**TABLE 2. Relationship between patient clinicopathologic characteristics and survival after resection of a solitary recurrent lesion**

| | Factors | No. | MST (mo) | 2-y survival (%) | 5-y survival (%) | Univariate analysis, *P* value | Multivariate analysis, *P* value |
|---|---|---|---|---|---|---|---|
| Age at recurrence resection (y) | ≥65 | 14 | 22 | 43 | 26 | .41 | — |
| | <65 | 14 | 27 | 75 | 38 | | |
| Sex | Male | 17 | 26 | 53 | 31 | .48 | — |
| | Female | 11 | 28 | 67 | 33 | | |
| RFI (y) | ≥2 | 13 | 26 | 68 | 45 | .25 | — |
| | <2 | 15 | 27 | 51 | 22 | | |
| CEA level at recurrence (ng/mL) | >5 | 9 | 22 | 30 | Not reached | .13 | .80 |
| | ≤5 | 19 | 30 | 70 | 38 | | |
| Symptoms at recurrence | + | 9 | 26 | 53 | 18 | .33 | — |
| | − | 19 | 28 | 60 | 36 | | |
| Site of recurrence | Intrapulmonary | 16 | 30 | 71 | 40 | .15 | .50 |
| | Extrapulmonary | 12 | 22 | 42 | 21 | | |
| Mode of recurrence | Locoregional | 10 | 26 | 57 | 46 | .41 | — |
| | Distant | 18 | 27 | 61 | 23 | | |
| Histology | Ad | 21 | 26 | 55 | 31 | .73 | — |
| | Non-Ad | 7 | 30 | 67 | 33 | | |
| Size of primary tumor (cm) | ≥4 | 14 | 26 | 51 | 17 | .18 | — |
| | <4 | 14 | 28 | 66 | 47 | | |
| Nodal status of primary tumor | N0 (N−) | 18 | 30 | 67 | 40 | .08 | .40 |
| | N1/N2 (N+) | 10 | 22 | 41 | 14 | | |
| p-Stage of primary tumor | I | 14 | — | 76 | 51 | .0045 | .04 |
| | II/III | 14 | 19 | 10 | 10 | | |
| Ly in primary tumor | + | 10 | 21 | 44 | Not reached | .32 | — |
| | − | 18 | 27 | 67 | 33 | | |
| V in primary tumor | + | 17 | 27 | 57 | 29 | .97 | — |
| | − | 11 | 26 | 61 | 36 | | |

*MST*, Median survival time; *RFI*, recurrence-free interval; *CEA*, carcinoembryonic antigen; *Ad*, adenocarcinoma; *Ly*, lymphatic permeation; *V*, vascular permeation.

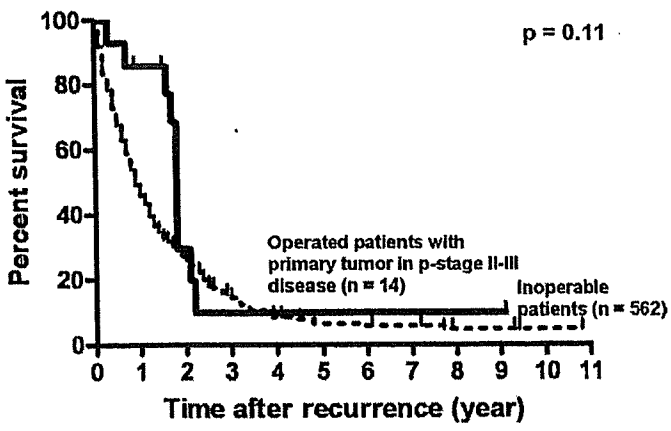


**Figure 2. Comparative survival curves among 14 resected patients with p-stage II or III primary non–small cell lung cancer and 562 patients without resection. There is no significant difference in survival probability after recurrence ($P = .11$).**

with p-stage II or III disease, however, had a poor outcome equivalent to that seen in patients with recurrent NSCLC in whom surgical intervention was not indicated. This suggests that advanced stage (ie, II or III) in the primary tumor is a contraindication for surgical intervention in patients with a solitary recurrence. Consistent with our result, Yoshino and coworkers[18] described a strong relationship between pathologic stage and clinical courses after recurrence in patients with NSCLC. They reported that the mean postrecurrent survival time was 590 days in pathologic stage I disease, 381 days in stage II disease, 257 days in stage IIIA disease, and 180 days in stage IIIB disease, with a significant difference being observed between stages I and IIIA ($P = .0215$). In patients with advanced and biologically aggressive NSCLC, a solitary recurrence might be just the beginning of progressive-disseminated disease.

In our series patients who underwent resection of the intrapulmonary recurrent lesion showed slightly but not significantly better survival than the extrapulmonary recurrence group ($P = .15$). This might be because some in-

trapulmonary lesions were actually metachronous second primary lung cancers. We can expect better prognosis for metachronous lung cancer compared with intrapulmonary recurrence.[19,20] It can often be hard to discriminate a solitary pulmonary recurrence from a metachronous second primary lung cancer if the 2 lesions are of the same histologic type.[21] Therefore aggressive surgical resection for an intrapulmonary lesion might be justified for patients with adequate pulmonary reserve, regardless of the primary tumor pathology.

Although we did not perform positron emission tomography (PET) with $^{18}$F fluorodeoxyglucose (FDG) for the patients in this study, FDG-PET has been reported to be a helpful adjunct in screening for distant metastases but not for brain metastases.[22] Several investigators have reported that FDG-PET could detect unexpected metastatic lesions in 10% to 20% of patients with newly diagnosed NSCLC.[23,24] PET imaging might also be helpful in avoiding surgical intervention in patients who have multiple recurrent lesions.[25] However, it is well known that FDG is not tumor specific and is also taken up in benign lseions.[22] Lardinois and colleagues[26] have reported that 46% of solitary extrapulmonary lesions detected by means of integrated PET and computed tomography were unrelated to lung cancer metastases. Further studies will be needed to clarify whether PET imaging is useful in identifying more clearly the population that benefits from additional surgical intervention and in prolonging subsequent survival.

The limitation of the current study is that the number of enrolled patients, especially in the surgical resection group, was obviously small. Therefore a multi-institutional study would be required to confirm our findings.

In conclusion, long-term survival can be achieved by means of resection of a solitary recurrent lesion in highly selected patients. However, surgical resection might be contraindicated if the primary NSCLC stage is II or III, especially when the recurrent lesion is extrapulmonary.

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# Carbonic anhydrase IX expression is associated with tumor progression and a poor prognosis of lung adenocarcinoma

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Summary Carbonic anhydrase (CA) IX catalyzes the hydration of carbon dioxide into carbonic acid and participates in a variety of physiological and biological processes. The aim of this study was to evaluate the prognostic significance of CA IX expression in patients with lung adenocarcinoma. Standard immunohistochemical techniques were used to study CA IX expression in 134 patients who underwent curative resection for adenocarcinoma of the lung at our hospital between January 1995 and December 1996. We evaluated the correlations between CA IX expression levels on cancer cells and clinicopathological factors. CA IX expression was not observed in normal lung tissue or specimens from non-invasive adenocarcinomas. CA IX immunostaining was detected in 33 (24.6%) invasive adenocarcinoma cases. Poor differentiated histological phenotype ($p=0.0015$), pathological stage ($p=0.0400$), vascular invasion ($p=0.0009$) and lymphatic permeation ($p=0.0050$) were significantly related to CA IX expression. On univariate analysis, CA IX positive cases showed significantly shorter overall survival ($p=0.0083$) and disease-free survival ($p=0.0122$). In particular, the overall and disease-free survivals in stages I+II were significantly shorter in the CA IX positive than in the CA IX negative cases ($p=0.0269$ and 0.0011, respectively). Our results suggest that CA IX expression is strongly associated with tumor progression and indicates a poor prognosis for patients with stages I+II lung adenocarcinoma.


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## 1. Introduction

Carbonic anhydrases (CAs) are zinc metalloenzymes that catalyze the reversible hydration of carbon dioxide for pH regulation and participate in a variety of physiological and biological processes [1–3]. Carbonic anhydrase IX (CA IX) is one of four transmembrane isozymes [4] and its expression can be induced in hypoxia via hypoxia-inducible factor 1 (HIF-1) protein binding to the hypoxia responsive element of the CA IX promoter [5,6]. CA IX was first identified in a human cervical carcinoma (HeLa) cell line and subsequently found to be expressed in several human carcinoma cell lines and clinical tumor specimens [7]. *In vitro* experiments revealed that transfection of CA IX can induce the transformation of 3T3 cells such that density-dependent growth arrest is lost [4]. Furthermore, overexpression of CA IX is associated with a poor prognosis in lung [8–12], breast [13], uterine cervix [14,15] and renal cell [16] carcinoma patients, whereas normal human tissues, except for the gastric mucosa [17,18], are not immunostained by CA IX antibody. Although CA IX has been reported to have diagnostic utility in various tumor types [19–22] and prognostic significance in head and neck [23], kidney [16,24] and lung [8–12] carcinomas, the role of CA IX in tumor progression remains unclear.

Lung cancer is associated with the highest cancer-related morbidity and mortality rate in the world. Non-small cell lung cancer (NSCLC) comprises approximately 80% of all lung cancers, among which adenocarcinoma and squamous cell carcinoma are the two major histological subtypes, although adenocarcinoma is the most common histological subtype of lung cancer in many countries. These two subtypes of carcinoma have different pathogenetic pathways and distinct biological characteristics [25]. As for CA IX expression in early stage NCSLC, recent reports have indicated CA IX expression in squamous cell carcinoma to be much higher than that in adenocarcinoma. This may be explained by squamous cell carcinomas being more necrotic than adenocarcinomas [8,9], suggesting the cancer microenvironment within squamous cell carcinomas to be more hypoxic.

Adenocarcinoma of the lung is well known to show histological heterogeneity which would reflect the biological diversity of adenocarcinoma. In this study, we examined CA IX expression in 134 consecutive cases that underwent curative resection of lung adenocarcinoma. The aim of this study was to examine the role of CA IX expression in lung adenocarcinoma.

## 2. Materials and methods

### 2.1. Patients

The study subjects were 140 patients with adenocarcinoma of the lung who underwent complete resection at the National Cancer Center Hospital East between January 1995 and December 1996. Five patients who died of non-cancer-related diseases were excluded from this study. One patient was excluded because the specimen obtained was of poor quality. Of the 134 stained specimens, 64 were from males and 70 from females. The mean age at surgery was 62 (range, 34–84). The median follow-up time was 5.2 years. Survival time was measured from the date of surgery.

### 2.2. Pathological studies

All surgical specimens were fixed with 10% formalin and embedded in paraffin. The tumors were cut at approximately 5-mm intervals, and serial 4-μm sections were stained with hematoxylin and eosin, the Alcian blue-periodic acid Schiff method to visualize cytoplasmic mucin production, or the Verhoeff van-Gieson method to visualize elastic fibers. Lymphatic spread and pulmonary metastases were evaluated on sections stained with hematoxylin and eosin. Vascular and pleural invasions were evaluated using the Verhoeff van-Gieson method. Two observers (H.K. and G.I.), both unaware of the clinical data, independently reviewed all pathological slides. The histological diagnoses were based on the revised World Health Organization (WHO) histological classification. In addition, the histological subtypes and percentage of each subtype present in the tumor were evaluated using the maximum cut surface of the tumor. The histological patterns were divided into four distinctive subtypes: bronchiolo-alveolar carcinoma (BAC), acinar subtype, papillary subtype, and solid adenocarcinoma with mucin. Tumor size was measured as the maximal diameter on the cut section of the lung. The pathological stage was determined according to the classification of the Union Internationale Contre le Cancer (UICC).

### 2.3. Antibodies

As we could not obtain anti CA IX monoclonal antibody (M-75 clone), we used commercially available rabbit (H-120) and goat (N-19) polyclonal antibodies against human CA IX, (Santa Cruz Biotechnology Inc., Santa Cruz, CA, USA).

### 2.4. Cells and cell cultures

The human pancreatic cancer cell line Capan-1 was purchased from American Type Culture Collection (Rockville, MD, USA). Capan-1 was maintained in DMEM medium with 20% heat-inactivated fetal bovine serum and antibiotics. Cells were maintained in a 5% $CO_2$ incubator at 37 °C. For the hypoxic treatments, the cells were seeded at a density of $5 \times 10^5$ cells per 6 cm dish. After 24 h, the cultures were transferred to a Napco 7000 incubator and maintained for 24 h in 0.5% $O_2$, 5% $CO_2$, balanced with $N_2$. Parallel samples were incubated under normoxic conditions. Finally, cells were extracted for Western blotting analysis.

### 2.5. Western blotting

Cells were solubilized in lysis buffer (300 mM NaCl, 0.5% Nonidet P-40, 1 mM EDTA, 20 mM HEPES–NaOH, PH 7.9, 50 μM leupepsin, 50 μM pepstain, 50 μM aprotinin, 10 mM sodium fluoride, 1 mM phenylmethysulonyl floride, 1 mM dithiothreitol, and 250 mM sodium orthovanadate). After incubation for 30 min at 4 °C, cell debris and nuclei were removed by centrifugation at 15,000 rpm for 10 min at 4 °C. The supernatants were mixed with concentrated Laemmli sample buffer, boiled for 5 min and centrifuged at 12,000 × *g* for 15 min. Samples of 20 μg of total cell lysate were electrophoresed on a 12% SDS-polyacrylamide gel,

and the separated proteins blotted electrophoretically onto a polyvinylidene difluoride membrane (Millipore Corp., Bedford, MA, USA). Nonspecific binding was blocked for 1 h with 5% nonfat dry milk containing 0.1% Tween 20 at room temperature. The membrane was incubated for an hour in the anti-CA IX antibody (H-120) (1:100) and HIF-1 α (H-206, Santa Cruz Biotechnology Inc., USA) (1:100) at room temperature and then for 1 h with peroxidase-labeled rabbit anti-rabbit antibody (1:3000; Zymed Laboratories Inc., San Francisco, CA, USA). Antibody binding was detected with an Amersham enhanced chemiluminescence system (Amersham Corp., Arlington Heights, IL, USA).

### 2.6. Immunohistochemistry

All tumor tissues used in this study were from routinely formalin-fixed pathological samples taken from resected lung specimen. One block containing the most extensive tumor component was selected from each specimen, following a review of hematoxylin and eosin-stained slides of the surgical specimens. Sections, 4 μm each, were cut from the paraffin blocks and mounted on silanized slides. The sections were deparaffinized in xylene, dehydrated in a graded ethanol series, and then immersed in methanol with 0.3% hydrogen peroxide for 15 min to inhibit endogenous peroxidase activity. After being washed with distilled water, the sections were placed in Retrieval Solution High pH (DakoCytomation, Carpinteria, CA, USA). For antigen retrieval, the slides were heated twice at 95 °C for 20 min in a microwave oven (H2800 Microwave Processor, Energy Beam Sciences Inc.) and then cooled for 1 h at room temperature. The slides were washed three times in phosphate-buffered saline (PBS). Nonspecific binding was then blocked by preincubation with 2% normal swine serum in PBS (blocking buffer) for 60 min at room temperature. Individual slides were next incubated overnight at 4 °C with primary anti-CA IX rabbit polyclonal antibody, (H-120, Santa Cruz Biotechnology Inc., USA), at a final dilution of 1:150 in the blocking buffer. The slides were washed three times with PBS then incubated with EnVision™ (DAKO, Denmark) for 1 h at room temperature. After extensive washing with PBS, the color reaction was developed in 2% 3,3′-diaminobenzidine in 50 mM Tris-buffer (pH 7.6) containing 0.3% hydrogen peroxidase for 5—10 min. The sections were finally counterstained with Meyer's hematoxylin, dehydrated, and mounted.

Two investigators (H.K. and G.I.), both blinded to the clinical outcomes, independently evaluated the staining results. Human renal cell carcinoma (RCC) was used as positive control for CA IX staining. When more than 20% of the cancer cells showed an unequivocally strong membranous and/or cytoplasmic reaction, we classified the case as CA IX positive.

### 2.7. Statistical analysis

The correlations between CA IX expression level and clinicopathological factors were evaluated by the $\chi^2$-test or Fisher's exact test, as appropriate. Overall survival was measured from the date of surgery to the date of death from any cause or the date the patient was last known to be alive. Survival curves were estimated by the Kaplan–Meier method, and differences in survival between subgroups were compared by the log-rank test. A $p$ value of less than 0.05 was considered significant. Statistical analysis software (StatView, Version 5.0) was used for the analyses.

## 3. Results

### 3.1. CA IX expression in normal lung and lung adenocarcinoma

We first performed immunohistochemical staining using two commercially available rabbit (H-120) and goat (N-19) polyclonal antibodies against human CA IX. Preliminary experiments showed the CA IX antibody H-120 to be more suitable than N-19 for immunohistochemical analysis in formalin-fixed paraffin-embedded sections. CA IX expression has previously been shown to upregulated by hypoxia in various cell lines. To test H-120 reactivity, the effect of the hypoxia on CA IX protein levels was examined by Western blot analysis. The human pancreatic carcinoma cell line Capan-1 was exposed to hypoxia (0.5% $O_2$ for 24 h). As expected, hypoxia-inducible transcription factor 1α (HIF-1α) was undetectable under normoxic, but readily stabilized, under hypoxic conditions. In the hypoxic state, marked upregulation of CA IX with a doublet having apparent molecular weights of 54 and 58 kDa was observed [8], whereas the level of CA IX was minimal in the normoxic state (Fig. 1A). The H-120 reactivity on Western blot analysis was identical to that in previous studies. Furthermore the immunohistochemical results that human renal clear cell carcinoma cells (clear cell carcinoma) (Fig. 1B) and carcinoma cells around the necrotic area (Fig. 1C) also showed positive reaction for H-120 antibody, which were identical to those obtained by monoclonal M-75 clone antibody [24,10,15], we used this antibody for further immunohistochemical study.

A series of 140 lung adenocarcinoma specimens were studied for CA IX expression. In normal human lung tissue,

Table 1 CAIX staining in lung adenocarcinoma

| | Cases (%) | CAIX staining | | $p$ value |
|---|---|---|---|---|
| | | Positive | Negative | |
| All cases | 134 (100) | 33 (24.6) | 101 (75.4) | |
| Non-invasive | 9 (6.7) | 0 (0) | 9 (100) | |
| Invasive | 125 (93.3) | 33 (26.4) | 92 (74.4) | 0.0214 |

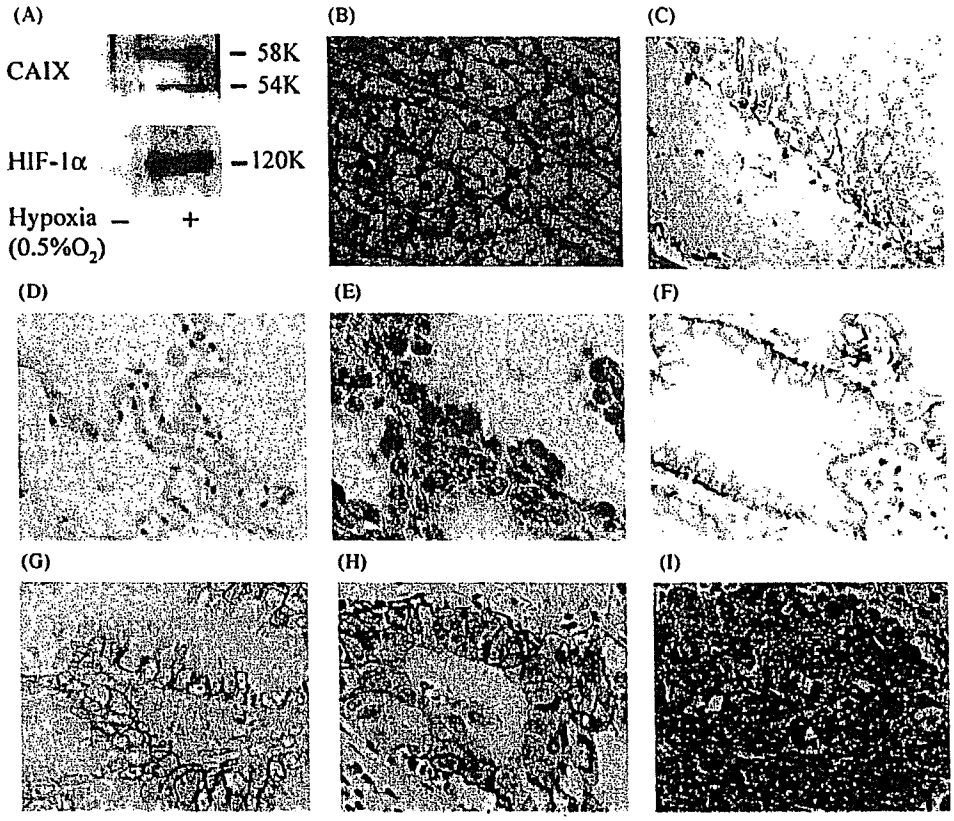


Fig. 1 Immunohistochemical analysis of CA IX expression in normal lung tissue and lung adenocarcinoma. (A) Evaluation of CA IX antibody (H-120). The effect of hypoxia on CA IX protein levels was examined by Western blot analysis. Strong CA IX expression was found in renal cell carcinoma (positive control) (B) and carcinoma cells around the necrotic area (C). There was no CA IX expression on type II alveolar pneumocytes (D) and on non-invasive adenocarcinoma cells (E). CA IX expression was observed in mucinous BAC component (F), papillary subtype (G), acinar subtype (H) and solid adenocarcinoma with mucin (I).

neither type II alveolar epithelium nor bronchial epithelium was immunostained by CA IX antibody (Fig. 1D). CA IX expression was present in 33 of 134 (24.6%) lung adenocarcinoma cases. All positive cases were invasive lung adenocarcinoma, however, no expression was observed in all nine cases with non-invasive adenocarcinoma (Table 1, Fig. 1E). Fig. 1F–I show representative CA IX immunostaining for invasive adenocarcinoma of the mucinous BAC (Fig. 1F),

Table 2 Correlation between CAIX staining and clinical factors

| Variables | Cases (%) | CAIX staining | | p value |
|---|---|---|---|---|
| | | Positive | Negative | |
| All cases | 134 (100) | 33 (24.6) | 101 (75.4) | |
| Age (years) | | | | |
| <65 | 73 (54.5) | 18 (24.7) | 55 (75.3) | 0.6939 |
| ≧65 | 61 (45.5) | 15 (24.6) | 46 (75.4) | |
| Gender | | | | |
| Male | 64 (47.8) | 13 (20.3) | 51 (79.7) | |
| Female | 70 (52.2) | 20 (28.6) | 50 (71.4) | 0.4796 |
| Smoking history | | | | |
| Smokers | 64 (47.8) | 17 (26.6) | 47 (73.4) | |
| Never-smokers | 70 (52.2) | 16 (22.9) | 54 (77.1) | 0.7675 |
| CEA (ng/ml) | | | | |
| ≦5 | 79 (59.0) | 15 (19.0) | 64 (81.0) | |
| >5 | 55 (41.0) | 18 (32.7) | 37 (67.3) | 0.0711 |

Table 3 Correlation between CA IX staining and pathological factors

| Variables | Cases (%) | CAIX staining | | p value |
|---|---|---|---|---|
| | | Positive | Negative | |
| Differentiation | | | | |
| Well | 69 (51.5) | 8 (11.6) | 61 (88.4) | |
| Moderately | 47 (35.1) | 18 (38.3) | 29 (61.7) | |
| Poorly | 18 (13.4) | 7 (38.9) | 11 (61.1) | 0.0015 |
| Tumor stage (pT) | | | | |
| T1 | 72 (53.7) | 16 (22.2) | 56 (77.8) | |
| T2 | 39 (29.1) | 9 (23.1) | 30 (76.9) | |
| T3 | 7 (5.2) | 2 (28.6) | 5 (71.4) | |
| T4 | 16 (12.0) | 6 (57.5) | 10 (62.5) | 0.6233 |
| Nodal involvement (pN) | | | | |
| N0 | 87 (64.9) | 16 (18.4) | 71 (81.6) | |
| N1 | 14 (10.5) | 4 (28.6) | 10 (71.4) | |
| N2 | 33 (24.6) | 13 (39.4) | 20 (60.6) | 0.0546 |
| Disease stage (p-stage) | | | | |
| I | 77 (57.5) | 13 (16.9) | 64 (83.1) | |
| II | 17 (12.7) | 4 (23.5) | 13 (76.5) | |
| III | 40 (29.8) | 16 (40.0) | 24 (60.0) | 0.0400 |
| Vascular invasion | | | | |
| Present | 72 (53.7) | 26 (36.1) | 46 (63.9) | |
| Absent | 62 (46.3) | 7 (11.3) | 55 (88.7) | 0.0009 |
| Lymphatic permeation | | | | |
| Present | 61 (45.5) | 21 (34.4) | 40 (65.6) | |
| Absent | 73 (54.5) | 12 (16.4) | 61 (83.6) | 0.0050 |
| Mitotic index (HPF) | | | | |
| 0–4/10 | 94 (70.2) | 19 (20.2) | 75 (79.8) | |
| 5–14/10 | 33 (24.6) | 13 (39.4) | 20 (60.6) | |
| >15/10 | 7 (5.2) | 1 (14.3) | 6 (85.7) | 0.7180 |

papillary (Fig. 1G), acinar (Fig. 1H) and solid (Fig. 1) growth pattern.

### 3.2. Relations between CA IX expression and clinicopathological factors

Patient characteristics are shown in Table 2. The mean age of our patients was 62 years (range, 34–84 years). There were 64 men and 70 women. Relationships between CA IX expression and clinical factors were analyzed, but none were found to be significant. Table 3 summarizes relationships between CA IX expression and pathological factors. Tumor differentiation ($p=0.0015$), pathological stage ($p=0.0400$), vascular invasion ($p=0.0009$), and lymphatic permeation ($p=0.0050$) were significantly related to CA IX expression. Nodal involvement showed a marginal association with CA IX expression.

### 3.3. Relationship of CA IX expression to overall survival and disease-free survival

Univariate analyses were performed using the Cox proportional hazards model to determine of the prognostic value of CA IX expression (Table 4). CA IX staining was significantly associated with shorter survival ($p=0.0083$). In addition, pathological tumor stage ($p=0.0001$), pathological nodal involvement ($p<0.0001$), vascular invasion ($p=0.0007$) and lymphatic permeation ($p<0.0001$) were also associated with shorter survival. Subsequently we performed a multivariate analysis to examine the importance of CA IX in survival when other prognostic factors were included (Table 5). Pathological tumor stage and nodal involvement were significant independent prognostic factors in the multivariate analysis of overall survival ($p=0.0111$ and $p<0.0001$, respectively). However, CA IX expression was not a significant independent prognostic factor ($p=0.1800$).

The disease-free survival results are presented in Table 6. The univariate analysis revealed CA IX staining to be significantly associated with shorter disease-free survival ($p=0.0122$). Pathological tumor stage ($p<0.0001$), pathological nodal involvement ($p<0.0001$), vascular invasion ($p=0.004$), lymphatic permeation ($p<0.0001$), tumor differentiation ($p=0.0028$) and the mitotic index ($p=0.0054$) were also associated with CA IX expression. Table 7 shows the multivariate analysis results for disease-free survival. Pathological tumor stage and nodal involvement were significant independent prognostic factors for disease-free survival ($p=0.0093$, $p<0.0001$, respectively). However, CA IX expression was not a significant independent prognostic factor ($p=0.4102$).

The overall survival and disease-free survival curves obtained by the Kaplan–Meier method, with statistical significance assessed using the log-rank test, are shown in Fig. 2. The cases positive for CA IX expression had significantly shorter overall and disease-free survival period than those who were negative ($p=0.0068$ and 0.0104, respectively). In addition, the overall and disease-free survivals of stages I + II cases were significantly shorter among those positive for CA IX than in the negative cases ($p=0.0269$ and 0.0011, respectively). In stage III, however, there were no differences between the two groups ($p=0.7422$ and 0.8540, respectively) (Fig. 3).

## 4. Discussion

Although prior studies reported that the overexpression of CA IX is associated with a poor prognosis in lung cancer, all of those dealt with non-small cell lung cancer, bringing together all variant histology [8–12]. Furthermore the large parts of the specimens, which the previous studies examined were squamous cell carcinoma. In our study, virtually none of the cancer cells in non-invasive adenocarcinoma cases expressed CA IX. Even in invasive adenocarcinoma cases, most cancer cells showing the lepidic growth (BAC component) were weak or negative for CAIX expression. Furthermore CAIX expression was related to a poor prognosis in term of overall and disease-free survival especially in early stage (p-stages I + II) than in late stage (p-stage III) disease. Our study was the first report considering about the role of CA IX expression in lung adenocarcinoma.

Although a study with a larger number of patients is warranted, these observations suggest that during lung adenocarcinoma progression, CA IX expression might be induced by factors characteristic of the cancer microenvironment [26]. Recently, Simi et al investigated the expression of

Table 4 Prognostic significance for overall survival (univariate analysis)

| Prognostic factor | *n* | Hazard ratio | 95% CI | *p* value |
|---|---|---|---|---|
| Age (years) | | | | |
| <65 | 73 | 1.0 | | |
| ≧65 | 61 | 1.415 | 0.785–2.548 | 0.2478 |
| Gender | | | | |
| Male | 64 | 1.013 | 0.571–1.798 | 0.9637 |
| Female | 70 | 1.0 | | |
| Differentiation | | | | |
| Well | 69 | 1.0 | | |
| Moderately, poorly | 65 (47+18) | 2.265 | 1.248 to4.111 | 0.0072 |
| Tumor stage (pT) | | | | |
| T1 | 72 | 1.0 | | |
| T2, 3, 4 | 62 (39+7+16) | 3.394 | 1.833–6.282 | 0.0001 |
| Nodal involvement (PN) | | | | |
| NO | 87 | 1.0 | | |
| N1, 2 | 47 (14+33) | 6.570 | 3.526–12.242 | <0.0001 |
| Vascular invasion | | | | |
| Present | 72 | 3.107 | 1.611–5.992 | 0.0007 |
| Absent | 62 | 1.0 | | |
| Lymphatic permeation | | | | |
| Present | 61 | 4.190 | 2.205–7.963 | <0.0001 |
| Absent | 73 | 1.0 | | |
| Mitotic index (HPF) | | | | |
| 0–4/10 | 94 | 1.0 | | |
| >5/10 | 40 | 1.749 | 0.970–3.152 | 0.0630 |
| CA IX staining | | | | |
| Positive | 33 | 2.210 | 1.226–3.983 | 0.0083 |
| Negative | 101 | 1.0 | | |
| Smoking history | | | | |
| Smokers | 64 | 1.116 | 0.630–1.977 | 0.7073 |
| Never-smokers | 70 | 1.0 | | |
| CEA (ng/ml) | | | | |
| ≦5 | 79 | 1.0 | | |
| >5 | 55 | 2.218 | 1.247–3.646 | 0.0067 |

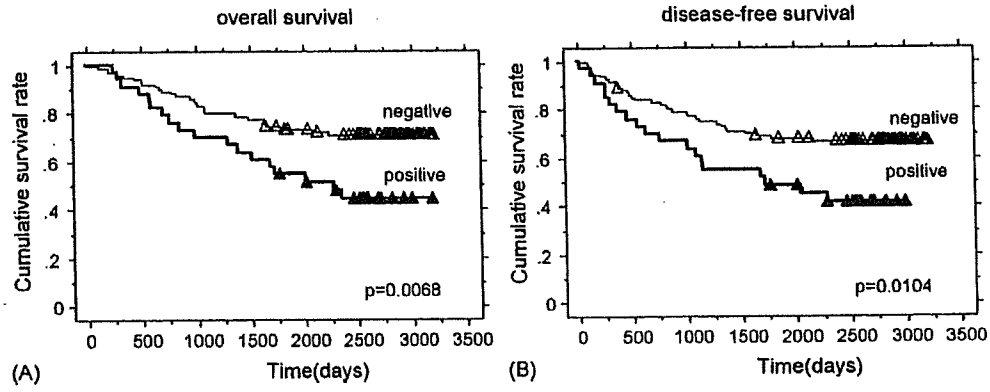


Fig. 2 Overall survival and disease-free survival in all lung adenocarcinoma stages; survival curve of CA IX positive patients.

**Table 5** Multivariate analysis for overall survival

| Prognostic factor | *n* | Hazard ratio | 95% CI | *p* value |
|---|---|---|---|---|
| Tumor stage (pT) | | | | |
| T1 | 72 | 1.0 | | |
| T2, 3, 4 | 62 (39+7+16) | 2.507 | 1.233—5.099 | 0.0111 |
| Nodal involvement (pN) | | | | |
| N0 | 87 | 1.0 | | |
| N1, 2 | 47 (14+33) | 4.797 | 2.219—10.372 | <0.0001 |
| Vascular invasion | | | | |
| Present | 72 | 0.921 | 0.416—2.040 | 0.8387 |
| Absent | 62 | 1.0 | | |
| Lymphatic permeation | | | | |
| Present | 61 | 1.644 | 0.779—3.470 | 0.1920 |
| Absent | 73 | 1.0 | | |
| CA IX staining | | | | |
| Positive | 33 | 1.549 | 0.817—2.938 | 0.1800 |
| Negative | 101 | 1.0 | | |
| CEA (ng/ml) | | | | |
| ≦5 | 79 | 1.0 | | |
| >5 | 55 | 0.805 | 0.408—1.587 | 0.5304 |

CAIX mRNA in 37 adenocarcinoma cases and demonstrated that the difference of CA IX mRNA expression was not able to discriminate different survival probability in adenocarcinoma patients, whereas in squamous and adenosquamous carcinoma, the difference was significant [12], which is at variance with our findings. There are possibilities for explaining this discrepancy. First, in cancer tissue, CAIX was expressed in both cancer cell and stromal cell, such

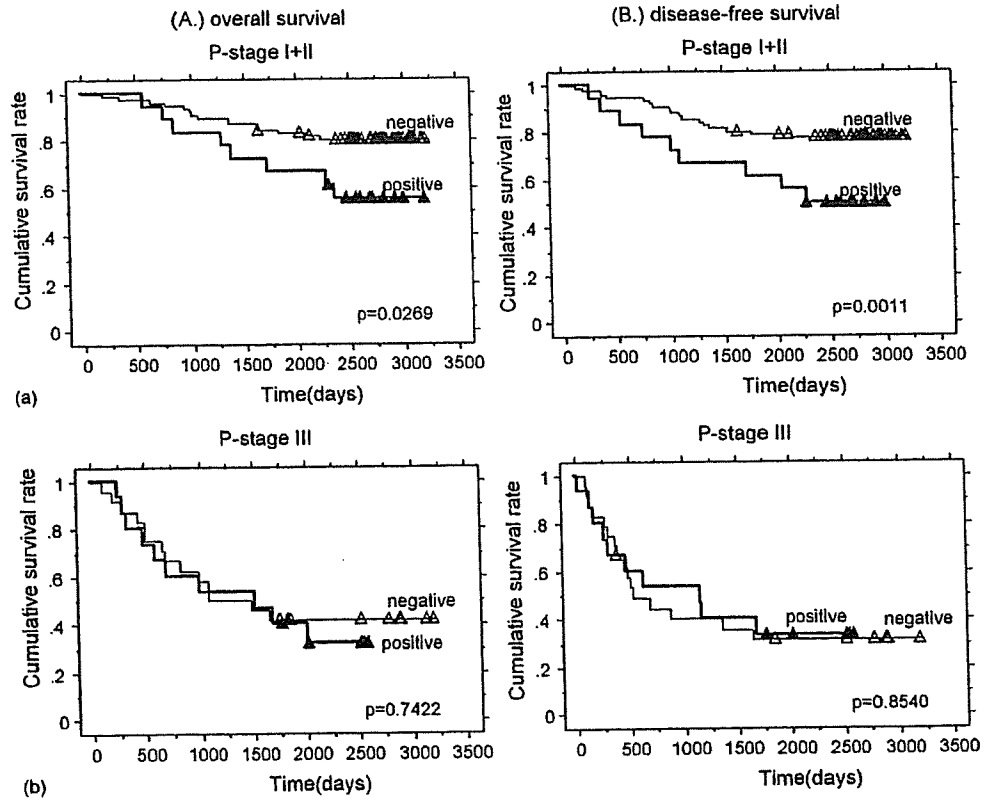


**Fig. 3** Overall survival and disease-free survival in (A) stages I+II, and (B) stage III; survival curve of CA IX positive patients.

Table 6 Prognostic significance for disease-free survival (univariate analysis)

| Prognostic factor | *n* | Hazard ratio | 95% CI | *p* value |
|---|---|---|---|---|
| Age (years) | | | | |
| <65 | 73 | 1.0 | | |
| ≧65 | 61 | 1.591 | 0.915–2.765 | 0.0996 |
| Gender | | | | |
| Male | 64 | 1.042 | 0.610–1.779 | 0.8801 |
| Female | 70 | 1.0 | | |
| Differentiation | | | | |
| Well | 69 | 1.0 | | |
| Moderately, poorly | 65 (47+18) | 2.369 | 1.434–4.170 | 0.0028 |
| Tumor stage (PT) | | | | |
| T1 | 72 | 1.0 | | |
| T2, 3, 4 | 62 (39+7+16) | 3.206 | 1.800–5.691 | <0.0001 |
| Nodal involvement (pN) | | | | |
| NO | 87 | 1.0 | | |
| N1, 2 | 47 (14+33) | 6.222 | 3.484–11.110 | <0.0001 |
| Vascular invasion | | | | |
| Present | 72 | 3.021 | 1.635–5.584 | 0.004 |
| Absent | 62 | 1.0 | | |
| Lymphatic permeation | | | | |
| Present | 61 | 3.654 | 2.022–6.602 | <0.0001 |
| Absent | 73 | 1.0 | | |
| Mitotic index (HPF) | | | | |
| 0–4/10 | 94 | 1.0 | | |
| >5/10 | 40 | 2.185 | 1.295–3.792 | 0.0054 |
| CAIX staining | | | | |
| Positive | 33 | 2.118 | 1.171–3.626 | 0.0122 |
| Negative | 101 | 1.0 | | |
| Smoking history | | | | |
| Smokers | 64 | 1.199 | 0.696–2.066 | 0.5123 |
| Never-smokers | 70 | 1.0 | | |
| CEA (ng/ml) | | | | |
| ≦5 | 79 | 1.0 | | |
| >5 | 55 | 2.340 | 1.353–4.049 | 0.0024 |

as cancer-associated fibroblast. Therefore, CAIX mRNA of cancer tissue would be derived from not only cancer cells but also stromal cells. On the other hand, our study focused on the CAIX expression in cancer cells only. Taking these results into consideration, CAIX expression in stromal cells also modify the cancer microenvironment. Alternatively, this discrepancy would relate to different case number and selection between these studies.

Although it has been reported that there was a significant positive correlation between hypoxia and CA IX expression in squamous cell carcinoma [14], it would be controversial whether this positive correlation applies to lung adenocarcinoma cases. Indeed, a certain population of carcinoma cells showing papillary growth also expressed CA IX in our study. Cancer cells forming a papillary structure usually line the fibrovascular stroma and are actually located on microvascular endothelial cells. This anatomical finding suggests that this type of cancer cells may not be present under hypoxic conditions. Alternative pathways for the upregulation of CA IX were recently reported. An *in vitro* experiment showed that CA IX expression can be upregulated by IFN-alpha and IFN-gamma in renal cell carcinoma cells [27]. On the other hand, it was shown that CA IX expression is, at least in part, regulated by methylation of the CA IX gene promoter and that hypomethylation of the CA IX promoter region is associated with increased CA IX expression in human renal cancer cell lines [28]. Thus, the CA IX expression on lung adenocarcinoma may be regulated by molecular mechanisms other than tumor hypoxia. By comparing the results of CA IX expression in squamous cell carcinomas cases under the same protocol, the current results will provide important insight into the biological role of CA IX on lung adenocarcinoma microenvironment.

CA IX expression was significantly associated with pathological nodular involvement (pN), lymphatic permeation and vascular invasion, which are surrogate markers for cancer

Table 7 Multivariate analysis for disease-free survival

| Prognostic factor | *n* | Hazard ratio | 95% CI | *p* value |
|---|---|---|---|---|
| Differentiation | | | | |
| Well | 69 | 1.0 | | |
| Moderately, poorly | 65 (47+18) | 1.205 | 0.615–2.363 | 0.5863 |
| Tumor stage (PT) | | | | |
| T1 | 72 | 1.0 | | |
| T2, 3, 4 | 62 (39+7+16) | 2.471 | 1.249–4.880 | 0.0093 |
| Nodal involvement (pN) | | | | |
| N0 | 87 | 1.0 | | |
| N1, 2 | 47 (14+33) | 4.475 | 2.185–9.164 | <0.0001 |
| Vascular invasion | | | | |
| Present | 72 | 0.863 | 0.392–1.901 | 0.7152 |
| Absent | 62 | 1.0 | | |
| Lymphatic permeation | | | | |
| Present | 61 | 1.424 | 0.708–2.862 | 0.3216 |
| Absent | 73 | 1.0 | | |
| Mitotic index (HPF) | | | | |
| 0–4/10 | 94 | 1.0 | | |
| >5/10 | 40 | 1.212 | 0.660–2.227 | 0.5357 |
| CA IX staining | | | | |
| Positive | 33 | 1.302 | 0.696–2.429 | 0.4102 |
| Negative | 101 | 1.0 | | |
| CEA (ng/ml) | | | | |
| ≦5 | 79 | 1.0 | | |
| >5 | 55 | 0.919 | 0.481–1.755 | 0.7969 |

metastasis, but not with the mitotic index, a cell proliferation marker. A previous *in vitro* study demonstrated that CA IX reduces E-cadherin-mediated cell–cell adhesion [26]. Therefore, it would be interesting to examine whether overexpression of CA IX might contribute to tumor metastasis by modulating cellular adhesiveness.

In summary, our findings indicate that CA IX expression is associated with tumor progression and is a factor predicting a poor clinical outcome in lung adenocarcinoma. By comparing the results of CAIX expression in squamous cell carcinoma cases under the same protocol, the current results will provide important insight into the biological role of CA IX on lung adenocarcinoma microenvironment. A prospective study with a larger number of patients is warranted, being positive for CA IX expression might indicate additional treatment before and/or after surgery.

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# Mediastinal Lymph Node Metastases and Visceral Pleural Invasion in Nonsmall Cell Lung Cancer Patients

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## Mediastinal Lymph Node Metastases and Visceral Pleural Invasion in Nonsmall Cell Lung Cancer Patients

*To the Editor:*

In their article on skip N2 metastases in nonsmall cell lung cancer (NSCLC) patients, Riquet and colleagues [1] suggest an explanation for the presence of skip metastasis. They suggest that skip metastasis is the result of desquamating tumor cells within the pleural space, which are reabsorbed by lymphatic vessels of the parietal or diaphragmatic pleura; such drainage involves mediastinal lymph node stations and theoretically induces (N1−) N2. This suggestion is based on the two previous findings of the authors. The presence of tumor cells in post-thoracotomy pleural lavage seemed to correlate with visceral pleural invasion (VPI) and particularly with a VPI subgroup, in which the tumor is exposed on the pleural surface and does not yet involve the parietal pleura [2]. Lymphatic drainage of the medial portion of the diaphragmatic pleura was made through the peri-tracheobronchial lymph node chains, explaining the greater frequency of mediastinal lymph node involvement observed in VPI cases [3, 4].

If this lymphatic drainage is truly the major tumor cell pathway of N2 metastasis in VPI patients, there should be more skip N2 cases than contiguous (N1+) N2 cases in VPI patients, because tumor cells would not travel through the hilar lymph nodes. However, their recent study did not demonstrate this possible role of VPI for (N1-) N2 metastasis (VPI+: [N1-] N2 25.9%, [N1+] N2 30.6%). This is consistent with our recent report on VPI in that there were significantly fewer skip N2 patients in the VPI group than in the non-VPI group (skip N2 patients: 17 of 73 VPI patients [23%] vs 36 of 90 non-VPI patients [40%]; $p = 0.0235$) [5]. Based on these findings, we suggest that the major VPI tumor cell pathway is through the subpleural lymphatics, hilar lymph nodes, and into the mediastinal lymph node. Such a pathway should result in less frequent skip N2 patients in the VPI patients, because VPI tumor cells would travel through the hilar lymph nodes rather than in non-VPI patients.

In our recent study, we assessed the relationship between VPI and other clinicopathologic characteristics, and we concluded that VPI is an independent indicator of NSCLC invasiveness and aggressiveness [5]. Riquet and colleagues attributed the poor prognosis of VPI NSCLC patients to desquamating tumor cells within the pleural space, which are reabsorbed by lymphatic vessels of the parietal or diaphragmatic pleura, drained to the mediastinal lymph node, and cause systematic dissemination. However, such a pathway is dubious. We postulate that the invasive and aggressive nature of VPI tumors is directly associated with their poor prognosis.

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# Risk Factors of Postoperative Respiratory Infections in Lung Cancer Surgery

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**Background:** Postoperative infections have been a major issue in lung cancer surgery. We changed our perioperative prophylactic antibiotic policy to a single dose of cefazolin before and after surgery in July 2002.

**Objective:** To identify the risk factors of postoperative pneumonia and empyema in lung cancer patients undergoing surgical resection.

**Methods:** From July 1992 through September 2003, 2105 patients underwent primary lung cancer resection at our division. We reviewed 1855 eligible patients for possible risk factors of pneumonia and empyema.

**Results:** Postoperative respiratory infections developed in 69 (3.7%) patients. There were 58 (3.1%) pneumonia cases and 18 (1.0%) cases of empyema. The mortality rate was 0.8% (15 patients). Nine (0.5%) patients died from postoperative respiratory infections. Multivariate analysis showed age 75 years or older, forced expiratory volume in 1 second as a percentage of forced vital capacity ($FEV_1$%) less than 70%, advanced pathologic stage, and induction therapy to be independent risk factors of pneumonia. For postoperative empyema, advanced age was the significant factor. Twelve of 18 patients (67%) with empyema were complicated with bronchopleural fistula. The infection incidence rate did not change significantly after we modified our prophylactic antibiotic policy to a single dose of cefazolin before and after surgery.

**Conclusions:** Lung cancer patients with advanced age, low $FEV_1$%, advanced pathologic stage, or induction therapy had a risk for pneumonia after lung cancer surgery. Postoperative empyema was associated with advanced age.

**Key Words:** Pneumonia, Empyema, Surgery, Lung cancer.

(*J Thorac Oncol.* 2007;2: 34–38)

Postoperative respiratory infection is a major issue in lung cancer surgery. Sok et al.[1] reported that 34 of 194 patients (18%) suffered from respiratory infections. The rates of pneumonia after pulmonary resection have been reported to range from 5.3%[2] to 22%.[3] Empyema is an intractable complication and can lead to a long hospital stay. Duque et al.[2] reported that 27 of 605 patients (4.4%) developed postoperative empyema after lung cancer surgery, and Belda et al.[4] reported an empyema rate of 6%.

Previous studies have shown smoking,[3,5,6] comorbid chronic obstructive pulmonary disease,[6,7] advanced age,[8] diabetes mellitus (DM),[9] and obesity[6] to be associated with postoperative infections. The aim of this study was to identify the risk factors of postoperative pneumonia and empyema and to ascertain the efficacy of a single dose of prophylactic antibiotics prophylaxis. We retrospectively reviewed patients who underwent surgical resection for lung cancer and identified risk factors by univariate and multivariate analyses. To our knowledge, there have been no reports evaluating the risk factors associated with postoperative respiratory infections after lung cancer surgery on a large scale by multivariate analysis.

## PATIENTS AND METHODS

From July 1992 through September 2003, 2105 consecutive patients underwent surgical resection for primary lung cancer at the Division of Thoracic Surgery, National Cancer Center Hospital East, Chiba, Japan. Retrospective data collection from their medical records and analyses were approved, and the need for informed consent from each patient was waived by the institutional review board in January 2005. Patients who underwent complete lung cancer resection and had complete perioperative data were enrolled, and 1855 were eligible. Their ages ranged from 22 to 90, with an average of 64 years. There were 1191 men and 664 women.

The surgical procedures were lobectomy in 1625 cases (87.6%), pneumonectomy in 100 (5.4%), wedge resection in 77 (4.2%), segmentectomy in 51 (2.7%), and bronchial resection in two (0.1%). We classified resection less than lobectomy as limited resection, and we classified combined resections with the chest wall, pericardium, great vessels, left atrium, vertebral bodies, or diaphragm as extended resection.

Cancers were pathologically staged according to the TNM classification system of the International Union Against Cancer staging system.[10] The pathological stages were as follows: 724 (39.0%) were IA, 376 (20.3%) IB, 72 (3.9%) IIA, 218 (11.8%) IIB, 266 (14.3%) IIIA, 167 (9.0%) IIIB, 28 (1.5%) IV, and four patients (0.2%) were stage 0. Of 31 patients who underwent induction therapy, 22 patients re-

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ceived chemotherapy, eight received chemoradiotherapy, and one received radiotherapy.

We performed resection under general anesthesia with an epidural catheter for postoperative pain control. All patients received local skin disinfection with povidone iodine immediately before surgery. The thoracic cavity was lavaged with 3 liters of warm saline immediately before chest closure. The ribs, muscles, and subcutaneous tissue were closed using absorbable sutures. Skin incisions were closed with buried sutures and suture strips.

Patients received uniform pain control. Epidural analgesia was continued for the first few days. Oral nonsteroidal antiinflammatory drugs were also administered for pain control, starting on the first postoperative morning. There was no attempt to assess levels of pain control.

All patients received perioperative prophylactic antibiotics. Until June 2002, the antibiotic regimen was at the discretion of each attending surgeon, mostly the first-generation cephalosporin for a few days starting immediately before surgery. From July 2002, patients received a single dose of 1.0 g of cefazolin immediately before skin incision and the same dose at 3 hours after surgery. If signs of postoperative infection, such as fever, purulent sputum, or infiltrative changes on a chest roentgenogram developed, additional antibiotics were allowed.

We documented postoperative pneumonia and empyema as postoperative respiratory infections. Postoperative pneumonia was defined as purulent sputum and pulmonary infiltrative changes on postoperative chest roentgenogram or computed tomography. Empyema was defined as purulent effusion in the postoperative thoracic cavity. These postoperative infections were not always be accompanied by a fever, leukocytosis, and/or elevated serum C-reactive protein level. Bronchoscopically proven bronchial stump failure with persistent air leakage was defined as bronchopleural fistula.

We investigated the incidence of postoperative pneumonia and empyema and analyzed the association between infection development and the following factors: age (<75 or ≥75 yr), sex, smoking history (positive or negative), body mass index <25 or ≥25), DM (positive or negative), forced expiratory volume in 1 second as a percentage of forced vital capacity ($FEV_1$%; <70 or ≥70%), surgical procedure (limited resection, lobectomy, or extended resection), induction therapy (positive or negative), and pathologic stage (I/II or III/IV). Operative mortality was defined as a death from any cause during the postoperative hospital stay.

## Statistical Analysis

In univariate and multivariate analyses, the logistic regression model was used to evaluate the risk factors associated with postoperative infections. The data were calculated using a StatView version 5 software package (SAS Institute Inc, Cary, NC). A $p$ value of less than 0.05 was defined as indicative of statistical significance.

## RESULTS

Postoperative respiratory infections developed in 69 (3.7%) of the 1855 patients. There were 58 (3.1%) pneumonia cases and 18 (1.0%) empyema cases. Seven (0.4%) patients developed both pneumonia and empyema. Eighteen of the 1855 patients (1.0%) developed bronchopleural fistula (BPF). Two of the 18 BPF patients underwent pneumonectomy. In the 18 empyema patients, 12 were complicated with BPF, and five were complicated with alveolar leakage or pneumonia. The mortality rate was 0.8% (15 patients). There were nine (0.5%) postoperative deaths from respiratory infections, and all of these patients were male smokers who had undergone a lobectomy. Of the 31 induction patients, seven (23%) underwent pneumonectomy, and 16 (52%) underwent extended resection. These rates were significantly higher ($p < 0.001$) than noninduction patients, indicating that induction patients had more advanced disease.

Table 1 shows the relationships between postoperative infections and risk factors. More than 5% of the patients 75 years or older with $FEV_1$% less than 70%, extended resection, induction therapy, or pathologic stage III/IV developed

**TABLE 1.** Postoperative Infections and Risk Factors

| Variable | *n* | Pneumonia (*n* = 58) | Empyema (*n* = 18) |
|---|---|---|---|
| Age (yr) | | | |
| ≥75 | 247 | 16 (6.5) | 6 (2.4) |
| <75 | 1608 | 42 (2.6) | 12 (0.8) |
| Sex | | | |
| Male | 1191 | 48 (4.0) | 16 (1.3) |
| Female | 664 | 10 (1.5) | 2 (0.3) |
| Smoking history | | | |
| (+) | 1259 | 50 (4.0) | 15 (1.2) |
| (−) | 596 | 8 (1.3) | 3 (0.5) |
| BMI | | | |
| <25 | 1468 | 45 (3.1) | 12 (0.8) |
| ≥25 | 374 | 13 (3.5) | 6 (1.6) |
| DM | | | |
| (+) | 142 | 2 (1.4) | 0 (0) |
| (−) | 1713 | 56 (3.3) | 18 (1.1) |
| $FEV_1$% | | | |
| <70% | 499 | 28 (5.6) | 6 (1.2) |
| ≥70% | 1356 | 30 (2.2) | 12 (0.9) |
| Limited resection | | | |
| (+) | 128 | 2 (1.6) | 0 (0) |
| (−) | 1727 | 56 (3.2) | 18 (1.0) |
| Lobectomy | | | |
| (+) | 1640 | 48 (2.9) | 17 (1.0) |
| (−) | 215 | 10 (4.7) | 1 (0.5) |
| Extended resection | | | |
| (+) | 87 | 8 (9.2) | 1 (1.2) |
| (−) | 1768 | 50 (2.8) | 17 (1.0) |
| Induction therapy | | | |
| (+) | 31 | 4 (12.9) | 0 (0) |
| (−) | 1825 | 54 (3.0) | 18 (1.0) |
| Pathologic stage | | | |
| I/II | 1398 | 33 (2.4) | 11 (0.8) |
| III/IV | 457 | 25 (5.5) | 7 (1.5) |

BMI, body mass index; DM, diabetes mellitus; $FEV_1$%, forced expiratory volume as a percentage of forced vital capacity. Numbers in parentheses are percentages.

postoperative pneumonia. In univariate analyses, advanced age, male gender, smoking history, low $FEV_1$%, surgical procedure induction therapy, and pathologic stage were the risk factors (Table 2). In multivariate analyses, advanced age, low $FEV_1$%, induction therapy, and pathologic stage were shown to be independent risk factors (Table 3).

For postoperative empyema, patients 75 years or older were at the highest risk (Table 1). In univariate analyses, advanced age and male gender were the risk factors (Table 4). Multivariate analysis showed that advanced age was a significant risk factor for empyema (Table 5).

To investigate the relationships between perioperative prophylactic antibiotics therapy and postoperative infections, we compared the incidence of postoperative respiratory infections between the two phases before and after July 2002, when we changed our prophylactic antibiotics therapy at our hospital (Table 6). After we changed our perioperative prophylactic antibiotic policy, 76 of 321 patients (23.7%) required additional antibiotics. Fifty (3.3%) of 1534 patients developed pneumonia before July 2002, and eight (2.5%) of 321 patients developed pneumonia after July 2002. For empyema, there were 16 (1.0%) of 1534 and two (0.6%) of 262 patients before and after July 2002, respectively. Although there were no statistically significant differences between the two phases, the infection incidence rates reduced after we changed the perioperative antibiotic policy to a single dose of cefazolin before and after surgery.

**TABLE 2.** Univariate Analysis of Relationships between Risk Factors and Postoperative Pneumonia

| Variable | Risk Ratio | 95% CI | *p* |
|---|---|---|---|
| Age (yr) | | | 0.0017 |
| ≥75 | 2.58 | 1.43–4.67 | |
| <75 | 1.00 | | |
| Sex | | | 0.004 |
| Male | 2.75 | 1.38–5.47 | |
| Female | 1.00 | | |
| Smoking history | | | 0.0038 |
| (+) | 3.04 | 1.43–6.45 | |
| (−) | 1.00 | | |
| Body mass index | | | 0.69 |
| ≥25 | 1.14 | 0.61–2.13 | |
| <25 | 1.00 | | |
| DM | | | 0.24 |
| (+) | 0.42 | 0.10–1.75 | |
| (−) | 1.00 | | |
| FEV1% | | | 0.0003 |
| <70% | 2.63 | 1.55–4.45 | |
| ≥70% | 1.00 | | |
| Surgery | | | |
| Extended | 6.38 | 1.32–30.8 | 0.021 |
| Lobectomy | 3.36 | 1.54–7.34 | 0.0024 |
| Limited | 1.00 | | |
| Induction therapy | | | 0.0043 |
| (+) | 4.86 | 1.64–14.4 | |
| (−) | 1.00 | | |
| Pathologic stage | | | 0.0013 |
| III/IV | 2.39 | 1.41–4.07 | |
| I/II | 1.00 | | |

DM, diabetes mellitus; CI, confidence interval; $FEV_1$%, forced expiratory volume as a percentage of forced vital capacity.

**TABLE 3.** Multivariate Analysis of Relationships between Risk Factors and Postoperative Pneumonia

| Variable | Risk Ratio | 95% CI | *p* |
|---|---|---|---|
| Age (yr) | | | 0.0008 |
| ≥75 | 2.91 | 1.56–5.44 | |
| <75 | 1.00 | | |
| Sex | | | 0.19 |
| Male | 1.85 | 0.74–4.65 | |
| Female | 1.00 | | |
| Smoking history | | | 0.43 |
| (+) | 1.51 | 0.55–4.18 | |
| (−) | 1.00 | | |
| Body mass index | | | 0.20 |
| ≥25 | 1.53 | 0.80–2.94 | |
| <25 | 1.00 | | |
| DM | | | 0.14 |
| (+) | 0.34 | 0.08–1.43 | |
| (−) | 1.00 | | |
| FEV1% | | | 0.026 |
| <70% | 1.89 | 1.08–3.30 | |
| ≥70% | 1.00 | | |
| Surgery | | | |
| Extended | 3.40 | 0.63–18.2 | 0.15 |
| Lobectomy | 1.94 | 0.79–4.74 | 0.15 |
| Limited | 1.00 | | |
| Induction therapy | | | 0.038 |
| (+) | 3.70 | 1.08–12.7 | |
| (−) | 1.00 | | |
| Pathologic stage | | | 0.0052 |
| III/IV | 2.23 | 1.27–3.92 | |
| I/II | 1.00 | | |

DM, diabetes mellitus; CI, confidence interval; $FEV_1$%, forced expiratory volume as a percentage of forced vital capacity.

## DISCUSSION

In patients undergoing surgery for lung cancer, postoperative pneumonia and empyema strongly correlate with outcome and constitute a major issue in patient management. Nagasaki et al.[11] showed a mortality associated with postoperative pneumonia of 25%, and mortality associated with postoperative empyema was 22%. Because of hypoventilation, secretory retention, pain, and inefficient cough, lung cancer patients are at a high risk of developing atelectasis and pneumonia after surgery.

We found that age 75 years or older was an independent significant risk factor for postoperative pneumonia and empyema. Iwamoto et al.[8] investigated postoperative pneumonia and revealed that elderly patients older than 65 years who underwent thoracic surgery showed the highest incidence of pneumonia. Smoking, obesity, and DM have been reported to

**TABLE 4.** Univariate Analysis of Relationships between Risk Factors and Postoperative Empyema

| Variable | Risk Ratio | 95% CI | p |
|---|---|---|---|
| Age (yr) | | | 0.018 |
| ≥75 | 3.31 | 1.23–8.91 | |
| <75 | 1.00 | | |
| Sex | | | 0.045 |
| Male | 4.51 | 1.03–19.7 | |
| Female | 1.00 | | |
| Smoking history | | | 0.17 |
| (+) | 2.38 | 0.69–8.27 | |
| (−) | 1.00 | | |
| Body mass index | | | 0.18 |
| ≥25 | 1.98 | 0.74–5.31 | |
| <25 | 1.00 | | |
| DM | | | 0.98 |
| (+) | — | — | |
| (−) | — | | |
| $FEV_1$% | | | 0.54 |
| <70% | 1.36 | 0.51–3.65 | |
| ≥70% | 1.00 | | |
| Surgery | | | |
| Extended | — | — | 0.98 |
| Lobectomy | 1.10 | 0.15–8.44 | 0.92 |
| Limited | 1.00 | | |
| Induction therapy | | | 0.98 |
| (+) | — | — | |
| (−) | — | | |
| Pathologic stage | | | 0.17 |
| III/IV | 1.96 | 0.76–5.09 | |
| I/II | 1.00 | | |

DM, diabetes mellitus; CI, confidence interval; $FEV_1$%, forced expiratory volume as a percentage of forced vital capacity.

**TABLE 5.** Multivariate Analysis of Relationships between Risk Factors and Postoperative Empyema

| Variable | Risk Ratio | 95% CI | p |
|---|---|---|---|
| Age (yr) | | | 0.0076 |
| ≥75 | 4.06 | 1.45–11.4 | |
| <75 | 1.00 | | |
| Sex | | | 0.06 |
| Male | 5.98 | 0.92–38.7 | |
| Female | 1.00 | | |
| Smoking history | | | 0.70 |
| (+) | 0.70 | 0.15–3.64 | |
| (−) | 1.00 | | |
| Body mass index | | | 0.09 |
| ≥25 | 2.40 | 0.87–6.62 | |
| <25 | 1.00 | | |
| DM | | | 0.98 |
| (+) | — | — | |
| (−) | — | | |
| $FEV_1$% | | | 0.94 |
| <70% | 0.96 | 0.34–2.71 | |
| ≥70% | 1.00 | | |
| Surgery | | | |
| Extended | — | — | 0.99 |
| Lobectomy | 1.18 | 0.14–9.56 | 0.88 |
| Limited | 1.00 | | |
| Induction therapy | | | 0.99 |
| (+) | — | — | |
| (−) | — | | |
| Pathologic stage | | | 0.12 |
| III/IV | 2.21 | 0.82–5.97 | |
| I/II | 1.00 | | |

DM, diabetes mellitus; CI, confidence interval; $FEV_1$%, forced expiratory volume as a percentage of forced vital capacity.

be definite risk factors of postoperative complications.[3,5–9] However, we could not find a significant correlation between postoperative pneumonia or empyema and these factors in multivariate analysis.

Patients with chronic obstructive pulmonary disease were shown to be at a high risk of postoperative pneumonia.[6,7] However, Cerfolio et al.[12] concluded that pulmonary complications were not associated with low $FEV_1$. In the present report, multivariate analysis revealed a preoperative $FEV_1$% of less than 70% was a significant risk factor for postoperative pneumonia. When low $FEV_1$ patients were 75 years or older, the risk of pneumonia was extremely high, at 10.7% (11/103; $p < 0.0001$). For these patients with impaired respiratory pulmonary function, preoperative rehabilitation might be needed to reduce the risk of postoperative respiratory complications.[13]

Busch et al.[3] showed that extended resection involving either the chest wall or other adjacent structures was associated with pulmonary complications. They also reported a significant increase in major pulmonary complications in patients who had received induction chemotherapy. Martin et al.[14] reported that, among patients who received induction therapy, the incidence of pneumonia and empyema were 12.1 and 1.3%, respectively. Although our multivariate analysis did not show a correlation between the extent of surgical resection and postoperative pneumonia, patients who underwent induction therapy and those with advanced pathologic stage were shown to have a significant risk of postoperative pneumonia. The incidence of pneumonia after induction therapy was similar to the results of Martin et al.,[14] at 13%. Induction therapy may be refrained in elderly patients.

**TABLE 6.** Rates of Postoperative Pneumonia and Empyema

| Period | Total | Pneumonia ($n = 58$) | p | Empyema ($n = 18$) | p |
|---|---|---|---|---|---|
| July 1992 to June 2002 | 1534 | 50 (3.3%) | 0.47 | 16 (1.0%) | 0.75 |
| July 2002 to September 2003 | 321 | 8 (2.5%) | | 2 (0.6%) | |

Deschamps et al.[15] studied empyema after pneumonectomy and indicated that the risk of empyema was associated with lower preoperative $FEV_1$, lower diffusion capacity of

lung to carbon monoxide, lower preoperative serum hemoglobin, right pneumonectomy, bronchial stump reinforcement, completion pneumonectomy, delayed chest tube removal, and the amount of blood transfusions. We identified age 75 years or older as a risk factor for empyema. Twelve of 18 empyema patients developed BPF. Had it not been for BPF, the majority of empyema cases might have been prevented. Although the role of bronchial stump coverage with a flap in preventing BPF remains controversial,[16] Taghavi et al.[17] recently reported that bronchial stump coverage with a pedicled pericardial flap was highly effective in postpneumonectomy BPF prevention. In this study, no patients receiving induction therapy developed empyema or bronchopleural fistula. This might be attributable to the protective pedicled flap we used for these high-risk patients. Further prospective study is necessary to confirm the role of bronchial stump coverage with a pedicled flap, but a randomized study would be unfeasible because of the rarity of BPF.

The Centers for Disease Control and Prevention recommended antimicrobial prophylaxis only when needed.[18] Ilves et al.[19] showed that prophylactic antibiotics were effective in reducing wound infections, but the incidence of pulmonary infections and empyema was not significantly decreased. Prolonged prophylactic antibiotics were reported not to be effective against postoperative pleuropulmonary infections. Olak et al.[20] revealed that six doses of cefazolin did not confer clinically important benefit beyond that obtained from a single dose as prophylaxis in elective general thoracic surgery. Our experience confirms these suggestions. A small dosage is also beneficial costwise.

In conclusion, we identified risk factors for postoperative respiratory infections in lung cancer patients. Elderly patients and patients after induction therapy need intensive perioperative management. Preoperative rehabilitation may be beneficial for those with impaired lung function.

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# Phase II Study of Weekly Paclitaxel for Relapsed and Refractory Small Cell Lung Cancer

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**Abstract.** *The purpose of this study was to evaluate the efficacy and toxicity of single-agent paclitaxel given weekly to patients with relapsed and refractory small cell lung cancer (SCLC). Patients were treated with 80 mg/m$^2$ paclitaxel administered weekly for 1 h for 6 weeks in an 8-week cycle. Twenty-two patients were enrolled, 21 of whom were eligible. The patient characteristics included: 20 males, 1 female; median age 66 years (range 48 - 75); performance status 0/1 in 19 and 2 in 5 patients. Grade 3/4 leukopenia and neutropenia occurred in 47.5% and 64%, respectively. Other grade 3/4 toxicities included infection, skin rash, neuropathy and pulmonary toxicity. There were 5 partial responses in 3 out of the 11 sensitive cases and 2 out of the 10 refractory cases, respectively. Paclitaxel, administered as a weekly infusion at a dose of 80 mg/m$^2$, was effective in treating relapsed and refractory SCLC.*

More than 95% of patients with small cell lung cancer (SCLC), who are initially treated with paclitaxel 80 mg/m$^2$, present a relapse and their response to a second-line therapy is poor. The responses obtained are usually brief, and the median survival is generally less than 4 months (1). Nevertheless, second-line chemotherapy may provide a significant palliation of symptoms and does result in a prolongation of survival in many patients.

The activity of paclitaxel as a single agent has been investigated in both previously-untreated and -treated SCLC patients. Two phase II trials were conducted to investigate its efficacy as a first-line treatment for SCLC. In a trial conducted by the Eastern Cooperative Oncology Group (ECOG), Ettinger *et al.* administered 250 mg/m$^2$ paclitaxel as a 24-h infusion to 36 patients (2), among whom 11 partial responses were observed. Kirschling *et al.* obtained a similar response rate, 41%, in a group of 37 patients on an identical paclitaxel dose-schedule (3). The results of a phase II study in previously treated patients were reported by Smit *et al.* (4). All 24 patients in that trial developed progressive disease within 3 months of receiving at least one previous chemotherapy regimen. Seven patients (29%) had a partial response to 175 mg/m$^2$ paclitaxel as a 3-h infusion. These data show that paclitaxel exhibits single-agent efficacy in SCLC comparable to that of the best agents. The results of Smit *et al.'s* study in patients with refractory SCLC are particularly impressive, since most response rates reported with single-agent or combination regimens in this population have been less than 15%. However, life-threatening toxicity occurred in 4 of these patients, 2 of whom experienced hematological toxicity.

Recent reports of the activity and tolerability of weekly doses of paclitaxel have generated a great deal of clinical interest. Weekly paclitaxel therapy has generally been quite well tolerated, causing minimal toxicity and no apparent cumulative myelosuppression. Substantial evidence from clinical trials indicates that weekly paclitaxel is effective and generally well tolerated as both a first- and second-line treatment for advanced NSCLC. A phase I/II trial by Koumakis *et al.* in a second-line setting tested weekly paclitaxel infused for the first 6 weeks of each 8-week cycle, and demonstrated that a paclitaxel dose escalation from 60 mg/m$^2$ to 90 mg/m$^2$ was tolerated (5).

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