PV branches. The ascending artery and truncus superior artery are often misidentified and confused with the apical segment vein or interlobar veins on 3D imaging. This might be due to the far more complicated ramification patterns of the PA in the upper lobe, particularly in the right upper lobe, than in the middle and lower lobes. However, we postulate that 3D imaging should be deemed acceptable because of the relatively low frequency of undetected PA branches.

The optimal strategy of managing postoperative complications of VATS anatomical resection is to prevent their occurrence. Perioperative complications and mortality with VATS lobectomy have been reported to occur at rates of ~5–32% and 0–7%, respectively [2, 18–20]. In the present study, postoperative complications in patients undergoing 3D imaging occurred in 8% with no mortality, and the risk of development of any complication in patients with 3D imaging was lower in comparison to those without 3D imaging. Notably, preoperative 3D simulation as well as intraoperative bleeding amount had significant association with total operative time. Possible reasons to explain these results are the assumptions that detailed surgical simulation and shared virtual lung anatomical information provided by 3D images between operating surgeons and a thoracoscopist might improve the safe and efficient performance of VATS, without causing vascular injuries due to unusual PA branching patterns, and thereby support a calm and efficient setting during lung resection.

The frequency of conversion from VATS lobectomy to open thoracotomy has been reported to range from 2% to as high as 23% [18, 19, 21, 22]. Although the most crucial concern with unexpected conversion to open thoracotomy are the possible increases in the risk of mortality and the development of complications, no postoperative complications arose in our 5 patients undergoing the 3D-CT (date not shown). Depending on the skill and ability to predict which patients are more likely to require conversion, the occurrence of serious complications can often be avoided. 3D information will be useful in training surgeons learning VATS procedures by shared real-time imaging with an experienced surgeon, influencing a surgeon's learning curve.

The limitations of this study are its retrospective nature and potential bias. Patient selection bias in conducting the preoperative 3D imaging may influence the result of short-term benefits in adverse events and operative time. To truly show the benefits of 3D software, a prospective randomized trial is needed.

In conclusion, this study demonstrated that preoperative simulations using 3D-CT angiography for the assessment of pulmonary vessel branching patterns appear to be beneficial for the safe and efficient performance of VATS anatomical resection and for further understanding of the surgical anatomy related to general thoracic surgery. Further advances in 3D-CT imaging technology will be useful in the development of not only VATS and open thoracotomy, but also robotic surgeries and cognitive and technical surgical education systems, without exposing patients to unnecessary risks.

SUPPLEMENTARY MATERIAL

Supplementary material is available at *EJCTS* online.

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Intractable Obstructive Endobronchial Granulation Caused by Surgical Materials After Sleeve Resection for Tracheal Carcinoma

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A 64-year-old woman underwent tracheal sleeve resection for adenocarcinoma. Thirteen months later minimal granuloma occurred at the anastomosis. Subsequently she had dyspnea from obstruction caused by the increasing size of the granuloma, which necessitated 4 repeated endobronchial debulking procedures and topical mitomycin C (MMC) application. However, the symptoms and granulation failed to resolve. Eventually, the granulation tissue and pledgeted sutures were removed from the anastomotic site using rigid bronchoscopy. Follow-up after 8 months showed no recurrence of symptoms, and the granuloma had resolved. Despite improvements in surgical suture material, removal of stitches should still be considered in the management of anastomotic obstruction caused by indolent and intractable granulation after tracheal resection or bronchoplasty.

(Ann Thorac Surg 2014;98:2200–2)

Despite changes in surgical techniques and increased experience in tracheobronchial sleeve resection, anastomotic complications remain a significant source of morbidity and occasional mortality. In certain cases, management of granulation tissue formation in the anastomosis is difficult despite all attempts. In this report, we describe a patient who experienced tracheal stenosis because of intractable benign granulation tissue after sleeve resection for tracheal carcinoma.

A 64-year-old woman who presented with cough was referred to our hospital for definitive therapy of tracheal adenocarcinoma. She had never smoked, her history was of hypertension and hyperlipidemia, and her family had no history of cancer. She underwent tracheal sleeve resection through a median sternotomy; 3 tracheal cartilaginous rings, which were located from rings 4 to 6 above the carina, were transected. End-to-end anastomosis was performed with polypropylene sutures (Prolene 3-0; Ethicon, Somerville, NJ), using a continuous stitch for the membranous wall and an interrupted single stitch for the cartilaginous part. During anastomosis, the needle cut through the cartilage rings on the right dorsal wall of the trachea. The injury was repaired using coated braided polyester sutures (Ticron with pledget 3-0, Covidien, Mansfield, MA) and was then wrapped circumferentially with a thymic flap. Pathologic diagnosis was adenocarcinoma, T2N0M0.

Surveillance bronchoscopy on the fourth postoperative day was unremarkable for stricture, granulations, or ischemic findings. On follow-up after 13 months, a swollen subcarinal lymph node was seen on a chest computed tomographic scan. Endobronchial ultrasonographically guided transbronchial needle aspiration of this lymph node did not show malignant cells. During bronchoscopy, there was an incidental finding of minimal granulation tissue on the right dorsal side of the tracheal anastomosis (Fig 1A). Subsequently there was further growth of the granulation tissue, necessitating 4 repeated debulking procedures through flexible bronchoscopy over a span of 6 months.

After 3 months of conservative management, she presented with stridor and dyspnea. Bronchoscopy revealed that the granulation tissue had grown, obstructing the tracheal lumen by 50% (Fig 1B). Topical mitomycin C (MMC) 0.4 mg/mL was applied to the granulation tissue. However, there was no change 3 weeks after MMC therapy (Fig 1C), so we removed the granulation tissue with an electrocautery snare using rigid bronchoscopy. After removal, a portion of the Ticron suture and white material were seen embedded in the granulation tissue (Fig 1D). These were removed by using flexible scissors and rigid forceps. Pathologic findings revealed granulation and an artificial foreign body with bacterial mass, not cartilage. On hindsight, this was the pledget used during the previous tracheobronchial tear repair. There was marked improvement in pulmonary function (Table 1) after pledgeted suture removal. Two months after suture removal, anastomotic granulation was significantly reduced (Fig 1E). Eight months later, the anastomotic granulation tissue had completely disappeared (Fig 1F).

Comment

Although the morbidity and mortality associated with tracheobronchoplasty have been decreasing with improvements made in surgical techniques and suture materials, anastomotic complications cannot be completely avoided [1]. Extensive necrosis and anastomotic dehiscence have become rare, but granulation tissue formation and stenosis persist. A therapeutic approach for airway stenosis caused by exuberant granulation tissue includes electrocautery, mechanical dilation, laser phototherapy, cryotherapy, and placement of stents. Debulking is one of the main treatment modalities for anastomotic granulation. However, controlling and treating the granulation tissue can be challenging in certain cases. One of the reasons is that debridement

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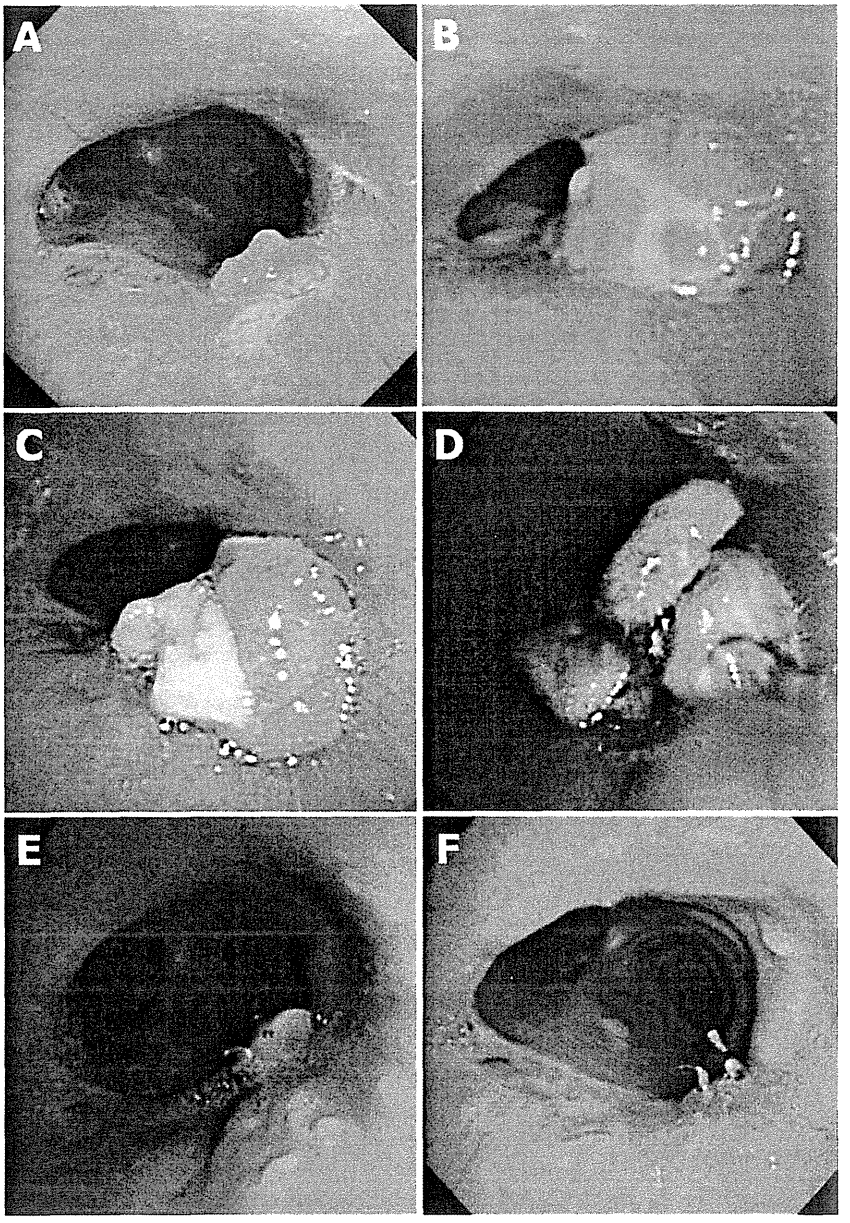

Fig 1. (A) Minimal granulation tissue formation on right dorsal membranous side of anastomosis 13 months after operation. (B) Excessive growth of granulation tissue on anastomosis 16 months after operation. (C) Anastomotic findings 2 weeks after topical mitomycin C (MMC) therapy. (D) Ticron with pledget embedded in granulation tissue, as seen under rigid bronchoscopy. (E) Anastomotic findings 2 months after suture removal, showing significant reduction in amount of granulation tissue. (F) Anastomotic findings 8 months after suture removal, showing that granulation tissue had resolved completely.

must be of the excess granulation tissue only to prevent perforation. In our patient, granulation tissue grew progressively despite repeated debulking.

Recently, topical MMC has been used with success as an adjunctive treatment for granulation tissue–related stenosis [2]. MMC is a cytotoxic agent that is isolated from

Table 1. Spirometry at 8 and 22 Months After Operation and on the Day After Suture and Pledget Removal

	Treatment Before Removal		
Variables	8 Months After Operation	22 Months After Operation	Day After Suture Removal
FEV_1 (L) (%)	1.91 (95.5)	0.84 (43.3)	1.87 (96.4)
FVC (L) (%)	2.59 (105.7)	2.52 (104.6)	2.45 (106.6)
FEV_1/FVC (%)	73.8	33.3	76.3

FEV_1 = forced expiratory volume in 1 second; FVC = forced vital capacity.

Streptomyces caespitosus and acts by DNA and RNA alkylation and cross-linkages, leading to decreased cell proliferation. Unfortunately, these benefits were not consistent with our results.

Anastomotic granulation tissue can be a consequence of airway ischemia or infection, and the resultant stenosis can lead to poor lung function and respiratory infections. In our patient, the postoperative clinical course during the first year was good; no anastomotic ischemic findings were detected. However, 13 months after the operation, anastomotic granulation started to worsen dramatically. Our patient showed a relatively indolent clinical course, which could probably be explained by the pledgeted sutures in the anastomotic site. There may have been no infection or ischemia that caused granulation tissue formation during the first year after anastomosis, but the pledgeted sutures used during tracheoplasty may have been pushed toward the luminal side during the healing process and eventually breached the bronchial wall. A similar report described a case of recurrent hemoptysis 18 months after lobectomy caused by the erosion of a pledgeted suture through the trachea [3]. Pledgeted sutures had been used to reinforce a bronchial stump or anastomotic site defect to prevent fistula formation. However, migration of these sutures into the airway can occur, leading to airway obstruction in addition to hemoptysis [4]. Coughing of blood or suture material resulting from suture migration and granulation tissue formation has been reported to occur in 4.4% of patients who undergo anastomotic surgical resection [5]. Although the migrated sutures are typically easily removed, care must be used when confronted with more complex anastomosis techniques, as in our patient. Similar to other authors, we found the use of flexible endoscopic scissors to be helpful in releasing the pledgeted suture once the granulation tissue had been adequately debrided and the suture line exposed [3, 6]. Therefore, we could avoid further invasive procedures such as endobronchial stenting or reoperation.

At present, we rarely find reports of postoperative granulation tissue formation in a tracheobronchial anastomosis, mainly because of the development of less reactive synthetic sutures. The type of suture material reportedly influences anastomotic complications in patients undergoing tracheobronchoplasty, especially when anastomotic stenosis caused by excessive granulation tissue is noted [1]. Synthetic sutures are reported to be superior to silk, and synthetic absorbable sutures reportedly minimize the risk of anastomotic complications [1]. For bronchial anastomosis, there have been contrasting reports on the choice of monofilament nonabsorbable sutures versus absorbable sutures [7, 8]. In our patient, we preferred to use braided suture instead of monofilament because we wanted to tie the pledgeted sutures at the anastomosis securely and gently to avoid further tearing.

Despite the aforementioned improvements, removal of surgical materials should still be considered in the management of airway anastomotic obstruction caused by excessive and intractable granulation tissue formation after bronchoplasty. In this case, pledgeted suture removal resolved the obstruction and symptoms of the patient. Furthermore, close postoperative follow-up of long duration is underscored, especially when pledgets are used at the site of anastomosis.

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FEATURE ARTICLES

Thymoma With Ring Calcification

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A 59-year-old man was referred to our department for evaluation of an asymptomatic abnormal mediastinal shadow detected on chest roentgenogram. Computed tomography (CT) of the chest showed an anterior mediastinal tumor with ring calcification. Thymectomy through a median sternotomy was performed. Pathologic examination showed an encapsulated type B2 thymoma with ring calcification. Microscopically, the calcified layer was within the fibrous capsule layer. A part of the fibrous capsule was thought to become calcified like a ring. Although focal calcification is sometimes found in thymomas, ring calcification is rare.

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Cytokeratin 19 expression in primary thoracic tumors and lymph node metastases

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ABSTRACT

Background: The use of one-step nucleic acid amplification (OSNA), which allows for the rapid intraoperative detection of lymph node (LN) metastasis, is becoming more widely accepted in breast cancer. To provide basic data for the development of this method for lung tumors, we conducted a large-scale investigation of cytokeratin (CK) 19 expression in thoracic tumors.
Patients and methods: We examined CK19 expression in specimens from a total of 801 surgically resected samples of primary lung adenocarcinoma (ADC), squamous cell carcinoma (SQC), large-cell carcinoma (LCC), pleomorphic carcinoma (PC), large cell neuroendocrine carcinoma (LCNEC), small cell carcinoma (SCC), and carcinoid tumor (CT) as well as pleural malignant mesothelioma and lung metastatic deposits from breast cancer using tissue microarrays (TMAs) and whole sections. We also compared the CK19 expression status between primary sites and LN metastatic deposits.
Results: The overall rate of CK19 expression as observed on TMAs and whole sections in the 801 analyzed cases was 88.0%. CK19 expression was detected in 94.6% of ADCs, 93.6% of SQCs, 54.5% of LCCs, 54.8% of PCs, 77.4% of LCNECs, 31.8% of SCCs, 34.0% of CTs, and 92.9% of malignant mesotheliomas. Expression of CK19 was also detected in 90.9% of lung metastatic deposits from breast carcinomas. CK19 expression was maintained between CK19-positive primary sites and the corresponding LN metastatic deposits. Of note, a portion of CK19-negative primary tumors showed upregulation of CK19 protein expression in LN metastases.
Conclusions: Most thoracic tumors, except for PCs, CTs, and SCCs, were positive for CK19. We also found that CK19 expression was maintained between CK19-positive primary tumors and the corresponding LN metastatic deposits. These results may be useful in the development of the OSNA method for the intraoperative detection of LN metastasis in non-small cell lung cancer (NSCLC).

1. Introduction

In non-small cell lung cancer (NSCLC), lymph node (LN) metastatic status is one of the most predictable adverse prognostic factors [1–3]. In particular, in deciding on the most appropriate surgical procedure for patients with NSCLC, the presence or absence of intrathoracic metastatic LNs is one of the most important factors. Identification of LN metastasis in decision-making for surgical procedures is also of importance in other tumor types. For example, in breast cancer, sentinel lymph node (SLN) biopsy has recently become a standard surgical procedure in the decision of whether to undertake axillary LN dissection. However, intraoperative diagnosis of SLNs for metastasis using frozen sections has a sensitivity with immunohistochemistry (IHC) of only 50–70% compared with the permanent histologic sections of the same LN [4,5].

Cytokeratins (CKs) are keratin-containing intermediate filament proteins found in the intracytoplasmic cytoskeleton of epithelial tissue. CKs are of two types: acidic type I CKs and basic or neutral type II CKs. CKs are usually found in pairs, comprising a type I CK and a type II CK. Among the 20 epithelial CKs, CK19 is the lowest molecular weight (40 kDa) acidic keratin and is a specific cytoskeletal structure of simple epithelia; its expression is observed in normal and cancerous epithelial cells of organs such as the breast, colon, and lung [6,13].

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The one-step nucleic acid amplification (OSNA) assay is a novel technique that utilizes a loop-mediated isothermal amplification (RT-LAMP) method of gene amplification [7,8]. The assay is characterized by the quantitative measurement of a target mRNA, a brief reaction time, a high specificity for the target mRNA, and an absence of genomic DNA amplification. On the basis of these advantages, the OSNA assay has been developed as an alternative intraoperative method for the detection of tumor metastasis, and has been validated as diagnosis of LN metastases in breast, gastric, and colorectal cancers [9–12]. Also, the potential OSNA utility to non-small cell lung cancer patients has been reported [13]. To provide basic data for the development of the OSNA method for the evaluation of LN metastasis in lung tumors, we used immunohistochemistry to evaluate the CK19 expression rate in several different types of thoracic tumors. In addition, we evaluated the concordance of CK19 expression between primary tumors and their LN deposits.

2. Materials and methods

2.1. Case selection

The Institutional Review Board of our hospital approved the current study (2010-0077). The specimens used in this study were from previously constructed tissue microarray (TMA) blocks, which utilized a core sample measuring 2 mm in diameter, and they included samples from a total of 801 cases seen at the National Cancer Center Hospital (Tokyo, Japan) between 1997 and 2007. The cases consisted of 726 primary lung tumors, including 294 adenocarcinomas (ADCs), 157 squamous cell carcinomas (SQCs), 11 large cell carcinomas (LCCs), 42 pleomorphic carcinomas (PCs), 106 large-cell neuroendocrine carcinomas (LCNECs), 66 small cell carcinomas (SCCs), and 50 carcinoid tumors (CTs), as well as 42 malignant mesotheliomas and 33 metastatic lung deposits from breast carcinomas. Histological diagnoses were based on the latest World Health Organization classification with the aid of immunohistochemical panels [14]. Among all ADC cases, the predominant histological patterns were classified based on the International Association for the Study of Lung Cancer/American Thoracic Society/European Respiratory Society (IASLC/ATS/ERS) classification [15].

2.2. Immunohistochemistry and evaluation

Heat-induced epitope retrieval with a target retrieval solution 9 (Dako, Carpinteria, CA, USA) was performed. Incubation with primary antibody to CK19 (RCK108, 1:50, Dako) was conducted using an automated stainer (Dako) following the vendor's protocol. ChemMate EnVision™ (Dako) detection kits were used. Immunohistochemical staining was scored independently by two observers (K.M. and K.T.). Discrepancies in judgment were resolved by means of a joint viewing of the slides under a multiheaded microscope. We defined immune-positive cases as those with a mean positive area ≥10%.

First, we performed CK19 staining on TMA sections. Then, the after mentioned 96 CK19-negative cases were subjected to CK19 immunostaining using the whole section from the largest area of the tumor blocks.

2.3. CK19 expression in LN metastasis

To elucidate the differences in CK19 expression between primary sites and their corresponding LN metastases, we selected the CK19-positive cases based on TMA data and the CK19-negative cases based on whole sections at primary tumor sites from patients with LN tumor deposits. CK19-positive cases were defined as those in which more than 5% of the tumor cells in the LN were positive.

2.4. Statistical analyses

Statistical analysis of CK19 expression among the different histological subtypes of ADCs was performed using Fisher's exact test with the SPSS Statistics 22 program (IBM Corporation, Somers, NY, USA).

3. Results

3.1. CK19 expression in TMA sections

CK19 expression was detected in 643 (80.3%) of all 801 analyzed cases. In primary lung and pleural tumors, CK19 expression was detected in 278 (94.6%) of 294 ADCs, 147 (93.6%) of 157 SQCs, 6 (54.5%) of 11 LCCs, 23 (54.8%) of 42 PCs, 82 (77.4%) of 106 LCNECs, 21 (31.8%) of 66 SCCs, 17 (34.0%) of 50 CTs, and 39 (92.9%) of 42 malignant mesotheliomas (Fig. 1). CK19 expression was also detected in 30 (90.9%) of 33 lung metastatic deposits from breast carcinomas (Figs. 1 and 2).

3.2. CK19 expression rates together with whole section data

Additional whole section analysis increased the CK19 expression from 80.3% to 88.0% of all cases. The expression rate in metastatic deposits from breast carcinoma remained unchanged (90.9%). Among primary lung and thoracic tumors, 62 (40%) of 155 negative cases were found to be changed to positive for CK19-expression using whole section analysis: 14 (87.5%) of 16 ADCs, 4 (40%) of 10 SQCs, 3 (60%) of 5 LCCs, 7 (36.8%) of 19 PCs, 11 (45.8%) of 24 LCNECs, 18 (40%) of 45 SCCs, 4 (12.1%) of 33 CTs, and 1 (33.3%) of 3 malignant mesothelioma (Fig. 3).

Final CK19 expression rates conjunction with TMA and whole sections was 99.3% ADC, 96.2% SQC, 81.8% LCCs, 71.4% PC, 87.7% LCNEC, 59.1% SCC, 42.0% CT, and 95.2% malignant mesotheliomas (Fig. 1).

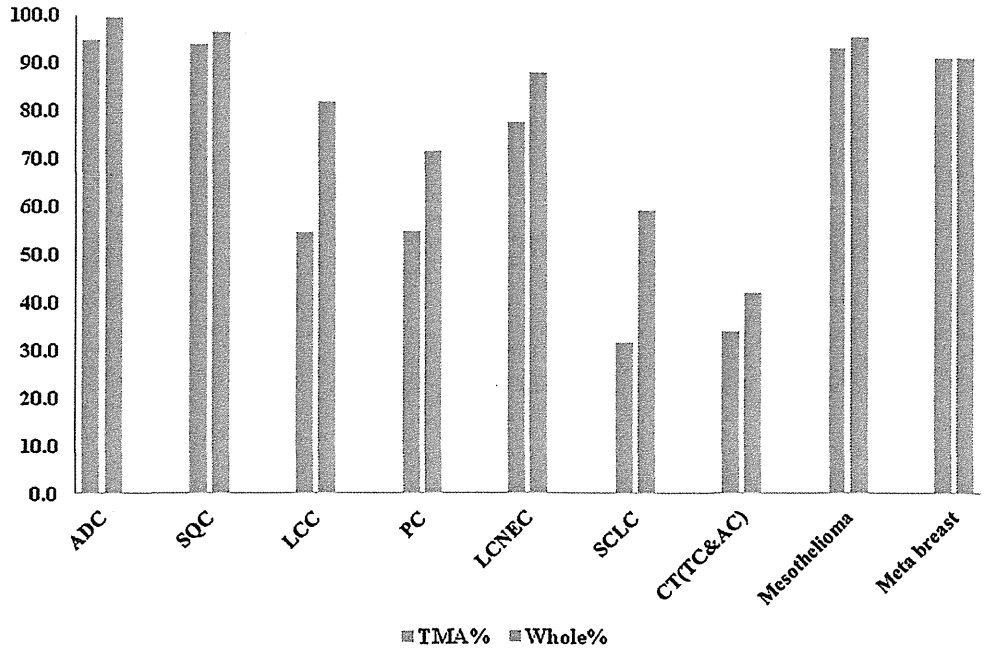
3.3. Correlation between CK19 expression and IASLC/ATS/ERS classification of lung ADCs

Among the 294 ADCs, the most common histological subtype was papillary-predominant, with 79 cases (26.9%), followed by 66 solid-predominant cases (22.4%), 54 acinar-predominant cases (18.4%), 46 invasive mucinous ADC cases (15.6%), 27 micropapillary-predominant cases (9.2%), and 22 lepidic-predominant cases (7.5%). There were no cases of mucinous adenocarcinoma in situ (AIS) or minimally invasive adenocarcinoma (MIA). When subdividing ADCs based on the IASLC/ATS/ERS classification, there were no statistical differences in CK19 expression ($P = 0.31$). Complete positivity was observed in papillary-, acinar-, micropapillary-, and lepidic-predominant ADCs. The other subtypes also demonstrated highly positive CK19 expression, with rates of 98.5% in solid-predominant and 97.8% in invasive mucinous ADCs.

3.4. CK19 expression in LN metastases

We also examined the CK19 expression in LN metastases from 38 lung tumors: 22 cases that were CK19-positive at the primary sites (10 ADCs, 7 SQCs, 1 LCC, 2 LCNECs, 1 SCC, and 1 CT) and 16 cases that were CK19-negative at the primary sites (1 ADC, 2 SQCs, 1 LCC, 3PCs, 4 LCNECs, 2 SCCs, and 3 CTs).

All LN deposits from cases that were CK19-positive at the primary sites were positive for CK19. In addition, 7 (43.8%) of 16 LN



ADC ; adenocarcinoma, SQC ; squamous cell carcinoma, LCC ; large cell carcinoma, PC ; pleomorphic carcinoma, LCNEC ; large-cell neuroendocrine carcinoma, SCC ; small cell carcinoma, CT ; carcinoid tumor, TC ; typical carcinoid, AC ; Atypical carcinoid, and Meta breast ; metastatic lung deposits from breast carcinomas.

Fig. 1. Cytokeratin 19 expression of thoracic tumor and metastatic breast cancer. Purple bar depicting the cytokeratin (CK) 19 expression rate based on tissue microarray and blue bar indicates the final conjunction with tissue-microarray and whole section samples. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

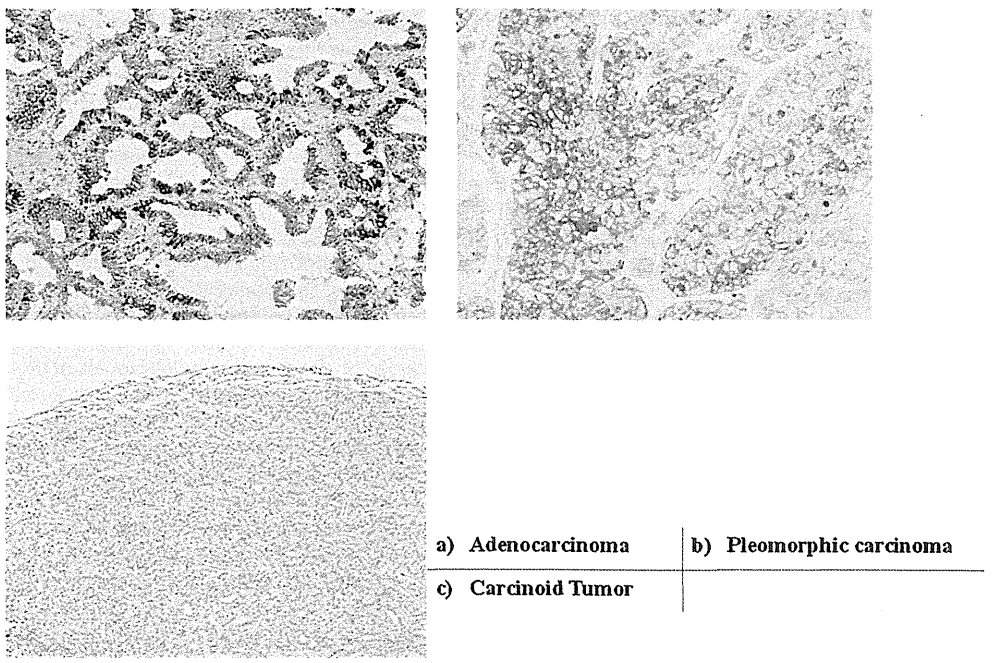


Fig. 2. Representative image of cytokeratin 19 expression. (a) Diffuse and strong staining in lepidic-predominant adenocarcinoma (magnification, 20×). (b) Diffuse and strong staining in pleomorphic carcinoma (magnification, 20×). (c) No staining in carcinoid tumor from tissue-microarray section (magnification, 20×).

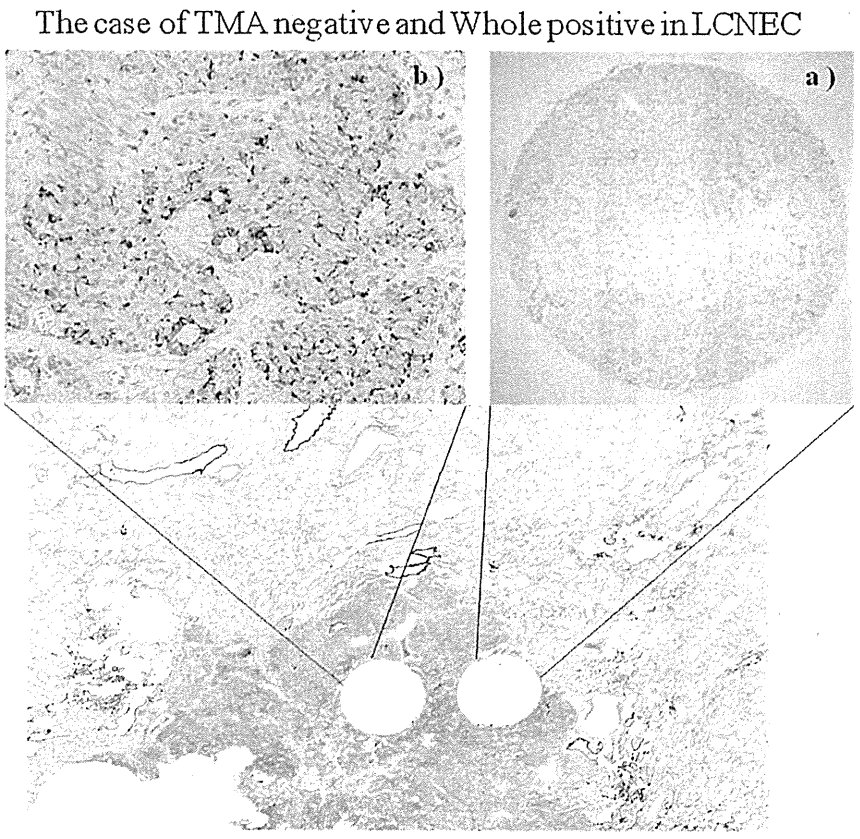


Fig. 3. Differences of cytokeratin 19 expression between tissue-microarray and whole section. (a) No cytokeratin 19 expression in large cell neuroendocrine carcinoma sample from tissue-microarray section. (b) Whole section from the same case showing the cytokeratin 19 negative of central areas including defects of tissue-microarray cores. Whereas, focal moderate staining distributing peripheral area of the tumor.

deposits from cases that were CK19-negative at the primary sites (1 of 1 ADC, 1 of 2 SQCs, 1 of 1 LCC, 3 of 3 PCs, and 1 of 4 LCNECs) demonstrated a change to positive for CK19.

Overall, some inflammatory cells including plasma cells and epithelioid macrophages showed weak CK19 staining in primary sites and LNs.

4. Comment

In this retrospective immunohistochemical study of CK19 expression in lung and pleural tumors, our findings demonstrated that most of the thoracic tumors except for PCs, CTs, and SCCs were positive for CK19. We also revealed that CK19 expression was maintained between CK19-positive primary sites and corresponding LN metastatic deposits. Of note, a portion of CK19-negative primary tumors displayed upregulation of CK19 protein expression in LN metastases.

The high prevalence rates we observed for CK19 expression in most of the lung and pleural tumors were consistent with previous reports, in which the positive rates for expression of CK19 ranged from 70% to 100% [16–19]. The sources of antibodies, dilutions, and the method of antigen retrieval must all be taken into consideration, since all of these methodological variations can significantly affect the results of protein expression using immunohistochemistry. Although there are no previous reports regarding the rate of expression of CK19 in lung metastatic deposits from breast carcinomas, our observed CK19 expression rate in metastatic deposits from breast carcinomas was similar to the range of expression reported in primary sites in previous reports [16,20,21]. Therefore, our method of CK19 detection does not indicate overrepresentation. On the other hand, the low rates of CK19 expression that we observed in PCs, CTs, and SCCs can be taken as genuinely indicative of low or no expression of CK19.

The various reasons for the low expression rates of CK19 in PCs and neuroendocrine carcinomas (NECs) most likely differ. The main reason for the low expression of CK19 in PCs may be epithelial–mesenchymal transition (EMT) [22–25]. During the EMT, epithelial cells lose epithelial phenotypes (downregulation of CK and loss of cell–cell adherence) and gain the mesenchymal phenotypes represented by vimentin expression. EMT is a natural process involved in development and wound healing. Additionally, EMT plays an important role in tumor invasion and metastasis. In PCs, the sarcomatoid component shows EMT [22,23].

On the other hand, the likely reason for the low CK19 expression rates we observed in NECs was that the tumor cells either did not express or expressed very low levels of CK19. Although most neuroendocrine organs are composed of simple epithelium, and thus endocrine tumors usually express the simple epithelial CKs such as CK19, the keratin profiles of these organs are complex due to the fact that some of them are considered to be derived from endoderm (islets of the pancreas, thyroid, parathyroid gland), some from mesoderm (adrenal cortex), and some from ectoderm (pituitary gland, pineal gland, adrenal medulla) [16]. In fact, 25 of 28 (89.3%) cases of pancreatic neuroendocrine tumors (NETs) were CK19 positive [26]. In contrast, low CK19 expression rates were reported in gastrointestinal carcinoid tumors and lung carcinoid tumors [16,27].

Another novel finding of our study was that all tumors that were positive for CK19 at the primary site maintained CK19 expression in LN metastases. Of note, we observed that a portion of

CK19-negative primary tumors also showed CK19 expression in LN metastases. Similar upregulation of CK19 expression between the primary site and LN metastases was observed in breast carcinomas; 67% of CK19-negative primary breast tumors demonstrated a change to CK19-positive status in the LN metastases[28–31]. One of the hypothesized reasons for this upregulation is reactivation of cytokeratin expression during metastasis [23,31] During the metastatic process, tumor cells at the primary site lose their cell polarity and cell–cell adhesion, and gain migratory and invasive properties to become mesenchymal cells. At the metastatic site, the tumor cells then return to a non-mesenchymal phenotype, via a mesenchymal–epithelial transition [23].

Similar to a previous study [13], we observed some inflammatory cells that reacted with CK19 in primary sites and LNs. Thus, it is necessary to carefully interpret immunostaining results to identify micro-metastases in lymph nodes. Furthermore, a previous report also revealed that CK19 reactivity in epithelioid macrophages induces a false positive result for the OSNA assay (i.e., no lymph node deposit but a positive result for the OSNA assay) [13].

The data from the current study indicate that performing immunohistochemistry for CK19 on biopsy specimens from the primary tumor site prior to OSNA analysis is of less importance. This is because most of the lung carcinomas for which surgery is indicated are positive for CK19 expression. In addition, not only did all CK19-positive tumors maintain their expression at LN metastatic deposits, but a portion of the CK19-negative primary tumors showed expression of CK19 in LN metastases, as well. There are conflicting reports regarding the preoperative evaluation of CK19 expression at the primary tumor site prior to OSNA assay in breast carcinoma [21]. In addition, CTs and SCCs that showed low expression of CK19 less frequently metastasized to LNs and were not indicated for surgical resection, respectively.

In conclusion, we performed a large-scale analysis of the expression of CK19 in thoracic tumor samples. Our data showed that most of the thoracic tumors except for PCs, CTs, and SCCs were positive for CK19. We also revealed that CK19 expression was maintained between CK19-positive primary sites and corresponding LN metastatic deposits. These results may be useful in the development of the OSNA method for the intraoperative detection of LN metastasis in NSCLC. However, since the OSNA assay is based on real-time amplification and quantitation of CK19 mRNA, our protein expression data are not directly applicable to the assay.

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Conflict of interest statement

All authors contributing to this work have no other conflict of interest to declare.

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Early-Stage Lung Cancer: 40s Anniversary

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Surgery remains the cornerstone in the early-stage non–small-cell lung cancer (NSCLC) treatment, but a lot of efforts have been focused on the use of systemic therapy in this setting, on technological advances in thoracic surgery and radiotherapy, and on better application of local therapeutic approaches to improve the survival rates in these patients.

The aim of this article is to provide a synthetic overview of the scientific achievements characterizing this setting during the past 40 years (Figure 1).

THORACIC SURGERY FOR EARLY-STAGE NSCLC

For early-stage lung cancer (mainly stages I and II), surgery has continued to be a mainstay treatment in the past 40 years or even longer period. However, there have been revision and improvement in many important procedures used for the complete resection of lung cancer. The present-day procedure for the curative resection is composed of the removal of lung parenchyma with primary tumor and sampling/dissection of locoregional lymph nodes. In relation to these, the determination of proper extent of parenchymal resection for lung cancer and assessment of prognostic significance of lymph node dissection have been two major issues. In addition to these, the management of earlier lung cancers and development of minimally invasive approach became the important challenge in the surgical community in the past 40 years.

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EVOLUTION OF LUNG CANCER SURGERY: EXTENT OF PARENCHYMAL RESECTION

The history of lung cancer surgery is that of minimization of the extent of parenchymal resection. Lung cancer surgery started as pneumonectomy, a removal of one entire lung of either side, in 1930s by the giants in surgical history such as Graham, Nissen, and Overholt.[1] In late 1950s and 1960s, pneumonectomy was gradually being replaced by lobectomy, and lobectomy became the standard by the mid-1960s.[2] The transition from pneumonectomy to lobectomy was based on the accumulation of surgeons' anecdotal but successful experiences. Further progress in lung-sparing resection was afforded by the development of "sleeve" resection in 1955.[3] Since that time, bronchoplastic and angioplastic resections became more widely adopted, as evidence accrued that these lung-sparing operations combined lower perioperative mortality,[4] better functional results,[5] improved quality of life,[6] and better long-term survival in suitable cases[7] compared with pneumonectomy. Then, the next step toward lesser resection was attempted through scientific way of randomized trial by North American Lung Cancer Study Group in late 1980s.[8] The trial compared the prognosis between lobectomy and limited resection for patients with T1N0 peripheral NSCLC, and the results indicated a 75% increase in recurrence rates and 30% increase in overall death rate. However, the data on postoperative pulmonary function were not given because of early funding termination, and the functional advantage of sublobar resection was not clearly demonstrated. It was concluded that lobectomy still must be considered the surgical procedure of choice for patients with peripheral T1N0 NSCLC. However, especially in these days, we are more often encountering earlier and smaller lung cancers with predominantly ground-glass appearance on high-resolution computed tomography (CT), and their superb prognosis has been shown.[9] Many case series that demonstrated the excellent prognosis after sublobar resection equivalent to that after lobectomy are accumulating, although these sublobar techniques were not novel.[10–12] The need of revision of randomized trial between lobectomy and sublobar resection has been evoked among thoracic surgeons. At present, two important randomized trials are actually underway across the Pacific Ocean. In the United States, the cancer and leukemia group B trial 140503 will randomize small peripheral tumors to lobectomy versus limited resection, wedge resection, or segmentectomy being allowed in the limited resection arm. In Japan, Japan Clinical Oncology Group (JCOG) 0802 study, a prospective randomized trial compares the prognoses between

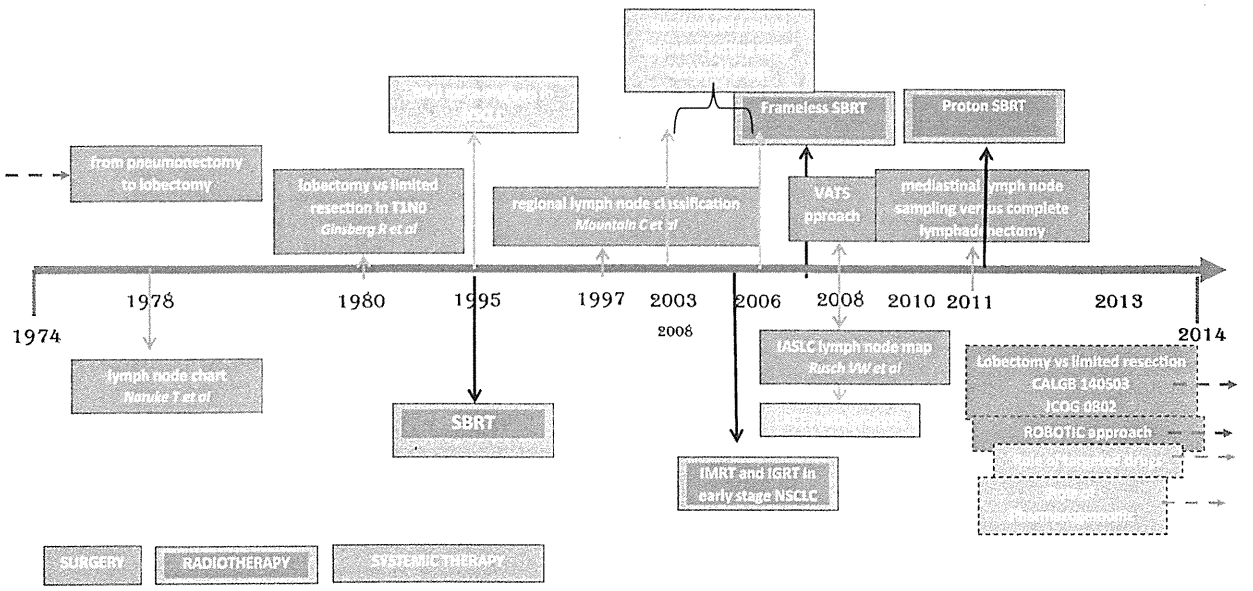

FIGURE 1. Early-stage non–small-cell lung cancer (NSCLC): overview of the scientific achievements characterizing this setting over the last 40 years.

lobectomy and segmentectomy in a noninferiority setting.[13] In cancer and leukemia group B trial, primary end point was noninferiority of disease-free survival (DFS), and secondary end points were noninferiority of overall survival (OS), local and systemic recurrence rates, and difference in spirometry at 6 months. Target accrual is 1297 patients. In Japan Clinical Oncology Group trial, the primary end point was noninferiority of OS, and secondary end points were difference in spirometry at 6 and 12 months, noninferiority of DFS, local recurrence rate, and others. Target accrual is 1100 patients. For both these trials, in case the prognosis after segmentectomy is not significantly inferior to that of lobectomy and pulmonary function after segmentectomy is significantly superior to that of lobectomy, segmentectomy is confirmed as a new standard.

Definitive answer to the question whether sublobar resection can replace lobectomy will be given soon. However, until then it is recommended that anatomical segmentectomy be reserved for the CT screening–detected pure ground-glass opacity lesions or part-solid lesions less than 2 cm located in the peripheral third of the lung, after frozen section of N1 and N2 lymph nodes has confirmed the T1aN0M0 status. In addition, frozen section or cytological evaluation of resection margins is recommended.[14]

LYMPH NODE DISSECTION FOR LUNG CANCER

Another important aspect of lung cancer surgery is the management of the locoregional lymph nodes, because we realize that metastasis to these lymph nodes is strongly prognostic. Naruke et al[15] published a landmark article in 1978, in which so-called lymph node map (chart) was introduced for the first time. He analyzed the prognosis of patients with metastasis at the specific lymph node site and showed a prognostic importance to describe the site of lymph nodes. Owing to this nodal chart, surgeons became able to speak in the same language of lymph nodes. There have been several revisions in lymph node map. In United States, Mountain–Dressler American Thoracic Society map has been mainly used.[16] However, the coexistence of different maps caused discrepancy in tumor–node–metastasis (TNM) staging worldwide. In 2009, the International Association for the Study of Lung Cancer (IASLC) map was promulgated as a part of IASLC staging project for the global use.[17]

An IASLC workshop in 1996 discussed the techniques available at that time for intrathoracic nodal evaluation.[18] The participants, including Dr Naruke, agreed the term "systematic nodal dissection" (SND) and defined the minimum standards for such an assessment. These included the labeling of all excised nodes using an internationally accepted nodal map, the excision of a minimum of three mediastinal nodal stations, one of which should be the subcarinal node, station 7, and excision of hilar and intrapulmonary nodal stations in a centrifugal manner until the extent of resection required has been established. Subsequently, a proposal was made that the definition of a complete resection should accept SND as a requirement for an R0 resection with a minimum of three mediastinal and three N1 nodes/stations excised/sampled and examined by the pathologist.[19] SND was shown to identify 18% "unexpected N2" disease after preoperative evaluation by CT scanning and selective mediastinal exploration.[20] The development of positron emission tomographic scanning may have reduced this incidence by as much as half, but the inaccuracy of preoperative nodal evaluation remains problematic.[21] An alternative approach was suggested by Japanese colleagues, Lobe-Specific Nodal Dissection.[22] The attraction of this technique was the demonstration that the subcarinal nodes in station 7 were rarely involved in the case of right upper lobe and left upper segment tumors if all other superior mediastinal nodal stations were clear of disease on frozen section. Although this may save time and a difficult dissection of station 7 nodes during video-assisted thoracoscopic surgery (VATS) lobectomy, most surgeons find that that removing all mediastinal nodes by SND is expedient.

There has been much debate as to whether such extensive nodal evaluation contributes to cure after complete resection. One study showed that survival after resection for stage I, node-negative, NSCLC improved with the number of lymph nodes resected and examined, with a statistically significant cut point at six lymph nodes,[23] a surprising result given that all such nodes were thought to be clear of disease on pathological examination. However, such an effect could be merely a reflection of "stage migration."[24] Studies confirmed that extensive nodal excision was more accurate in determining the correct nodal category compared with "sampling,"[25,26] and one randomized prospective trial showed a survival for SND compared with sampling.[27] To further evaluate the prognostic significance of systematic lymph node dissection in comparison with sampling, American College of Surgeons Oncology Group conducted a large-scale randomized trial with 1100 patients undergoing resection for T1-2, N0, or nonhilar N1 NSCLC, randomized between mediastinal lymph node dissection (MLND), roughly equivalent to systematic nodal dissection and an extensive systematic sampling (ACOSOG Z0030).[28,29] The results indicated that in 4% of N2 patients lymph node metastasis was overlooked in sampling and there was no difference in perioperative indicators such as blood loss and operative time between sampling and dissection. Most importantly, lymph node dissection did not improve OS or DFS in early-stage lung cancer. It was concluded that "MLND provides patients with the most accurate staging and the opportunity for adjuvant therapy if occult metastatic disease is present. Because current preoperative staging cannot definitively identify patients with mediastinal lymph node involvement and because patients with known hilar or mediastinal disease (N2) or with T3 or T4 tumors may benefit from MLND because the pretest probability of N2 disease is higher, we still recommend that all patients with resectable NSCLC undergo MLND because the procedure does not increase mortality or morbidity." The discussion regarding the prognostic impact of SND has become superfluous since the finding that node-positive cases benefit from adjuvant chemotherapy.[30] In the adjuvant era, it is of paramount importance that the surgeon and pathologist collaborate to ensure that the determination of the postsurgical/pathological N category is conducted using the most rigorous techniques available. In the 7th edition of TNM for lung cancer, the IASLC lymph node map and table of nodal definitions were acknowledged as the recognized method for describing regional node involvement in lung cancer.[22] The definition of an R0 resection has been expanded to incorporate the resection and pathological examination of the minimum number of lymph nodes proposed in the definition of SND.[31]

TREND TOWARD MINIMALLY INVASIVE SURGICAL APPROACH

Apart from the extent of parenchymal resection, surgeons used various types of chest wall incisions in the past few decades. Since the late 1990s, the VATS has been extensively used especially for early-stage lung cancers.[32] Perioperative parameters in patients who underwent lobectomy by either open or VATS approach were extensively analyzed, and the consensus has been established that the VATS major lung resection is feasible and gives the shorter hospital stay, less morbidity, and less cost.[33,34] According to the retrospective, multinstitutional database analyses of 3961 patients who underwent either open lobectomy or VATS lobectomy, VATS procedure was significantly superior to open procedure in hospital costs, length of stay, and risk of adverse events. Only operative time was longer for VATS procedures.[32] Similar results were reproduced by a propensity-matched analysis of Society of Thoracic Surgeons database regarding 5042 open thoracotomy lobectomy and 1281 VATS lobectomy. Again, VATS lobectomy was associated with lower incidence of arrhythmias, reintubation, blood transfusion, shorter stay (4.0 versus 6.0days), and shorter chest tube duration. There was no difference in mortality between two surgical modalities.[35] However, it should be realized that the prognostic equivalency between VATS and open procedures has not been definitively proved especially in advanced lung cancers because the comparison between different modalities cannot be completely free from selection bias. Actually, surgeons prefer open procedures to VATS procedures in dealing with difficult, time-consuming, risky cases. Furthermore, the surgical robot is being introduced and applied for the resection of lung cancer recently. The robotic procedures are expected to facilitate the dissection phase of the resection by providing a high-resolution binocular view, wrist-like action of the instruments, and ease of fine dissection in a confined space. Some early reports addressed the low mortality, morbidity, and the advantage that it can achieve a good dissection in difficult situations.[36] However, the disadvantage that there is no tactile feedback to surgeons must be mentioned. The feasibility is to be evaluated from technical and economical viewpoints.[37]

STAGING OF LUNG CANCER

Staging of lung cancer is the basis for clinicians to make treatment plans and anticipate the outcome. In the past, two great leaders played very important roles in revisions of recent staging systems: Mountain for 6th edition and Goldstraw for 7th edition. The IASLC decided to take responsibility for the revision of TNM staging system in lung cancer and initiated IASLC staging project in 1998.[38] Previous revisions of the TNM classification for lung cancer had been based on iterative analysis of a single institutional database in the United States. The cases had been accrued during a period of 40 years and were predominantly referred for a surgical opinion. The IASLC was able to develop a data set of more than 100,000 cases, donated by 46 data centers from more than 20 countries around the globe, treated by all modalities of care and accrued during a 10-year period. Collection and analysis of these data were undertaken by Cancer Research and Biostatistics, a not-for-profit organization in the United States. Such a huge increase in the database available for analysis permitted validation, both internal and external, to a degree not possible previously,[39] more closely aligning stage with prognosis than ever before. The IASLC proposals for revision[38,40–48] were accepted by the Union for International Cancer Control and the American Joint Committee on Cancer and formed the basis of the 7th edition of TNM for Lung Cancer.[49,50] The IASLC Staging and Prognostic Factors committee is presently working toward the 8th edition of TNM due to be published late in 2016. The project will be

expanded to include other tumor sites in the thorax: malignant pleural mesothelioma, in collaboration with the International Mesothelioma Interest Group, and thymic malignancies, in association with the International thymic Malignancies Interest group. The proposals for the staging classification of lung cancer and these other tumor sites will be published in the *Journal of Thoracic Oncology* during the next 12 to 18 months.

Radiotherapy for Early-Stage NSCLC

Surgery remains the cornerstone in the early-stage NSCLC treatment, and concerning the role of radiotherapy in the adjuvant setting, there is no indication that postoperative radiotherapy (PORT) improves outcome in patients with completely resected N0 or N1 disease, with a meta-analysis in fact demonstrating a detrimental effect on survival in these cases. For patients in whom unsuspected mediastinal nodal metastases are discovered during surgery, PORT has not been shown to improve OS in prospective randomized studies.[51] Ongoing trials (LUNGART, NCT00410683) are currently evaluating the contribution of PORT delivered through modern techniques in N2 resected patients.

A lot of efforts during the last four decades have been focused on technological advances in radiotherapy and on a better application of such an alternative local therapeutic approach to improve the survival rates in patients considered at risk for surgery.[52]

The apparent reluctance to refer such patients for conventional radiotherapy was partly due to the requirement for 30 or more once-daily treatments, which is cumbersome for frail elderly patients. Moreover, outcomes of conventionally fractionated radiotherapy (CFRT) in early-stage NSCLC was poor despite treatment to doses ranging from 60 to 66–70 Gy because local tumor recurrences were seen in approximately 40% of patients, with OS at 3 years of approximately 20% to 30%.[53] Also, hyperfractionation and accelerated hyperfractionation, used to intensify radiation dose from a biological point of view, were ineffective in improving these outcome figures, even if not extensively studied in early-stage NSCLC.

When compared with observation, a Surveillance, Epidemiology, and End Results registry study demonstrated that CFRT alone leads to only modest improvements in outcomes (median OS = 1.7 years with CFRT versus 1.2 years with observation and 5-year OS = 15% versus 14%, respectively).[54]

In the mid-1990s, the principles of cranial stereotactic radiosurgery were transferred to extracranial sites by pioneering work at the Karolinska Hospital in Sweden.[55] This so-called stereotactic body radiotherapy (SBRT) approach, also known as stereotactic ablative radiotherapy, was further developed by centers in Japan,[56] Germany,[57] and North America.[58] In the United States, preliminary results from Indiana University led to the Radiation Therapy Oncology Group 0236 trial, a phase II study that enrolled medically inoperable patients with T1-T2 (less than 5 cm), peripherally located NSCLC. All patients received 60 Gy (20 Gy × 3). Fifty-five patients were enrolled, and the results demonstrated a 3-year actuarial LC rate of 98%, with OS at 3 years of 56% (median OS of 4 years).[59]

In subsequent years, encouraging results from both prospective and retrospective studies resulted in rapid adoption of SBRT for early-stage NSCLC.[60] The rationale of SBRT for early-stage NSCLC is that higher radiotherapy doses are more effective in locally controlling the tumor (local tumor control rates of 90% and higher are achieved, with rates of severe toxicity below 10%), which in turn translates into longer OS.

Due to large differences in single fraction and total doses between different studies, a comparison of physical doses is less meaningful. The current recommended tumor dose for SBRT of lung tumors is a minimum of 100 Gy BED, prescribed to the target volume encompassing isodose.[61–63] Total doses are typically delivered in between one and five fractions. Treatment of tumors in the proximity of critical normal organs has led to the used of so-called risk-adapted fractionation schemes that deliver the minimal required dose of 100 Gy BED in a larger number of lower treatment fractions.[64,65]

Fractionation appears especially valuable for centrally located tumors (tumors located either adjacent to the proximal bronchial tree or ≤1 cm from the heart or mediastinum) because it allows for radiobiological sparing of critical organs.

A higher incidence of complications has been initially reported after SBRT for central tumors.[66] However, a systematic review of the literature indicates that SBRT is a relatively safe and effective curative treatment, provided that appropriate fractionation schedules are used for central tumors.[67]

A favorable therapeutic ratio of high local control and simultaneously low toxicity has been maintained even after a more widespread adoption of SBRT outside of clinical trials and specialized radiotherapy centers.[32,68] This finding of reproducible clinical outcome despite relevant variability and time trends in SBRT practice suggests that clinical SBRT outcomes are fairly robust. Safety profile of SBRT is certainly quite good; symptomatic radiation pneumonitis (RP) is uncommon after the treatment of peripheral lung tumors measuring 5 cm or less, irrespective of the presence of common findings of RP/fibrosis on follow-up CT scans.

SBRT is safely practiced also in patients with severe pulmonary comorbidities, in patients with and very poor pretreatment pulmonary function, and in patients older than 75 years. A higher incidence of severe RP has been reported only in patients with pre-existent idiopathic pulmonary fibrosis.

Milder and risk-adapted fractionation schedules have to be used in larger (more than 5 cm) and centrally located tumors. More uncommon toxicities reported after SBRT include chest wall toxicity such as rib fracture and/or neuropathic pain. At present, SBRT is the guideline-recommended nonsurgical treatment of choice for early-stage NSCLC.[69]

Guidelines for SBRT have been reported by several professional groups: very shortly, SBRT is a technique for delivering external beam radiotherapy with a high degree of accuracy, using high doses of irradiation, which are delivered in one or few treatment fractions to an extracranial target.[32]

SBRT can be adequately performed using either traditional linear accelerators equipped with suitable image-guidance technology or linear accelerators specifically adapted for SBRT and using dedicated delivery systems. The SBRT procedure was initially defined by the use of stereotactic frame-based patient set-up. However, frame-based stereotactic patient set-up has been replaced by image-guidance

(frameless SBRT with image-guidance technologies). With nonframe-based patient set-up, external stereotactic coordinates are replaced by visualization of a patient's anatomy using images acquired on-table and subsequently compared with pretreatment planning images (image-guided radiotherapy). Several technologies for image guidance are commercially available, and superiority of one method over the other has not been demonstrated. Use of volumetric imaging (cone beam CT), as opposed to only implanted fiducials, has the advantage of enabling assessment of changes in target shape and position, relative to the position of organs at risk.

SBRT: SIMULATION, PLANNING, AND TREATMENT DELIVERY

Four-dimensional CT is the recommended technique for SBRT simulation, due to its ability to accurately target moving thoracic tumors and define patient's specific internal target volume .[70]

For planning, all published prospective trials have used three-dimensional conformal treatment planning. Intensity-modulated radiation therapy (IMRT) and advanced rotational techniques such as volumetric modulated arc therapy (VMAT) have the potential to increase dose conformity and homogeneity and reduce treatment delivery times.[71]

VMAT is a form of IMRT in which the gantry continuously moves around the patient with a varying speed and rate of dose delivery. The maximal dose rate on some of the current linear accelerators (flattening filter free) using this approach is up to four times faster than the standard dose rates most often used.

When using IMRT planning, larger volumes of normal pulmonary tissue, including contralateral lung, can be exposed to low radiation doses (V_5); especially when treating larger tumors with VMAT-based approach, doses to the contralateral lung may predict for the risk of pneumonitis.[72]

Regarding delivery phase and active motion management strategies, continuous irradiation in free breathing is performed using the internal target volume concept, the mean target position concept, or real-time tumor tracking. Noncontinuous irradiation of the tumor in a reproducible position is performed using gated beam delivery in predefined phases of the breathing cycle.[73]

FUTURE DIRECTIONS

Currently, the safest dose and fractionation for SBRT in centrally located tumors are not known: some ongoing trials will certainly contribute to clarify this issue. The role of SBRT in so-called borderline operable patients will also be better clarified.

The use of proton radiotherapy is receiving increased attention as a modality that is just as effective as photon therapy, but with improved dose distribution in terms of better therapeutic ratio; the role of proton SBRT will be promptly evaluated, especially for centrally located tumors.

Finally, despite high rates of primary tumor control, the rate of distant failure remains consistently high; more work is needed to identify biomarkers that may predict those patients at risk of developing systemic failures such that these patients can be eventually offered adjuvant treatments.

SYSTEMIC TREATMENT FOR EARLY-STAGE NSCLC

At the end of the 1970s, it became clear that many early-stage NSCLC patients already had occult distant metastases at the time of surgery, which led to predominantly distant recurrence and death.[74] Effective adjuvant therapies thus might improve outcome, even if small studies with the available agents at that time were not effective to demonstrate a benefit.[75]

In the 1980s, several randomized controlled trials (RCTs) further studied adjuvant chemotherapy. They were small (less than 100 patients per arm), and many used the so-called cyclophosphamide, adriamycin, cisplatin (CAV) regimen. Some effects on distant metastasis and DFS could be demonstrated, but side effects—emesis in particular—impeded delivery of doses that could affect long-term outcome.[76,77]

In the 1990s, a landmark individual patient–based meta-analysis on the effect of chemotherapy in NSCLC reported the potential benefit of postoperative cisplatin-based chemotherapy.[78] In the adjuvant setting, the comparison of surgery alone versus surgery and chemotherapy gave a hazard ratio (HR) for (OS) of 0.87 (13% reduction in the risk of death, estimated absolute benefit of 5% in 5-year OS, $p = 0.005$). This meta-analysis was the basis for the statistical design of the International Adjuvant Lung Cancer Trial.

The landmark International Adjuvant Lung Cancer Trial results were reported in the *2000* decade and were the first to indicate the benefit of adjuvant cisplatin-based chemotherapy in 1867 patients with completely resected stage I to III NSCLC.[79] This trial, together with several others, both with positive[80,81] and negative[82,83] outcomes, was entered into the individual patient–based Lung Adjuvant Cisplatin Evaluation meta-analysis.[29] In this study of the five largest trials (4584 patients), the HR of death was 0.89 (95% confidence interval (CI) 0.82–0.96; $p = .005$), corresponding to a 5-year absolute benefit of 5.4% with chemotherapy. The benefit varied with stage and was documented in stage II, associated with N1 disease in the 6th edition of TNM used in these studies, and stage III, patients with N2 disease in these studies.

In the same decade, several RCTs examining the value of neoadjuvant cisplatin-based chemotherapy, started based on promising signals in very small-sized trials from the *1990s*,[84,85] were reported.[86–88] These trials were in general of smaller size and could not demonstrate a significant improvement in OS. A recent meta-analysis on 2385 patients, however, reported a very similar HR compared with the adjuvant approach: 0.87 (95% CI 0.78–0.96, $p = 0.007$), with an absolute 5-year OS improvement of 5%.[89]

In Japanese patients, several studies looked at adjuvant use of oral uracil-tegafur for 2 years, based on promising findings from the *1990s*. In a RCT with 999 resected stage I adenocarcinoma patients, OS was significantly better with uracil-tegafur versus standard follow-up: HR 0.71 (95%CI 0.52–0.98, $p = 0.04$).[90]

Postoperative cisplatin-based chemotherapy remains limited by toxicity because the most often used regimen of cisplatin-vinorelbine is not well tolerated by patients, which led to delivery of the planned number of cycles in only 50%

to 75% of the patients in the phase 3 studies. Moreover, even if clearly significant, effect on OS remains limited. Several ways to improve are currently being studied. Ways to improve are depicted in Figure 2. With surgery alone (left part), around 40% of the patients will be cured, around 40% will relapse and die of lung cancer, and around 20% will die of comorbidity (often smoking-related cardiovascular or lung disease). With adjuvant chemotherapy (right part), we have brought this to 45%, 35%, and 20%, respectively.

A first idea is to improve tolerability and thus drug delivery of adjuvant chemotherapy. Better anti-emetics such as the neurokinin-1 antagonists were an important step. Using better tolerated chemotherapy has been examined as well, and several trials reported a far lower toxicity and better drug delivery with, for example, cisplatin-pemetrexed.[91,92]

A second way is to use adjuvant therapy only in those who are more likely to benefit based on prognostic factors. Indeed, nowadays we administer adjuvant treatment to 100 patients to have an extra cure in 5% of these (number needed to treat 20). Examples are the 15-gene signature reported in the BR.10 adjuvant trial[93] or the 14 gene quantitative polymerase chain reaction –based assay.[89]

A third strategy is enlarging the therapeutic ratio, that is, the risk-benefit profile, based on predictive factors. Several predictive factors for better activity of chemotherapy have been described in retrospective reports, such as expression of the excision repair cross-complementation 1 expression (ERCC1) for sensitivity to cisplatin,[94] thymidilate synthase expression for pemetrexed,[95] or ribonucleotide reductase M1 expression for gemcitabine.[96] This principle of so-called pharmacogenetic-driven adjuvant chemotherapy is currently being explored in several prospective trials. A recent report on the French study showed multicenter feasibility of such an approach, but further development was stopped because of unreliable ERCC1 readouts, due to different antibodies reacting with different isoforms.[59] Moreover, recent phase 3 confirmatory pharmacogenetic studies in the setting of advanced NSCLC were disappointingly negative.[97,98] So, albeit attractive, the principle of biomarker-driven adjuvant chemotherapy clearly needs further technical refinement before patient benefits can be expected.

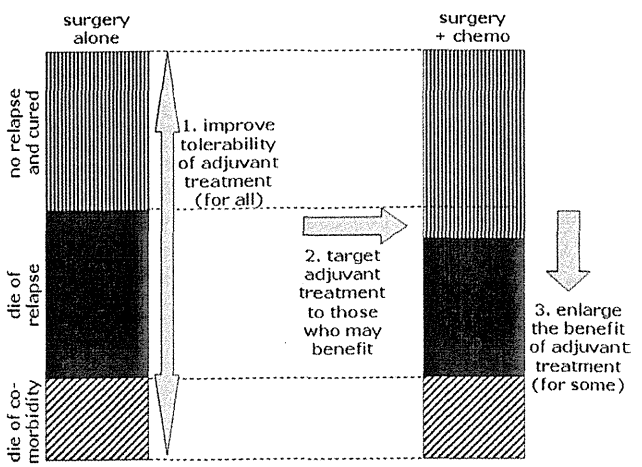

FIGURE 2. Ways to improve adjuvant therapy for resected non–small-cell lung cancer (NSCLC). Adapted with permission from Spiro SG, Tanner NT, Silvestri GA, et al. Lung cancer: Progress in diagnosis, staging and therapy. *Respirology* 2010; 15: 44–50.

The past decade has also seen the marked increase in the development of novel therapeutic strategies targeting signaling pathways, such as epidermal growth factor receptor (EGFR), angiogenesis, and, more recently, immunotherapy in stage IV NSCLC. The potential contribution of these strategies in the adjuvant setting is still a matter of debate. More than 1000 patients were included in a phase III trial (NCIC BR.19) originally designed to evaluate the efficacy of gefitinib versus placebo in unselected patients with resected stage IB to IIIA NSCLC disease; unfortunately this study was prematurely stopped because of the negative results of other gefitinib studies.[99–101] No overall survival benefit was detected with adjuvant gefitinib in the 503 patients included, and results were also inconclusive among EGFR mutant patients. Another phase III study (RADIANT) is comparing erlotinib with placebo in patients with resected stage IB to IIIA NSCLC after being treated with adjuvant chemotherapy (ClinicalTrials.gov, NCT00373425). Eligible patients include those with EGFR mutation, gene amplification, or protein expression.[102] Erlotinib did not prolong disease free survival in these NSCLC completely resected population.

The role of bevacizumab (added to cisplatin-based chemotherapy) in the same disease setting is currently under evaluation in Eastern Cooperative Oncology Group 1505 study, a randomized phase III trial in completely resected stage IB to IIIA NSCLC.

MAGE-A3 vaccine is a cancer immunotherapy that is being developed specifically in the adjuvant setting in patients with resected NSCLC, where MAGE-A3 antigen is expressed in 33% of the tumor samples. On the basis of a phase II study in which patients who received MAGE-A3 vaccine had a nonsignificant improvement in DFS and OS compared with placebo, a large phase III trial was designed and 2270 patients were enrolled. Unfortunately, at the beginning of 2014 it was announced that the trial did not meet its first and second coprimary end points. There was no significant improvement in DFS compared with placebo in either the overall MAGE-A3–positive population (first co-primary end point) or in those MAGE-A3–positive patients who did not receive chemotherapy (second co-primary end point). Also a third end point based on a previously identified gene signature predicting efficacy of MAGE-A3 vaccine was not reached.

CONCLUSIONS

Surgery remains the standard treatment for early-stage NSCLC. In the past 40 years, a lot of improvements have been made in this setting with the introduction of modern surgical techniques and radiotherapeutic approaches, alternative treatments to sublobar resection—in patients with borderline medical criteria for surgery—based on SBRT, and the complementary role of systemic treatments has been definitively established.

Nevertheless, many issues are still a matter of debate, and OS improvements are clearly needed in this curative

