Original article: Clinical study

Altered lymphatic structure and function in pleural anthracosis: Negative role in skip N2 metastasis

Running head: Altered lymphatics in anthracosis

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Visual abstract

Key question

Is pleural anthracosis impacted on changes in lymphatic structures and function, which interferes with skip metastasis?

Key findings

Pleural anthracosis induced abnormal pleural lymphatics, reduced lymph drainage, and infrequent skip metastasis

Take-home message

Intraoperative assessment of anthracosis helps planning the best surgical strategy in a case-specific manner in patients with lung cancer.

Abstract

Objectives: The present study investigated whether or not pleural anthracosis is associated with changes in the pleural lymphatic structures or function, which would interfere with nodal skip metastasis.

Methods: This study comprised two different case series. In the first series, we observed pleural lymphatic drainage using near-infrared fluorescent endoscopy by the subpleural injection of indocyanine green immediately after thoracotomy for lung cancer. We also performed a histological assessment of the pleura. In the second series, we reviewed the nodal metastatic pattern (skip or non-skip metastasis) in pathological N2 lung cancer involving the pleura. These findings were compared with the severity of pleural anthracosis, which was quantified by thoracoscopic vision and a software-based imaging analysis.

Results: In the first series (n=42), pleural lymphatic drainage was not visualized in 19 (45%) patients who predominantly had severe anthracosis, while it was visualized in the remaining 23 (55%) patients who predominantly had minimal anthracosis. Histologically, severe anthracosis was associated with pleural thickening accompanied by a decreased incidence of straight-running lymphatic vessels and, in turn, an increased incidence of short lymphatic vessels, which was suggested to be the result of pleural remodeling. In the second series (n=53), a skip metastatic pattern was found in 24 (45%) patients who predominantly had less-severe anthracosis, while a non-skip metastatic pattern was found in 29 (55%) patients who predominantly had severe anthracosis.

Conclusions: These results suggest that pleural anthracosis may induce pathological changes in the pleural lymphatics and decreased pleural lymphatic drainage, thereby interfering with nodal skip metastasis.

Key words: lung cancer; pleural lymphatics; indocyanine green fluorescence; anthracosis; skip lymph node metastasis

Abbreviations and Acronyms

ICG = indocyanine green

Introduction

Pulmonary lymph is mainly drained via lymphatics running along the bronchi[1-3]. Thus, cancer cells generally metastasize to the hilar (N1) nodes and mediastinal (N2) nodes, sequentially. However, pulmonary lymph is also drained via lymphatics running within the visceral pleura, which can flow directly into the mediastinum[4-7]. Accordingly, cancer cells can metastasize via pleural lymphatics to the N2 nodes directly, without metastasizing to the N1 nodes, a possible mechanism underlying skip N2 metastasis[8-12].

We previously attempted to visualize the pleural lymphatic drainage using near-infrared fluorescent endoscope after the subpleural injection of indocyanine green (ICG) immediately following thoracotomy for lung cancer[13]. As a result, pleural drainage pathways were observed in 58% of our patients who predominantly had no smoking history, while they were not observed in the remaining patients who predominantly had a history of heavy smoking. Unfortunately, we did not evaluate the relationship among smoking exposure, the structure of pleural lymphatics, and the occurrence of nodal skip metastasis. In general, long-term smoking exposure results in some deposition of carbon dust in the lung tissue (anthracosis), although most inhaled dust is excreted via the airways or lymphatics[14-16]. Although the susceptibility to anthracosis following tobacco or environmental smoking exposure differs greatly among individuals, the deposited dusts may adversely influence the maintenance of the normal lung structure.

Based on these findings, we hypothesized that pleural anthracosis is associated with an impaired pleural lymphatic flow and altered pleural lymphatic vessel structure, thereby interfering with lymph node metastasis via pleural lymphatics. To address these issues, we conducted two different case series.

In the first series, we evaluated the *in vivo* pleural lymphatic drainage using ICG fluorescence imaging and assessed the pleural lymphatics structure microscopically. In the second series, we evaluated the lymph nodes metastasis

patterns (skip or non-skip metastasis). These results were then compared with the severity of pleural anthracosis as well as other clinicopathological factors. Based on these findings, we will be able to determine the appropriate extent of lymph node dissection in a case-specific manner if we can identify patients who are likely or unlikely to develop skip metastasis based on macroscopic grading of pleural anthracosis.

Patients and Methods

Patients

This study was approved by the Institutional Review Board of Kagoshima University (#22-147; March 14, 2011. #180333; April 1, 2019. #210059; June 25, 2021). We obtained informed consent from each patient.

We performed two different case series in this study. The first case series comprised 42 patients with non-small cell lung carcinoma who underwent lobectomy or segmentectomy and lymphadenectomy at our institution between 2013 and 2020. The pleural lymph flow was examined prospectively by ICG fluorescence imaging, as described below. We then evaluated the relationship among the degree of anthracosis, the presence or absence of pleural lymph flow, and the construction of pleural lymphatic vessels. The second case series comprised 53 patients with pathological N2 who underwent lobectomy with systematic ipsilateral mediastinal lymphadenectomy between 2010 and 2019, including 24 with skip metastasis and 29 with non-skip metastasis. Patients without pleural invasion were not included. According to the method described below, we examined the relationship between the degree of anthracosis and the pattern of mediastinal lymph node metastasis (skip or non-skip metastasis).

Visualization of pleural lymphatic drainage

We observed pleural lymphatic drainage in a modified procedure, as we reported previously [13]. In brief, under general anesthesia with single-lung ventilation, we selectively inflated the specific segment of the affected lobe by jet ventilation and outlined the segment. We then let the lung collapse and injected ICG (25 mg/10 mL) by 0.5 ml in 3-5 portions with a 23-G or thinner needle into the subpleura of the specific segment. After bilateral ventilation for five minutes, two surgeons conducted observations with a near-infrared camera (IMAGE1 S^{TM} FI; STORTZ, Tokyo, Japan) in real time and judged whether or not ICG had moved along the pleura from the injected site (Video).

Quantification of pleural anthracosis

In the first series, we observed the visceral pleura under thoracoscopy just after thoracotomy and scored the degree of anthracosis as either 0, 1, or 2 (Fig. 1A), as follows:

0 = No or dotted anthracosis

1 = Linear anthracosis

2 = Patchy anthracosis

Likewise, in the second series, we scored the degree of anthracosis in similar fashion by reviewing the operative video. Two board-certified surgeons (TA and UK) independently assessed the degree of anthracosis without knowledge of the clinical information of the patients. In cases of disagreement, a final decision was reached by consensus of the same two surgeons.

In addition to the scoring of anthracosis, we calculated the ratio of the anthracosis area in the resected lung using the Image J software program (National Institutes of Health, Bethesda, MD, USA). We converted the color photograph of the resected lung to a grayscale image. We then obtained the total pleural area by outlining the resected lung. Finally, we extracted the black area (anthracosis area) and calculated the anthracosis ratio (%) using the following formula (Fig. 1B):

Anthracosis ratio (%) = Anthracotic area / Pleural area.

Immunohistochemistry assessments

Resected lung tissues from the first case series were immediately fixed in 10% buffer formalin and embedded in paraffin. To highlight lymphatic vessels, 3-μm-thick sections were prepared from each block (1 block per case), and immunohistochemical staining for Monoclonal Mouse Anti-Human Podoplanin (Dako, Carpinteria, CA, USA) was performed as the primary antibody.

Under the supervision of the pathologist (TK), immunohistochemistry and archived Hematoxylin-Eosin (H&E) slides were reviewed by two authors (TA and UK) at 40× magnification independently in a random order, without knowledge of the patients' clinical data. The final decisions were reached by consensus.

The assessment of pleural lymphatics

Pleural length: The length of the visceral pleura within each slide was measured by tracing the surface of the pleura using the Image J software program (Fig. 2).

Pleural cross-sectional area: The cross-sectional area of the visceral pleura within each slide was measured by outlining the visceral pleura using the Image J software program (Fig. 2).

Pleural thickness: The pleural thickness was calculated as follows: Pleural cross-sectional area / Pleural length.

Pleural lymph vessel density: The total number of pleural lymphatic vessels possessing D2-40 (total lymph vessel count) within the entire pleura in each slide was counted. The lymph vessel density was defined as follows: Total lymph vessel count / Pleural length.

Pleural vessel length: The transversal length of pleural lymphatics (lymph vessel length) was measured in all lymphatics within each slide. The median and upper quartile values of the lymph vessel length in each patient were obtained (Fig. 2).

Pleural lymph vessel cross-sectional area: The cross-sectional area of each lymph vessel, including the luminal space was obtained by outlining each vessel using the Image J software program. The lymph vessel cross-sectional area was calculated as follows: Sum of the cross-sectional areas of lymph vessels within the slide / pleural length (Fig. 2).

Statistical analyses

A chi-square test was used to compare categorical variables, a non-parametric test was used to compare numerical variables between the groups, and a linear regression analysis was used to compare numerical variables. A *p*-values less than 0.05 was considered statistically significant. All statistical analyses were performed using IBM SPSS Statistics software program, version 22 (SPSS Inc., Chicago, IL, USA).

Results

Pleural lymphatic flow

We observed the movement of ICG fluorescence from the injection site along the pleura in 23 of the 42 patients (55%) (Table 1, Fig 3). ICG reached the adjacent segment or further in 11 of the 23 patients with any ICG movement. ICG reached as far as the adjacent lobe in four patients with any ICG movement. ICG fluorescence was not detected in any hilar or mediastinal lymph nodes. No adverse events occurred after ICG administration.

The patient characteristics according to the presence or absence of ICG movement are shown in Table 1. There were no significant differences between the groups regarding age, sex, smoking history, pulmonary function test results, or pathological type of lung cancer (Table 1, Supplementary Table 1). However, patients with ICG movement had significantly lower anthracosis scores and anthracosis ratios

than those without ICG movement (Table 1). Likewise, patients with ICG movement had emphysematous changes (intrapulmonary air space on computed tomography ≥1 cm) less frequently than those without ICG movement. According to the multivariate regression analysis, the anthracosis score was the only significant variable to predict ICG movement among the anthracosis score and emphysematous changes (odds ratio: 0.346, 95% confidence interval: 0.143-0.838, *P* = 0.019).

Lymph node metastasis was found in 4 of the 23 (17%) patients with ICG movement and in 4 of the 19 (21%) patients without ICG movement (*P* = 1.0). Skip N2 metastasis was found in 3 patients with ICG movement but not found in any patients without ICG movement (*P* = 0.239).

Severity of pleural anthracosis

The mean anthracosis score and the mean anthracosis ratio in 90 patients from both case series (5 patients were excluded for lacking surgical videos) was 1.01 (range, 0.0-2.0) and 5.24 (range, 0.02-24.76), respectively. The anthracosis score was significantly dependent on the anthracosis ratio (R = 0.683, *P* <0.001) (Fig. 1C).

Histopathological findings

We assessed visceral pleura for 4.4±1.6 cm length (range, 0.6 - 7.2 cm) in each slide. The total number of pleural lymphatic vessels per slide was 103 ± 57 (range, 17-244). According to a linear regression analysis, an increased anthracosis rate (%) was associated with increased pleural thickness, increased lymph vessel density, decreased median lymph vessel length, decreased upper quartile lymph vessel length, and increased lymph vessel cross-sectional area (Table 2).

Representative images of patients with minimal anthracosis and those with severe anthracosis are shown in Figure 4. Patients with severe anthracosis had an increased incidence of short lymphatics, resembling small fragments of vessels or meandering vessels, while patients with minimal anthracosis had relatively

straight-running lymphatics, findings that were compatible with the results of the regression analysis (Fig. 4).

Pleural anthracosis and skip metastasis

Patient characteristics according to the nodal metastatic pattern (skip and non-skip N2 metastasis) are shown in Table 3. Female gender (P = 0.001) and non-smokers (P = 0.005) were more predominant among patients with skip N2 metastasis than those with non-skip N2 metastasis. There were no significant differences with regard to the age, computed tomography findings, or pathological type of lung cancer between the groups (Table 3, Supplementary Table 2), although a computed tomography assessment was not done in one patient due to obstructive pneumonia. The anthracosis score (P = 0.035) and anthracosis ratio (P = 0.012) were significantly higher in patients with non-skip N2 metastasis than in those with skip N2 metastasis.

Discussion

According to the current fluorescence imaging study, the pleural lymphatic flow appeared to have suffered interference in patients with relatively severe anthracosis. The results were also supported by the findings of a histopathological study: patients with relatively severe anthracosis had markedly different lymphatics structures from patients with minimal anthracosis. Finally, according to the retrospective review of patients with N2 metastasis, patients with minimal anthracosis predominantly developed skip N2 metastasis, in contrast to patients with relatively severe anthracosis. These results suggest that pleural invasion of tumors likely led to skip metastasis via the pleural lymphatics in patients with minimal anthracosis, while such a scenario was unlikely to lead to skip metastasis in patients with relatively severe anthracosis. We believe that the lymphatics along the bronchovascular bundle may be dominant in patients with relatively severe anthracosis. Thus, our results may be

clinically valuable in intraoperatively determining the appropriate extent of lymph node dissection in a case-specific manner.

In our first case series, pleural lymph flow was detected in 55% of patients, which was comparable to our previous study results[13]. In the current study, we conducted detailed evaluations regarding the relationship between the pleural lymph flow and the underlying pulmonary disease. As a result, pleural anthracosis and emphysematous changes (air space ≥1 cm) were associated with interfering with the pleural lymph flow. Interestingly, a smoking history was not associated with the pleural lymph flow. According to a multivariate analysis, pleural anthracosis was the only significant factor associated with interfering with the pleural lymph flow. We believe that susceptibility to pleural anthracosis is not necessarily dependent to the amount of smoking exposure, although this differs greatly among individuals. Therefore, intraoperative findings regarding pleural anthracosis are the most important point to consider when determining the grade of pleural lymph flow.

Previous investigators found an increased lymph vessel density in the lung tissues of various lung diseases, such as chronic obstructive pulmonary disease, interstitial pneumonia, and pulmonary tuberculosis[17-19], although the role of the increased lymph vessel density in the pathogenesis and lymphatic function remains unclear. While our results appeared to be compatible with the previous study results, concern remains about whether or not our findings were indeed attributable to the chronic lung disease, as previous investigators focused mainly on the intrapulmonary lymphatics, not the pleural lymphatics. However, Takano et al.[20] focused on pleural anthracosis in their autopsy study, finding that the amount of intrapulmonary carbon spots was significantly dependent on the amount of pleural carbon spots. They also reported that carbon particles contributed to a chronic inflammatory response, characterized by the recruitment of inflammatory cells and remodeling of the lung tissue, including the pleura, which was accompanied by interstitial fibrosis. We thus believe that the increased lymph vessel density in the anthracotic pleura is an

adverse reaction to carbon particles and associated with an impaired lymphatic function. Interestingly, we also found straight-running lymphatics more frequently in patients with minimal anthracosis than in patients with severe anthracosis (Fig. 4), which was supported by the regression analysis (Table 2). We believe that patients with severe anthracosis had infrequent straight-running lymphatics because lymphatics in such patients are frequently fragmented or meandering as a result of pleural remodeling, which can be a reason for the increased lymph vessel density in patients with severe anthracosis. An assessment with a three-dimensional pathological examination might help clarify these issues.

We compared the prognostic outcome between patients with skip N2 metastasis and those with non-skip N2 metastasis. Patients with skip N2 metastasis ultimately showed a significantly better prognosis than those with non-skip N2 metastasis in both the disease-free and overall survival (P = 0.010, P = 0.047, respectively) (Fig 5). These results suggest that lobectomy with systematic lymph node dissection may be recommended in order to avoid missing metastatic skip N2 nodes in patients with minimal pleural anthracosis. This suggestion is also supported by our observation that the injected ICG moved to an adjacent segment at a considerable rate (48%) in patients with any pleural ICG movement. In contrast, conventional selective lymph node dissection can be indicated in patients with relatively severe pleural anthracosis, as these patients are unlikely to develop skip metastasis. Furthermore, we believe that segmentectomy can be indicated in these patients if N1 metastasis is denied by a frozen section diagnosis. Segmentectomy may be particularly beneficial in patients with severe pleural anthracosis, as a high pleural anthracosis rate (%) was associated with a poor FEV1/FVC (R = -0.333, P = 0.031), according to our first series (n = 42). A previous report found that segmentectomy was associated with a comparable five-year overall survival rate to lobectomy in patients with small peripheral lung cancer[21-24]. Unfortunately, segmentectomy is not recommended in patients with peripheral lung cancer with

pleural invasion. We believe that segmentectomy can be applied even in lung cancers invading the pleura, given the findings of a large-scale validation study based on an intraoperative N1 node assessment in patients with relatively severe pleural anthracosis.

Several limitations associated with the present study warrant mention. First, we did not perform a pathological assessment of the pleural lymphatics that drained ICG; instead, we randomly sampled the normal lung tissue to evaluate pleural lymphatics. We believe that the ICG movement at the lung surface is caused by pleural lymphatic drainage, as was already proven by Riquet et al.[6] by the subpleural injection of dye. We also believe that changes in the pleural lymphatics in the sampled tissue are not a regional change but a global finding. Second, although skip N2 metastasis was identified only in patients with pleural ICG drainage (n=3), not in patients without it, the difference was not statistically significant. We therefore reviewed additional case series of patients who had pathologically proven N2 disease, so whether or not patients with skip metastasis in this series indeed developed pleural lymphatics remains unclear.

Conclusion

The current study suggested that pulmonary anthracosis induces pathological changes in the pleural lymphatics (infrequent straight-running lymphatics) and reduces pleural lymphatic drainage, thereby interfering with nodal skip metastasis. These results may be clinically valuable in intraoperatively determining the appropriate extent of lymph node dissection and lung resection in a case-specific manner in patients with lung cancer invading the visceral pleura.

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Author contribution statement:

Conception and design: TA and SM

Collection and assembly of data: TA, UK, AM, NT, KG, UT, and TT

Data analysis and interpretation: TA, UK, TA, TK, and SM

Manuscript writing: All authors.

Final approval of manuscript: All authors.

Data Availability Statement: The data underlying this article will be shared on reasonable request to the corresponding author.

FIGURE LEGENDS

Figure 1. Definition of the anthracosis score, which was scored based on the thoracoscopic view (A), and the anthracosis ratio (%), which was quantified by the imaging analysis software program (B). The the anthracosis score was significantly correlated with anthracosis ratio (%) (R = 0.683, *P* <0.001) (C). Dot = mean anthracosis ratio (%), Bar = standard deviation

Figure 2. Explanation of the parameters used in the current histological assessment (A: Immunohistochemical staining of D2-40, B: Explanatory panel). The following parameters were measured in each lymph vessel and each slide: pleural length (blue bold line), pleural cross-sectional area (dotted area), pleural lymph vessel length (black two-way arrow line), pleural lymph vessel cross-sectional area (red area).

Figure 3. Representative image (A: white-light image, B: near-infrared light image, C: schematic illustration of ICG drainage) of indocyanine green fluorescent movement via the pleural lymphatics of a patient with mild anthracosis (anthracosis score = 1, anthracosis ratio = 8.7%).

Figure 4. Images of immunohistochemical staining of D2-40 in two representative patients with minimal pleural anthracosis (A, B) and another two patients with severe pleural anthracosis (C, D). The patients with minimal anthracosis had a thinner pleura (A, B) than those with severe anthracosis (C, D). The patients with severe anthracosis (C, D) had more lymphatics (per pleural length), particularly short lymphatics, than those with minimal anthracosis (A, B). Accordingly, straight-running lymphatics, which were seen in patients with minimal anthracosis (A, B), were rarely seen in patients with severe anthracosis (C, D).

Figure 5. The disease-free and overall survival curves based on the Kaplan-Meier method, according to patients with a skip N2 metastasis pattern (n=24) and a non-skip N2 metastasis pattern (n=29).

Video. Video shows the detection of pleural lymphatic drainage of ICG from the injected site. After thoracotomy, ICG was injected at the subpleura of the resected lung. Five minutes after bilateral ventilation, the movement of ICG from the injected site was observed with a anear-infrared thoracoscope in a real-time manner. Note that the ICG moved from the injected site toward the mediastinum.

Table 1. Patient characteristics according to the presence of pleural lymphatic flows

Variables	All n=42	Pleural lymphatic flow		*P* value
		Yes n=23	No n= 19	
Age (years)	69.2±8.3	68.0±9.1	70.7±7.4	0.463
Sex (M/F)	26/16	11/12	15/4	0.057
Smoking history (Yes/No)	27/15	13/10	14/5	0.337
LAA on CT (%)	3.74±7.94	1.60±3.65	6.33±10.70	0.054
Air space (≥1 cm) on CT (Yes/No)	8/34	1/22	7/12	0.015
Injected side (Right/Left)	25/17	13/10	12/7	0.663
Injected lobe (Upper/Lower)	31/11	17/6	14/5	0.987
Tumor location (Central/Peripheral)	35/7	18/5	17/2	0.428
Anthracosis score	0.93±0.87	0.57±0.73	1.37±0.83	0.003
Anthracosis ratio (%)	3.84±3.05	1.49±1.88	2.83±1.84	0.005
Nodal involvement (Yes/No)	8/34	4/19	4/15	1.0
Skip N2 pattern (Yes/No)	3/39	3/20	0/19	0.239

LAA = low attenuation area less than -950 Hounsfield Units / total lung area

CT = computed tomography

Values are expressed as the number or mean ± standard deviation.

Table 2. Relationship between the percentage of anthracosis and various morphological parameters regarding the visceral pleura.

Variables	R	*P* value
Pleural thickness (μm)	0.410	0.007
Pleural lymph vessel density (/mm)	0.452	0.003
Median pleural lymph vessel length (μm)	-0.304	0.050
Upper quartile pleural lymph vessel length (μm)	-0.358	0.027
Pleural lymph vessel cross-sectional area (/mm)	0.392	0.010

Table 3. Patient characteristics of pathological N2 non-small cell lung cancer with pleural invasion

Characteristic	All n=53	skip N2 (N1-N2+) n=24	Non-skip N2 (N1+N2+) n=29	*P* value
Age (years)	68.1±9.3	66.5±11.7	69.4±6.8	0.265
Sex (M/F)	35/18	10/14	25/4	0.001
Smoking history (Yes/No)	39/14	13/11	26/3	0.005
LAA on CT (%)	3.74±7.94	1.60±3.65	6.33±10.70	0.054
Emphysematous change (Yes/No)	12/40	8/16	4/24	0.186
Affected side (Right/Left)	30/23	12/12	18/11	0.416
Affected lobe (Upper/Lower)	34/19	18/6	16/13	0.160
Tumor size (mm)	33.8±15.0	32.4±13.9	34.9±16.0	0.635
Histology (Adenocarcinoma/Others)	29/24	15/9	14/15	0.300
Pleural invasion (1/2 or 3)	27/26	13/11	14/15	0.669
Lymphatic invasion (Yes/No)	48/5	23/1	25/4	0.233
Vascular invasion (Yes/No)	39/14	18/6	21/8	0.832
Number of metastatic nodes	3.58±2.77	1.75±1.36	3.62±4.55	0.267
Anthracosis score	1.08±0.79	0.82±0.80	1.31±0.74	0.035
Anthracosis ratio (%)	2.61±3.16	1.79±2.82	3.30±3.30	0.012

LAA = low attenuation area less than -950 Hounsfield Units / total lung area

CT = computed tomography

Values are expressed as number or mean ± standard deviation.

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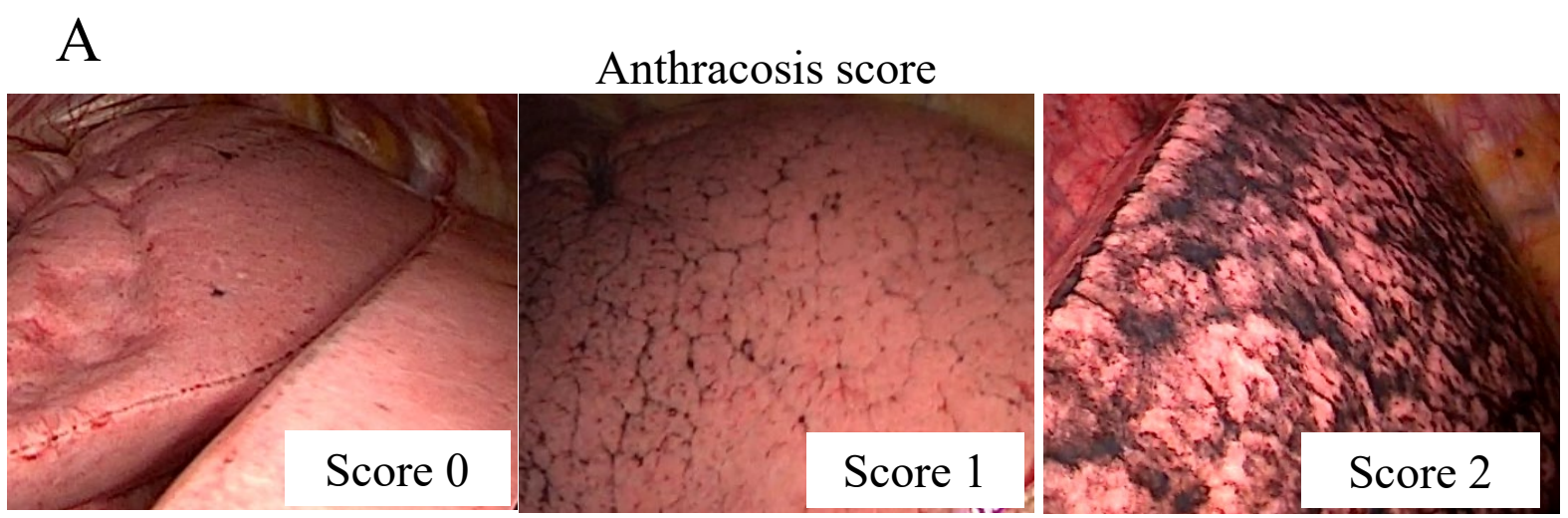
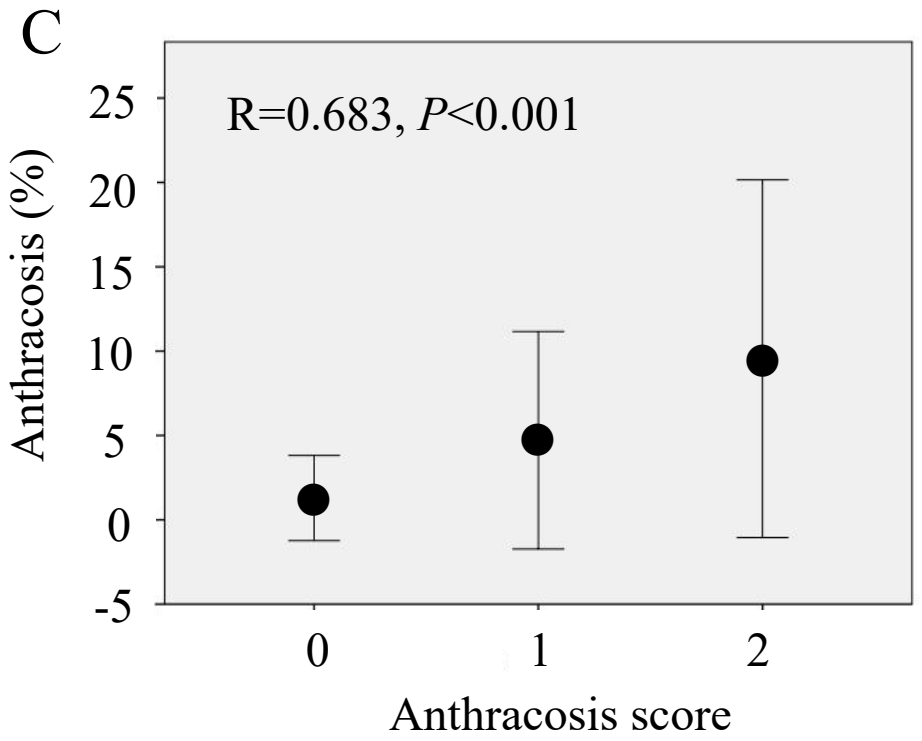
Figure 1


A
Anthracosis score
Score 0
Score 1
Score 2


C
R=0.683, P<0.001
Anthracosis (%)
25
20
15
10
5
0
-5
0
1
2
Anthracosis score

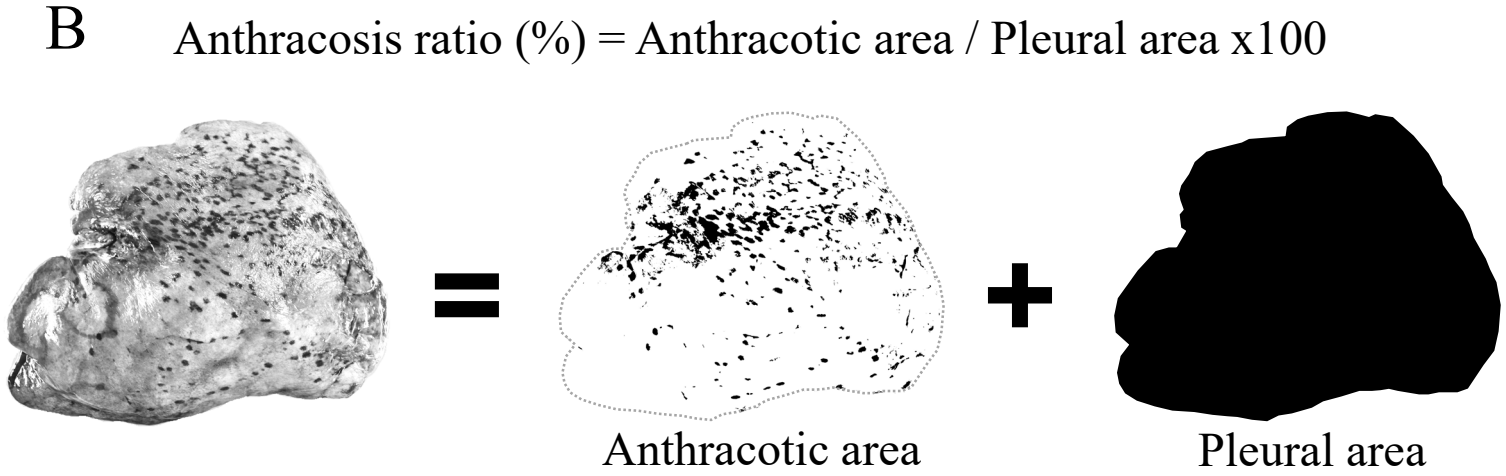

B
Anthracosis ratio (%) = Anthracotic area / Pleural area x100
=
+
Anthracotic area
Pleural area

Figure 2

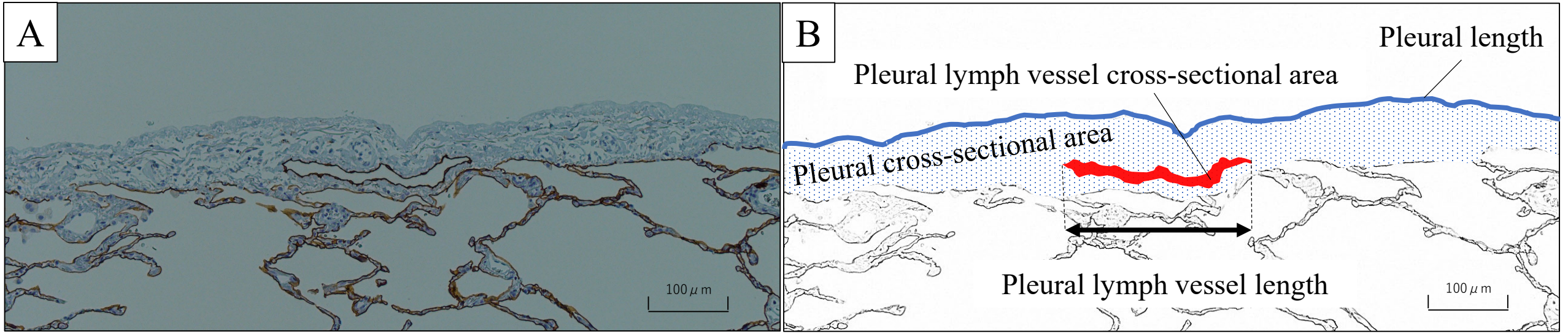

A
100 μ m
B
Pleural length
Pleural lymph vessel cross-sectional area
Pleural cross-sectional area
Pleural lymph vessel length
100 μ m

Figure 3

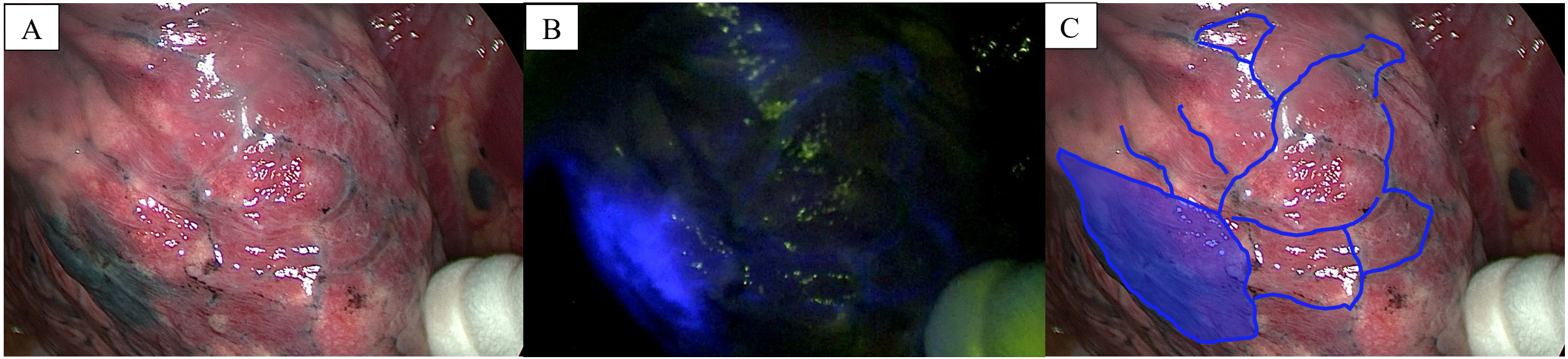
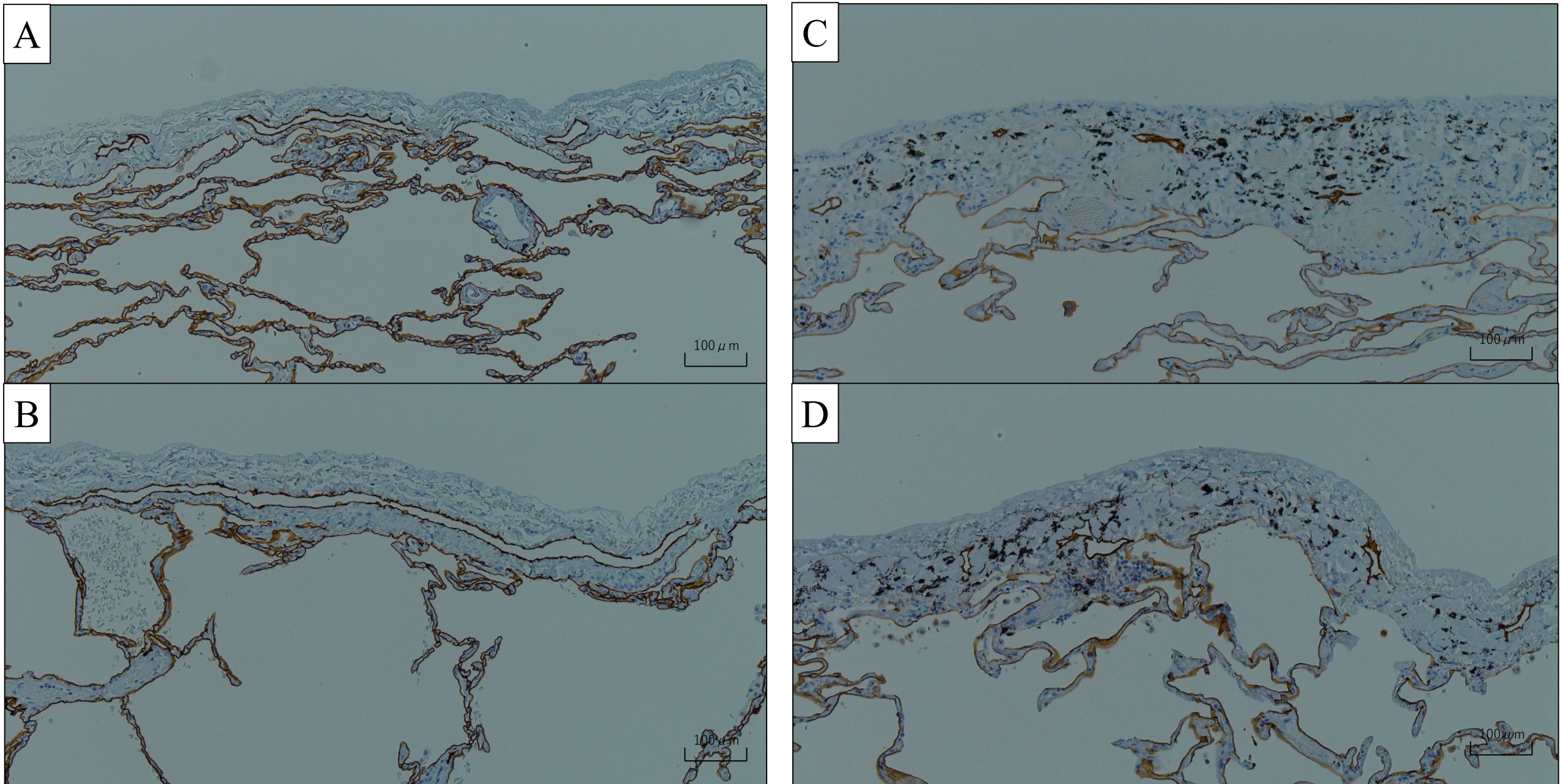
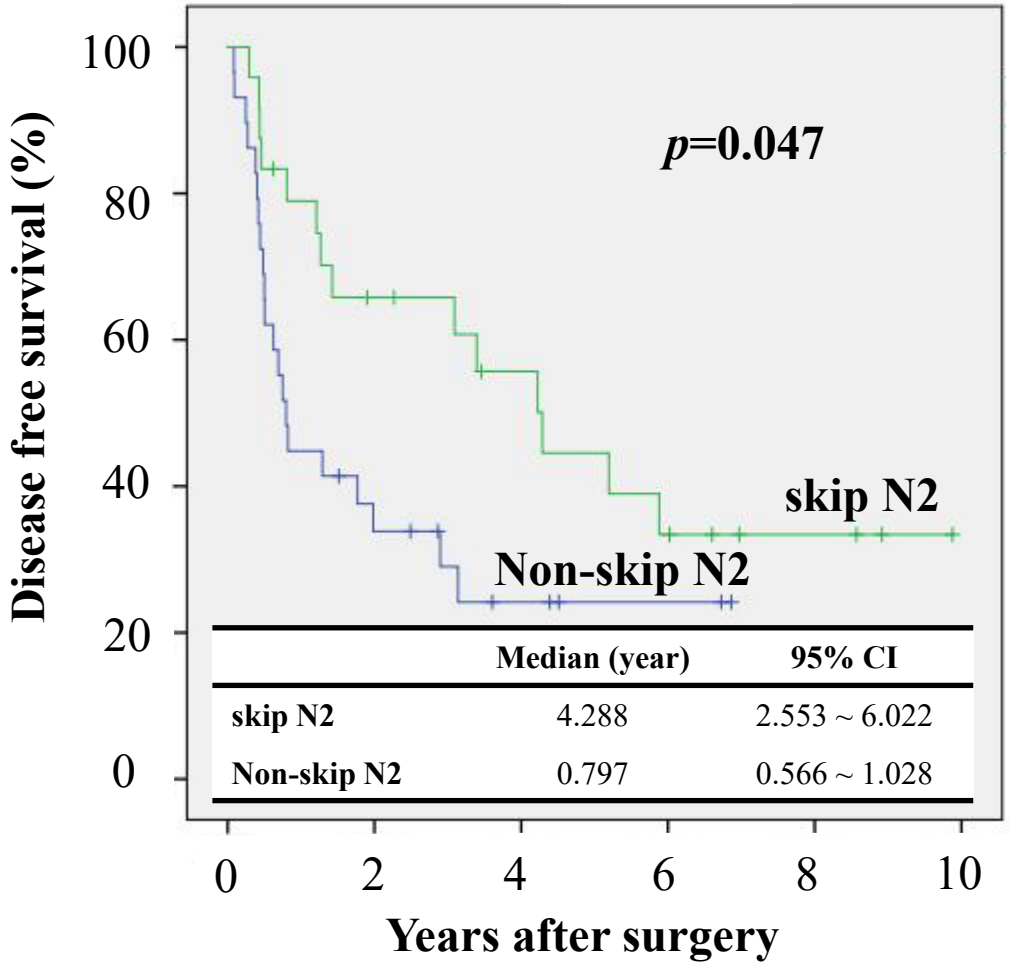


Figure 4



Supplementary Figure 1

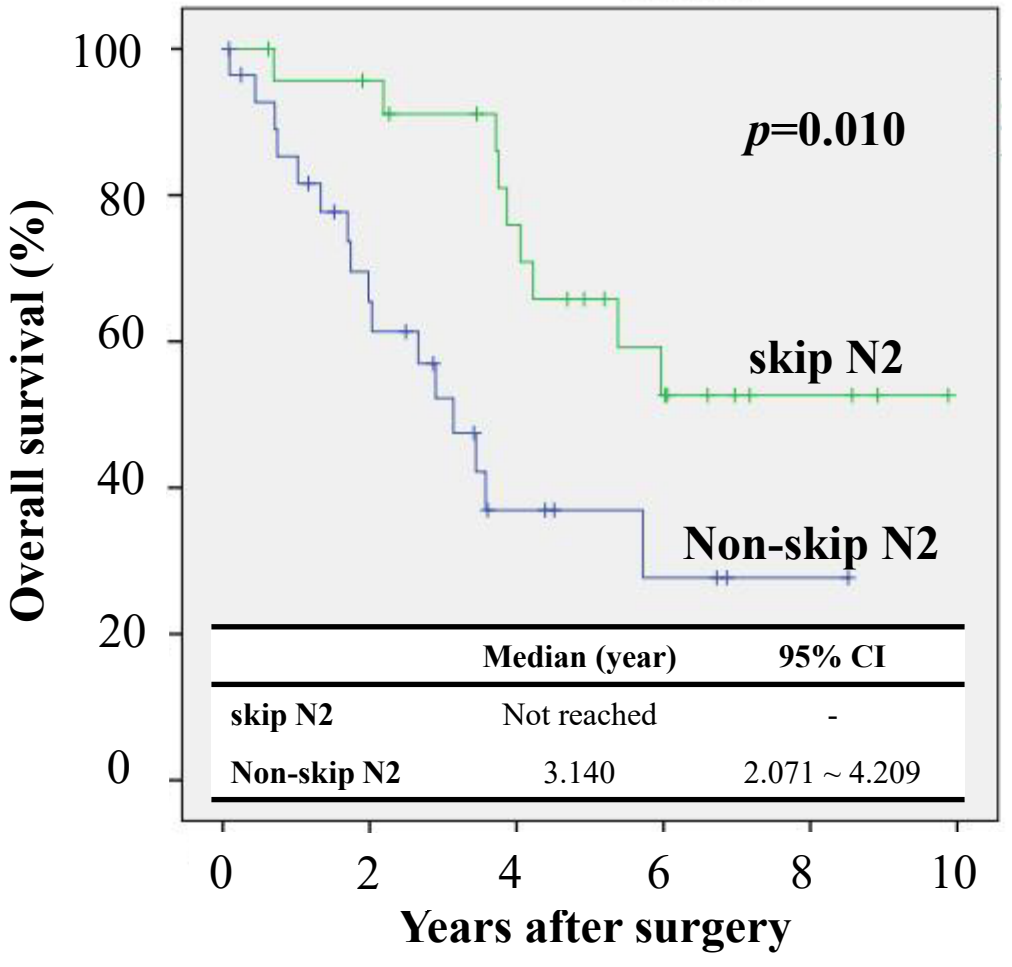
A



	Median (year)	95% CI
skip N2	4.288	2.553 ~ 6.022
Non-skip N2	0.797	0.566 ~ 1.028

No. at risk						
skip N2	24	14	10	6	3	0
Non-skip N2	29	9	4	2	0	0

B



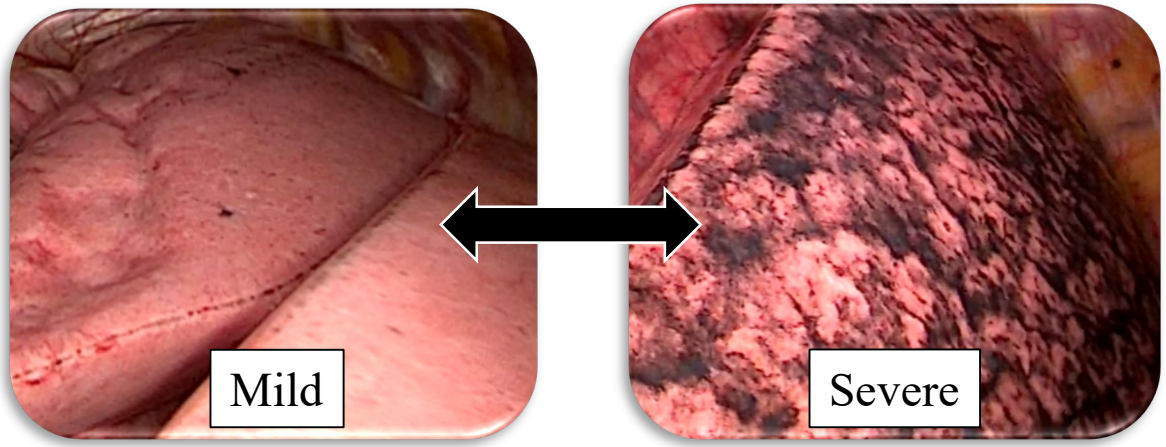
	Median (year)	95% CI
skip N2	Not reached	-
Non-skip N2	3.140	2.071 ~ 4.209

No. at risk						
skip N2	24	21	15	8	3	0
Non-skip N2	29	16	6	3	1	0

Central Picture

Study of visceral pleural lymphatics

Anthracosis



Case series #1

■ In vivo lymphography

Lymphatic drainage: Good ⟷ Poor

■ Microscopic study

Lymphatic vessel structure: Normal ⟷ Remodeling

Case series #2

■ Case control study (N2 lung cancer with pleural invasion)

Skip N2 metastasis: Likely ⟷ Unlikely

Supplementary Tables

Supplementary Table 1. Computed tomography findings according to the presence of pleural lymphatic flow

Characteristic	All n=42	Pleural lymph flow		*P* value
		Yes n=23	No n= 19	
GGO (Yes/No)	2/40	0/23	2/17	0.199
Honeycomb lung (Yes/No)	0/42	0/23	0/19	N/A
Pleural thickening (Yes/No)	2/40	0/23	2/17	0.199
Interlobular septal thickening (Yes/No)	2/40	0/23	2/17	0.199
Traction bronchiectasis (Yes/No)	3/39	0/23	3/16	0.084

GGO = ground-glass opacity

N/A = not applicable

Supplementary Table 2. Univariable and multivariable analysis of factors associated with pleural lymphatic ICG movement

Variables	Univariable analysis			Multivariable analysis		
	OR	95% CI	P	OR	95% CI	P
Emphysematous change	0.078	0.009-0.710	0.024	0.120	0.012-1.226	0.074
Anthracosis score	0.294	0.127-0.684	0.004	0.346	0.143-0.838	0.019

ICG = indocyanine green

OR = odds ratio

CI = confidence interval

Supplementary Table 3. Computed tomography findings according to the N2 metastasis pattern

Characteristic	All n=53	skip N2 n=24	Non-skip N2 n=29	*P* value
GGO (Yes/No)	2/50	1/23	1/27	1.0
Honeycomb lung (Yes/No)	2/50	1/23	1/27	1.0
Pleural thickening (Yes/No)	13/39	6/18	7/21	1.0
Interlobular septal thickening (Yes/No)	3/49	2/22	1/27	0.59
Traction bronchiectasis (Yes/No)	8/44	4/20	4/24	1.0

GGO = ground-glass opacity

Supplementary Table 4. Relationship among anthracosis score, the presence of pleural lymphatic ICG image, and skip N2 metastasis

Variables	Anthracosis score		
	0	1	2
Pleural lymphatic ICG image, Yes/No (%)	13/4 (76.5%)	7/4 (63.6%)	3/11 (21.4%)
Skip N2 in the first series, Yes/No (%)	2/15 (11.8%)	1/10 (9.1%)	0/14 (0%)
Skip N2 in the second series, Yes/No (%)	9/4 (69.2%)	8/10 (44.4%)	5/12 (29.4%)

ICG = indocyanine green