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THORACIC

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

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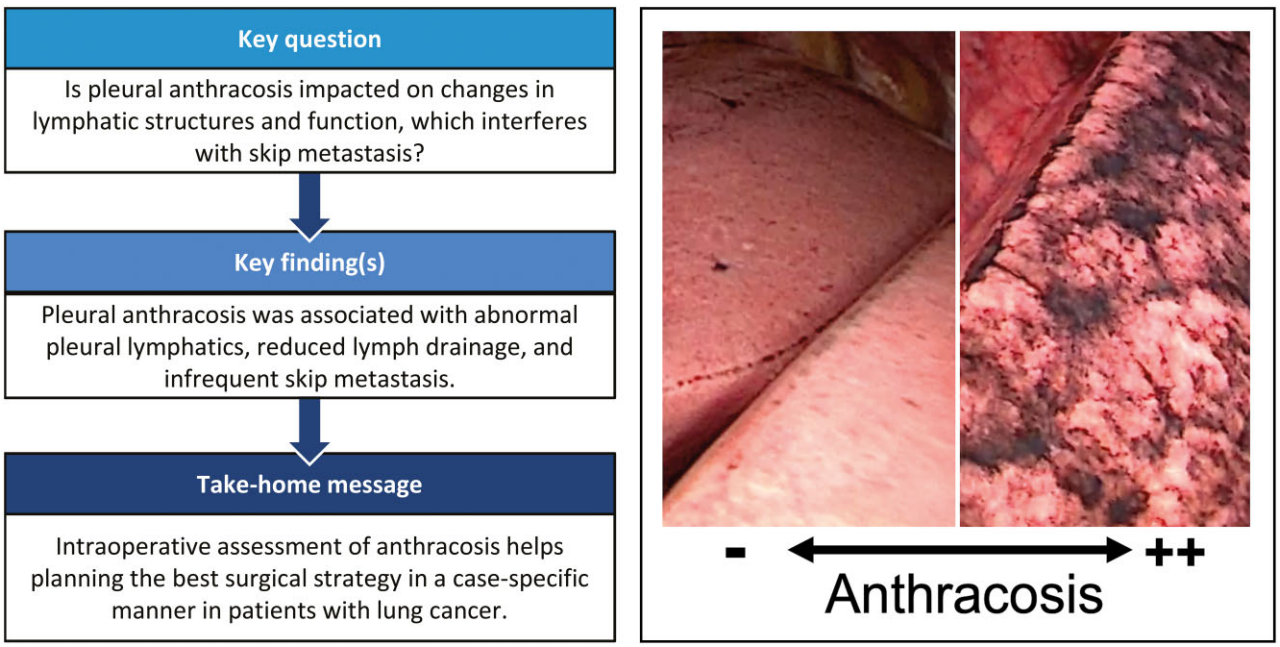


Abstract

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 2 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 had relatively severe anthracosis, while it was visualized in the remaining 23 (55%) patients who had relatively 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 remodelling. 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: Pleural anthracosis was associated with pathological changes in the pleural lymphatics and decreased pleural lymphatic drainage, thereby interfering with nodal skip metastasis.

Keywords: Lung cancer • Pleural lymphatics • Indocyanine green fluorescence • Anthracosis • Skip lymph node metastasis

ABBREVIATION

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 2 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 the macroscopic grading of pleural anthracosis.

PATIENTS AND METHODS

Ethical statement

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

Patients

We performed 2 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 anaesthesia 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 5 min, 2 surgeons conducted observations with a near-infrared camera (IMAGE1 S^{TM} FI; STORTZ, Tokyo, Japan) in real time and judged whether or not ICG-derived fluorescence had moved along the pleura from the injected site (Video 1).

Quantification of pleural anthracosis

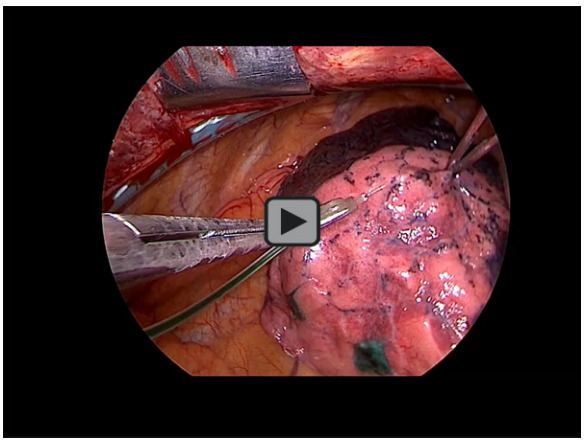
In the first series, we observed the visceral pleura under thoracoscopy just after thoracotomy and scored the degree of anthracosis (anthracosis score) as either 0, 1 or 2 (Fig. 1A), as follows:

0 = no or dotted anthracosis,

1 = linear anthracosis and
2 = patchy anthracosis.

Likewise, in the second series, we scored the degree of anthracosis in a similar fashion by reviewing the operative video. Two board-certified surgeons (AT and Kazuhiro Ueda) 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 2 surgeons.

In addition to the scoring of anthracosis, we calculated the ratio of the anthracosis area in the resected lung using the ImageJ software programme (National Institutes of Health, Bethesda, MD, USA). We converted the colour photograph of the resected lung to a greyscale 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 × 100.



Video 1: The detection of pleural lymphatic drainage of indocyanine green from the injected site. After thoracotomy, indocyanine green was injected at the subpleura of the resected lung. Five minutes after bilateral ventilation, the movement of indocyanine green from the injected site was observed with a near-infrared thoracoscope in a real-time manner. Note that the indocyanine green moved from the injected site towards the mediastinum.

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 (Kazuhiro Tabata), immunohistochemistry and archived haematoxylin–eosin slides were reviewed by 2 authors (Aya Takeda and Kazuhiro Ueda) 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 ImageJ software programme (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 ImageJ software programme (Fig. 2).

Pleural thickness. The pleural thickness was calculated as follows: pleural cross-sectional area/pleural length (Fig. 2). This is simply based on the following formula: pleural cross-sectional area = pleural thickness × 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

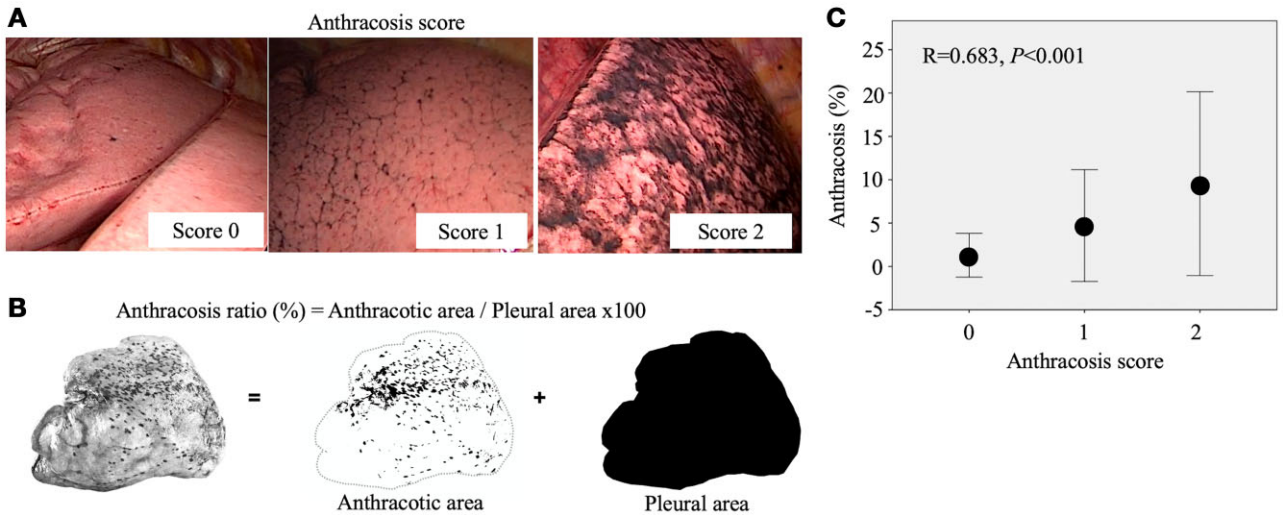


Figure 1: Definition of the anthracosis score, which was scored based on the thoracoscopic view (**A**), and the anthracosis ratio (%), which was quantified by the imaging analysis software programme (**B**). The anthracosis score was significantly correlated with anthracosis ratio ($R = 0.683$, $P 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

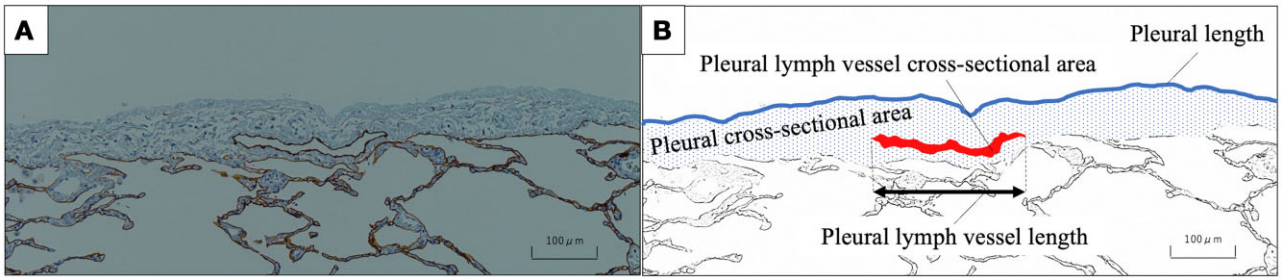


Figure 2: Explanation of the parameters used in the current histological assessment (**A**: immunohistochemical staining of D2-40, **B**: explanatory panel). The following parameters were measured in each lymph vessel and each slide: pleural length (blue bold line), pleural cross-sectional area (dotted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

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	0.46
Sex (M/F)	26/16	11/12	15/4	0.039
Smoking history (yes/no)	27/15	13/10	14/5	0.25
LAA on CT (%)	3.74 ± 7.94	1.60 ± 3.65	6.33 ± 10.70	0.16
Emphysematous change on CT (yes/no)	8/34	1/22	7/12	0.010
Injected side (right/left)	25/17	13/10	12/7	0.66
Injected lobe (upper/lower)	31/11	17/6	14/5	0.62
Tumour location (central/peripheral)	35/7	18/5	17/2	0.29
Anthracosis score	0.93 ± 0.87	0.57 ± 0.73	1.37 ± 0.83	0.003
Anthracosis ratio (%)	2.09 ± 1.96	1.49 ± 1.88	2.83 ± 1.84	0.005
Nodal involvement (yes/no)	8/34	4/19	4/15	0.53
Skip N2 pattern (yes/no)	3/39	3/20	0/19	0.15

LAA less than -950 HU/total lung area. Values are expressed as the number or mean ± standard deviation.
CT: computed tomography; F: female; LAA: low attenuation area; M: male.

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 ImageJ software programme. 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 Mann–Whitney *U*-test was used to compare numerical variables between the groups and a linear regression analysis was used to compare numerical variables. Fisher's exact test was used to compare categorical variables in situations where the chi-square test was not valid. A multivariable logistic regression analyses were used to determine the relationship between clinical factors and the pleural lymphatic ICG movement. Using multivariable regression, independent factors of pleural lymphatic flow were determined using 2 variables with the lowest *P*-value because of the limited number of events ($n = 23$). We selected the anthracosis score in the multivariable analysis, rather than the anthracosis ratio, because the anthracosis score is more applicable than the anthracosis ratio in the clinical setting. A *P*-value of <0.05 was considered statistically significant. All statistical analyses were performed using IBM SPSS Statistics software (IBM Corp. Released 2013. IBM SPSS Statistics for Windows, Version 22.0. Armonk, NY: IBM Corp.).

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 and 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 4 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, smoking history or radiological findings of the tumour and the lung (Table 1 and Supplementary Material, Table S1). 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

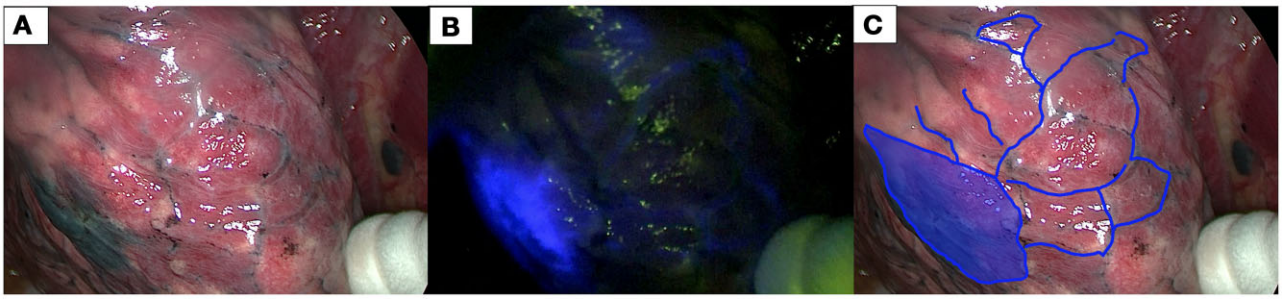


Figure 3: Representative image (**A**: white-light image, **B**: near-infrared light image, **C**: schematic illustration of indocyanine green drainage) of indocyanine green fluorescent movement via the pleural lymphatics of a patient with mild anthracosis (anthracosis score = 1, anthracosis ratio =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

Table 2: Relationship between the anthracosis ratio and various morphological parameters regarding the visceral pleura

Variables	*R*	*P*-Value
Pleural thickness (μm)	0.410	0.007
Pleural lymph vessel density (1/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 (1/mm)	0.392	0.010

R: correlation coefficient.

emphysematous changes (intrapulmonary air space on computed tomography ≥1 cm) less frequently than those without ICG movement. According to the multivariable 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, $P = 0.019$) (Supplementary Material, Table S2).

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 ($P = 0.53$). Skip N2 metastasis was found in 3 patients with ICG movement but not found in any patients without ICG movement ($P = 0.15$).

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) were 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 ratio 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 Fig. 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 ($P = 0.004$) 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 and Supplementary Material, Table S3), although a computed tomography assessment was not done in 1 patient due to obstructive pneumonia. There were also no significant differences in the pleural lavage cytology results between the groups (Table 3), suggesting the limited effect of cancer cells in the pleural cavity on skip N2 metastasis. 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.

Pleural lymphatic flow, anthracosis and skip metastasis

In the first case series, the identification rate of pleural lymphatic ICG movement in patients with anthracosis score 0, 1 and 2 was 77%, 64% and 21%, respectively (Supplementary Material, Table S4). Accordingly, the identification rate of skip N2 metastasis in patients with anthracosis score 0, 1 and 2 was 12%, 9% and 0%, respectively. Likewise, in the second case series, the identification rate of skip N2 metastasis in patients with anthracosis score 0, 1 and 2 was 69%, 44% and 29%, respectively. With regard to the anthracosis ratio, the potential of the anthracosis ratio on diagnosing skip N2 metastasis was proven by a receiver operating characteristic curve analysis: the area under the curve was 0.703. The best threshold value of anthracosis ratio was 0.887 (sensitivity = 86%, specificity = 54%) (Supplementary Material, Fig. S1).

DISCUSSION

According to the current fluorescence imaging study, the pleural lymphatic flow appeared to have suffered interference in patients

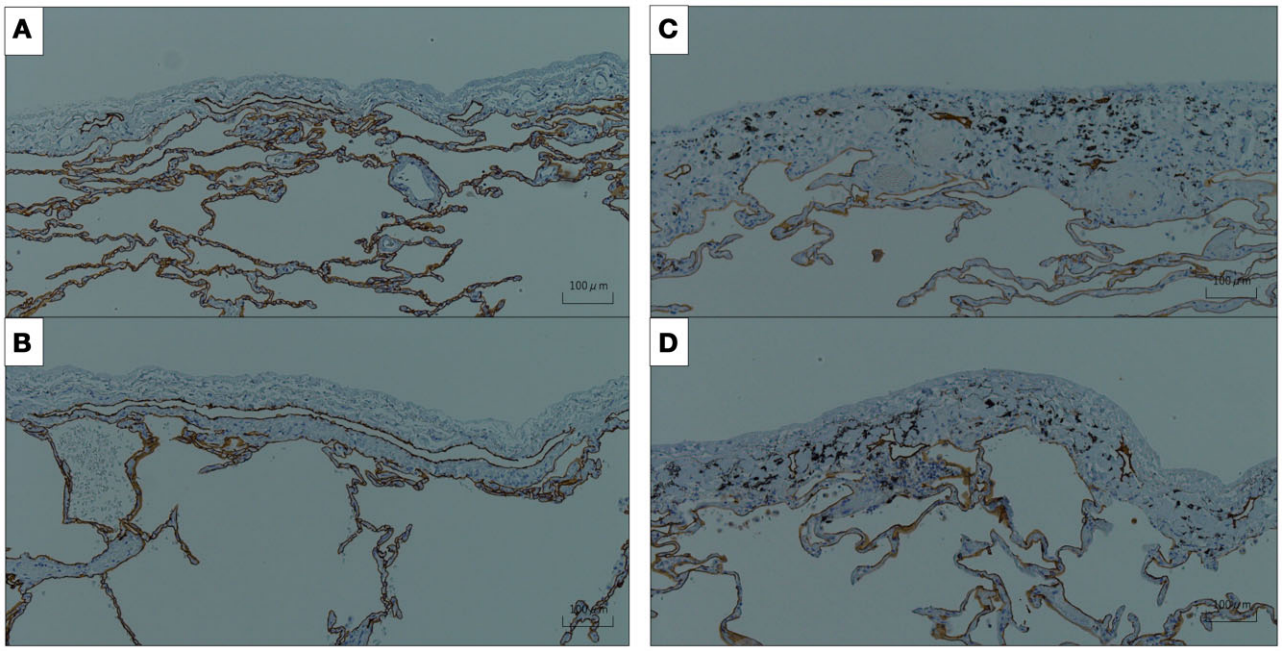


Figure 4: Images of immunohistochemical staining of D2-40 in 2 representative patients with minimal pleural anthracosis (**A** and **B**) and another 2 patients with severe pleural anthracosis (**C** and **D**). The patients with minimal anthracosis had a thinner pleura (**A** and **B**) than those with severe anthracosis (**C** and **D**). The patients with severe anthracosis (**C** and **D**) had more lymphatics (per pleural length), particularly short lymphatics, than those with minimal anthracosis (**A** and **B**). Accordingly, straight-running lymphatics, which were seen in patients with minimal anthracosis (**A** and **B**), were rarely seen in patients with severe anthracosis (**C** and **D**).

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 ± 9.4	66.5 ± 11.7	69.4 ± 6.8	0.78
Sex (M/F)	35/18	10/14	25/4	0.001
Smoking history (yes/no)	39/14	13/11	26/3	0.004
LAA on CT (%)	3.59 ± 4.88	3.24 ± 4.71	4.04 ± 5.17	0.95
Emphysematous change (yes/no)	12/40	8/16	4/24	0.1
Affected side (right/left)	30/23	12/12	18/11	0.38
Affected lobe (upper/lower)	34/19	18/6	16/13	0.13
Tumour size (mm)	33.8 ± 15.0	32.4 ± 13.9	34.9 ± 16.0	0.64
Histology (adenocarcinoma/others)	29/24	15/9	14/15	0.30
Pleural invasion (1/2 or 3)	27/26	13/11	14/15	0.67
Lymphatic invasion (yes/no)	48/5	23/1	25/4	0.23
Vascular invasion (yes/no)	39/14	18/6	21/8	0.83
Number of metastatic nodes	2.77 ± 3.58	1.75 ± 1.36	3.62 ± 4.55	0.27
PLC (positive/negative)	6/47	2/22	4/25	0.43
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 less than -950 HU/total lung area. Values are expressed as number or mean ± standard deviation.

CT: computed tomography; F: female; LAA: low attenuation area; M: male; PLC: pleura lavage cytology.

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 tumours 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. Considering that the current subjective evaluation of the pleural anthracosis (anthracosis score) was accurately associated with the objective findings (anthracosis ratio) and the pathological findings on pleural lymphatics, our results may be clinically valuable in 'intraoperatively' determining the appropriate extent of lymph node dissection in a case-specific manner.

According to the receiver operating characteristic curve analysis for the potential of anthracosis ratio on diagnosing skip N2 metastasis pattern, skip metastasis occurred in some patients with high anthracosis ratio. Thus, we could not define a threshold

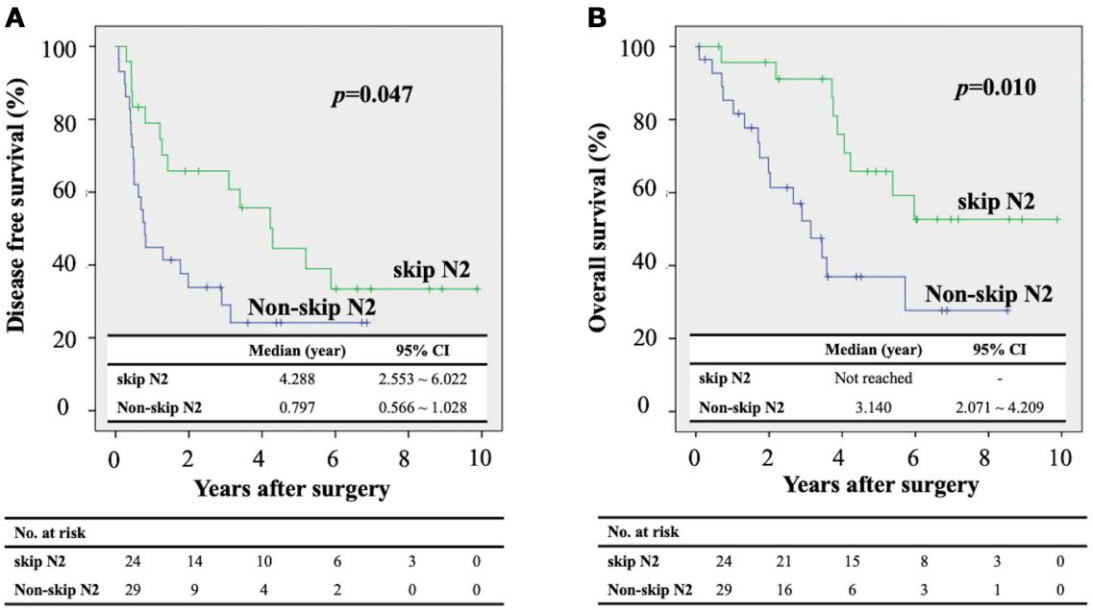


Figure 5: The disease-free and overall survival curves based on the Kaplan–Meier method, according to patients with a skip N2 metastasis pattern ($n = 24$) and a non-skip N2 metastasis pattern ($n = 29$).

value of anthracosis ratio to intraoperatively deny skip metastasis. There are some possible explanations for this result. First, skip metastasis still occurs in rare occasion via pleural lymphatics in patients with severe pleural anthracosis. Second, skip metastasis can develop via lymphatics along the bronchovascular bundle via direct lymphatic communications between tumour and the mediastinal node or via the lymphatic shunt within the hilar nodes. Because the current ICG imaging system cannot contribute to clarifying these speculations, we must make further study with large sample size by comparing the severity of anthracosis with lymphatic structures including hilar lymph nodes.

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 multivariable 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 remodelling 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 remodelling, 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. Nonetheless, we must keep in mind that there is a possibility that the anomaly in the pleural lymphatics caused abnormal accumulation of carbon debris to the pleura, leading to reduced incidence of skip N2 metastasis.

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 complete resection without missing metastatic skip N2 nodes is important in patients with minimal pleural anthracosis. In addition, segmentectomy is not recommended in these patients because the injected ICG moved to an adjacent segment at a considerable rate (48%) in patients with any pleural ICG movement. In contrast, we believe that segmentectomy can be indicated in patients with relatively severe pleural anthracosis if N1 metastasis is denied by a frozen section diagnosis. Segmentectomy may be particularly beneficial

in patients with severe pleural anthracosis, because anthracosis ratio was negatively associated with forced expiratory volume in 1 second / forced vital capacity (R = -0.333, P = 0.031), according to our first series (n = 42).

Limitations

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. Third, the main results regarding the grade of anthracosis and the presence or absence of pleural lymphatic flow were relied on subjective evaluation, which can compromise reproducibility of our study results. The long study period can also contribute to compromise consistency of evaluation. To dispel these concerns, 2 authors (AT and Kazuhiro Ueda) reviewed surgical movies to evaluate the subjective parameters in a random order, without knowledge of the patients' clinical data.

CONCLUSION

The current study suggested that pulmonary anthracosis was associated with pathological changes in the pleural lymphatics (infrequent straight-running lymphatics) and reduced 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.

SUPPLEMENTARY MATERIAL

Supplementary material is available at *EJCTS* online.

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Conflict of interest: none declared.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author (Kazuhiro Ueda), without limitations, upon reasonable request.

Author contributions

Aya Takeda: Data curation; Formal analysis; Investigation; Methodology; Visualization; Writing—original draft; Writing—review & editing. **Kazuhiro Ueda:** Conceptualization; Data curation; Formal analysis; Investigation; Methodology; Writing—original draft; Writing—review & editing. **Masaya Aoki:** Formal analysis; Investigation; Methodology; Writing—review & editing. **Toshiyuki Nagata:** Investigation; Methodology; Writing—review & editing. **Go Kamimura:** Investigation; Methodology; Writing—review & editing. **Tadashi Umehara:** Investigation; Writing—review & editing. **Takuya Tokunaga:** Investigation; Methodology; Writing—review & editing. **Kazuhiro Tabata:** Conceptualization; Investigation; Methodology; Writing—review & editing. **Akihide Tanimoto:** Data curation; Supervision; Writing—review & editing. **Masami Sato:** Conceptualization; Investigation; Supervision; Writing—review & editing.

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