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Application of Cluster Analysis to Distant Metastases from Lung Cancer

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Abstract. *Background/Aim: In patients with lung cancer, there has been no study that treated 'distant metastases' as 'metastatic patterns'. This study aimed to evaluate if specific 'metastatic patterns' exist in lung cancer patients. Patients and Methods: Data were collected from lung cancer patients between 2009 and 2018. Metastatic patterns were analyzed using cluster analysis in patients with epidermal growth factor receptor (EGFR) mutation-positive lung adenocarcinoma, those with small cell lung cancer (SCLC), and those with squamous cell lung cancer (SqCLC). Results: In 313 patients (127 patients with EGFR mutation, 87 patients with SCLC, and 99 patients with SqCLC), metastatic patterns existed in each of the three subset groups, and metastatic patterns of these groups were statistically different. Conclusion: The knowledge of the metastatic patterns might be useful for clinical practice in the foreseeable future, as it enables a more efficient detection of metastatic disease through imaging, and a more effective treatment at predicted metastatic sites.*

There are several dominant and conflicting theories, based upon studies of autopsied patients, to explain metastatic distribution (1-3). The mechanical theory, proposed by Ewing, states that the distribution of tumor metastases is explained entirely on the basis of blood flow patterns (1). However, blood flow patterns do not aid the prediction of distant metastases (2). By contrast, the 'soil-seed' hypothesis, originally proposed by Paget in 1889, states that the distribution of tumor metastases can be explained on the basis of favorable microenvironments in certain organs (3). Paget suggested that there was more to the patterns of metastasis than factors that involved blood flow (3).

Distant metastasis almost always develops in lung cancer. Initial staging evaluations report that 40-60% of patients present with metastatic disease (4, 5). The most common sites of metastasis encountered in pretreatment evaluations are the lung, bone, brain, liver and adrenal gland (6-9). Most studies on lung cancer metastasis have examined the frequency of specific organ metastasis. Relatively few studies have examined 'metastatic patterns' in lung cancer, focusing largely on clinical data (10-13) or experimental methods (14, 15). The following two studies are noteworthy in terms of previous studies using clinical data: an evaluation of metastatic patterns in 4399 adenocarcinoma patients by Hess *et al*. (10); and metastatic patterns in 537 small-cell lung cancer (SCLC) patients by Elliot *et al*. (11). The former study, however, included adenocarcinomas of 11 primary tumor sites (10), and the latter study evaluated autopsied patients (11). Therefore, much remains to be clarified. Whether through experimental or clinical research, increased knowledge of the 'metastatic patterns' of lung cancer will be useful to perform efficient imaging tests and inform on effective treatment for the predicted metastatic sites.

Cluster analysis is a multivariate analysis method that classifies target groups by creating clusters that are similar to each other, from groups of subjects with different properties complicated together (16, 17). In clinical research, this type of analysis has been used in infectious diseases to classify pathogens (18) and to explore the relationship between genotypes and phenotypes of bronchial asthma (19).

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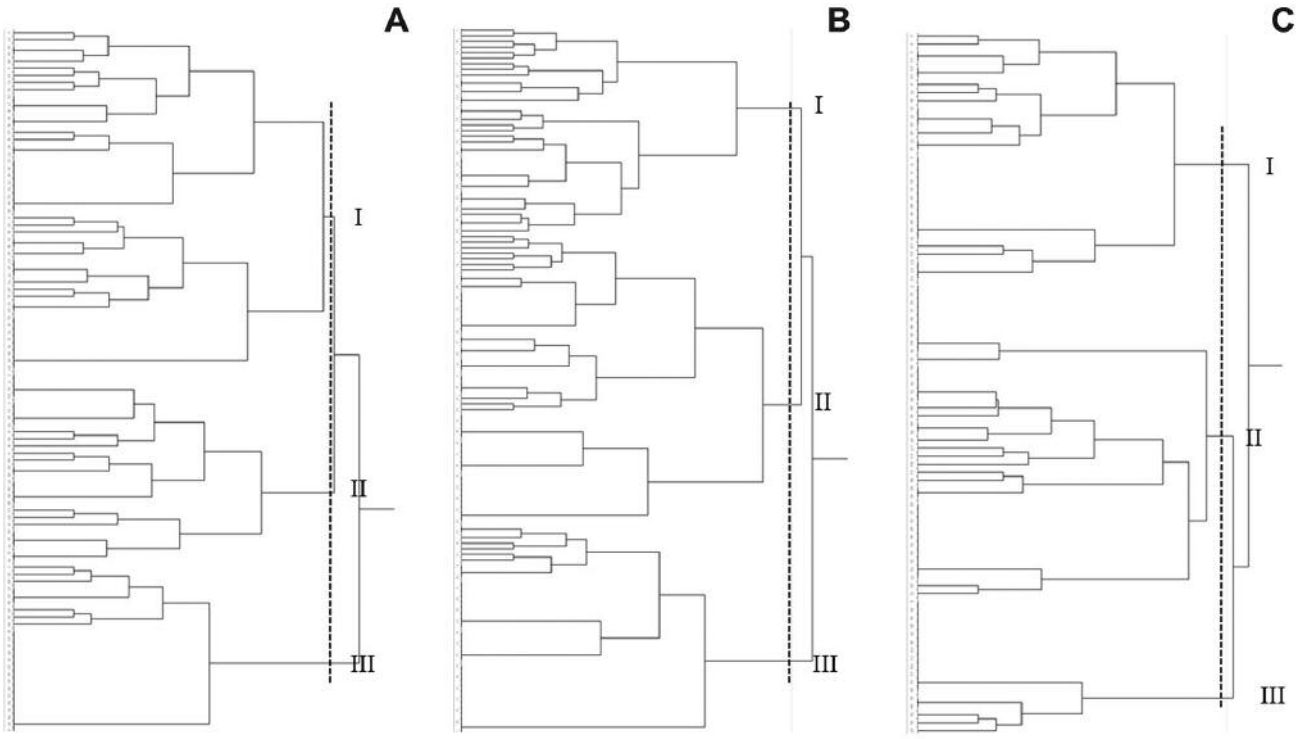

Figure 1. *Dendrogram indicating the three-cluster model. The cutoff line for the cluster selection process is indicated by the dotted line. (A) Lung adenocarcinoma patients with an epidermal growth factor receptor (EGFR) mutation. Cluster I (bone-liver-other sites type, number of patients=48), cluster II (lung-liver type, number of patients=24), and cluster III (lung-brain type, number of patients=27). (B) Small cell lung cancer (SCLC) patients. Cluster I (brain-adrenal gland type, number of patients=37), cluster II (lung-distant lymph nodes-other sites type, number of patients=52), and cluster III (bone-liver type, number of patients=38). (C) Squamous cell lung cancer patients. Cluster I (bone type, number of patients=31), cluster II (brain type, number of patients=39), and cluster III (lung-adrenal gland type, number of patients=17).*

A recent study using cluster analysis in lung cancer patients identified prognostic factors other than tumor-node-metastasis classification (TNM) factors (20). In regards to cluster analyses of distant metastasis, Lee *et al*. at the Johns Hopkins reported on the metastasis of Kaposi's sarcoma in autopsied patients with human immunodeficiency virus infection (21). Yet, the study did not describe their statistical methods in detail and significant differences were not statistically evaluated. Therefore, to our knowledge, there have been no studies aimed at clarifying 'metastatic patterns' that appropriately use cluster analysis.

We performed this study to determine if there are specific metastatic patterns in lung cancer patients. Furthermore, if patterns were detected, we clarified differences of metastatic patterns amongst specific groups of lung cancer patients: those with epidermal growth factor receptor (*EGFR*) gene mutation-positive lung adenocarcinoma (as a representative of cancers with clear driver mutation), and those with SCLC and squamous cell carcinoma (as two representative histological types of lung cancer).

Patients and Methods

Patients. Patients who presented with pathologically diagnosed lung cancer between April 2009 and October 2018 at four tertiary hospitals in Japan; University of Tsukuba Mito Medical Center-Mito Kyodo General Hospital, University of Tsukuba Hitachinaka Medical Center-Hitachinaka General Hospital, Ryugasaki Saiseikai General Hospital, and Tsukuba Kinen General Hospital, were identified retrospectively *via* computerized searches of tumor registry data. Medical record information from diagnostic imaging, including chest computed tomography (CT), brain magnetic resonance imaging (MRI) or enhanced head CT, bone scan and ultrasonography and/or CT of the abdomen, was used to identify the location of metastatic tumors. Information on distant metastases was collected in detail, with the most common metastatic sites being lung, bone, brain, liver, adrenal gland, distant lymph nodes, and other sites. Clinical data for age, gender, smoking habit, primary site of lung cancer, maximum diameter of the primary tumor, and N-stage of lung cancer were also collected.

This study was approved by the institutional ethics committee of each Hospital (Project approval number: NO16-66-NO18-17). Written comprehensive informed consent at the time of admission for obtaining pathological specimens was obtained from each patient.

Table I. *Characteristics in three cluster groups of patients with epidermal growth factor receptor (EGFR) mutated lung adenocarcinoma.*

Characteristic	Cluster I	Cluster II	Cluster III	*p*-Value
Age				
40-59	2	5	3	
60-79	33	13	22	0.0566
80-	13	6	2	
Gender				
Male	20	5	8	
Female	28	19	19	0.1869
Smoking (pack-year)				
0-5	38	20	20	
5-50	7	3	6	
50-125	2	1	1	0.9103
Unknown	1	0	0	
Primary site of lung cancer				
Right upper	14	5	4	
Right middle	3	1	3	
Right lower	8	3	6	0.5622
Left upper	8	10	7	
Left lower	14	5	6	
Unknown	1	0	1	
N-factor				
0	15	8	6	
1	6	3	5	0.9136
2	13	7	6	
3	14	6	10	
EGFR				
Ex19del	25	11	17	
Ex21L858R	20	12	10	0.2720
Others	3	1	0	
Metastasis present/absent				
Lung	35/13	0/24	13/14	0.0001
Bone	18/30	23/1	18/9	0.0001
Brain	38/10	23/1	2/25	0.0001
Liver	30/18	18/6	26/1	0.0054
Adrenal gland	45/3	23/1	21/6	0.0478
Lymph nodes	42/6	23/1	21/6	0.1601

Statistical analysis. Cluster analysis was performed to classify patients (16). Briefly, pre-clusters to reduce the size of the matrix that contained the distances between all possible pairs of cases were performed. Then, the standard hierarchical clustering algorithm was applied to the pre-clusters to explore a range of solutions with different numbers of clusters. At this point, hierarchical cluster analysis was performed using Ward's method to generate a dendrogram for estimation of the number of likely clusters within the population. Cluster boundaries were defined by large differences between successive fusion levels (17). At each cluster, samples were merged into larger clusters to minimize the within-cluster sum of squares or to maximize the between-cluster sum of squares in Euclidean distance. Variables for cluster analysis included the common metastatic sites described above. The type of *EGFR* mutation was also included as a variable in patients with *EGFR* mutant tumors. Statistical analyses were performed using BellCurve for Excel (version 3.0). Differences in proportions between two and among three independent groups were compared using the chi-square test. $p<0.05$ was considered statistically significant.

Results

Patient characteristics. All 313 consecutive, pathologically diagnosed lung cancer patients with metastatic disease (127 patients with *EGFR* mutation, 87 patients with SCLC, and 99 patients with squamous cell lung cancer) were included in this study. The most common metastatic sites were the lung, bone, brain, liver, and adrenal gland.

Metastatic clusters in EGFR mutant tumors. A dendrogram illustrating the three-cluster model is shown in Figure 1A. In this cluster model, metastatic groups were identified as follows; cluster I (bone-liver-other sites type, number of patients=48), cluster II (lung-liver type, number of patients=24), and cluster III (lung-brain type, number of patients=27). Demographic and baseline clinical and pathological characteristics of the identified clusters are shown in Table I. There were no significant differences between the three clusters in regards to age, gender, smoking habit, primary site, size of primary tumor, and regional lymph nodes examined.

Metastatic clusters in SCLC. A dendrogram illustrating the three-cluster model is shown in Figure 1B. In this cluster model, metastatic groups were identified as follows; cluster I (brain-adrenal gland type, number of patients=37), cluster II (lung-distant lymph nodes-other sites type, number of patients=52), and cluster III (bone-liver type, number of patients=38). As shown in Table II, there was no statistically significant difference in clinical and pathological characteristics such as age, gender, smoking habit, and primary site, except for maximum size of the primary tumor.

Metastatic clusters in squamous cell lung cancer. A dendrogram illustrating the three-cluster model is shown in Figure 1C. In this cluster model, metastatic groups were identified as follows; cluster I (bone type, number of patients=31), cluster II (brain type, number of patients=39), and cluster III (lung-adrenal gland type, number of patients=17). Except for age, there was no statistical difference among these clusters in the clinical and pathological characteristics (Table III).

Comparison of clusters in three types of lung cancers. Figure 2 shows the differences in metastatic patterns among the nine clusters found in the three types of lung cancer studied here: *EGFR* mutant lung tumors, and small cell and squamous cell histological subtypes. There was a statistically significant difference among them ($p=0.0001$).

Table II. *Characteristics of three cluster groups of patients with small cell lung cancer.*

Characteristic	Cluster I	Cluster II	Cluster III	*p*-Value
Age				
50-59	3	3	1	
60-69	19	17	9	
70-79	14	18	21	0.0515
80-89	1	13	6	
90-	0	1	1	
Gender				
Male	31	42	32	
Female	6	10	6	0.8931
Smoking (pack-year)				
0-25	3	10	9	
25-75	23	27	24	0.4720
75-150	11	15	5	
Primary site of lung cancer				
Right upper	15	13	10	
Right middle	1	2	3	
Right lower	9	13	8	0.4369
Left upper	4	14	7	
Left lower	6	10	10	
Unknown	2	0	0	
Maximum size of the primary tumor (mm)				
≤20	4	9	5	
21-60	20	22	25	
61-100	7	17	8	0.0092
≥101	0	3	0	
Unknown	6	1	0	
N-factor				
0	0	2	2	
1	3	0	0	
2	10	20	13	0.1331
3	23	30	23	
Unknown	1	0	0	
Metastasis present/absent				
Lung	30/7	33/19	32/6	0.0472
Bone	26/11	43/9	11/27	0.0001
Brain	21/16	33/19	30/8	0.1105
Liver	18/19	51/1	14/24	0.0001
Adrenal gland	6/31	49/3	36/2	0.0001
Lymph nodes	16/21	36/16	38/0	0.0001
Other sites	27/10	31/21	38/0	0.0001

Table III. *Characteristics of three cluster groups of patients with squamous cell lung cancer.*

Characteristic	Cluster I	Cluster II	Cluster III	*p*-Value
Age				
50-69	5	18	5	
70-79	12	17	8	0.0093
90-	14	4	4	
Gender				
Male	25	32	16	
Female	6	7	1	0.4368
Smoking (pack-year)				
0-5	4	2	1	
5-50	10	22	9	
50-100	14	11	5	0.5857
100-150	1	3	1	
Unknown	2	1	1	
Primary site of lung cancer				
Right upper	5	7	3	
Right middle	2	1	0	
Right lower	6	11	7	0.6216
Left upper	11	15	3	
Left lower	7	5	4	
Maximum size of the primary tumor (mm)				
≤30	4	3	3	
31-60	13	19	5	
61-100	12	14	12	0.0776
≥101	2	0	0	
Unknown	0	3	0	
N-factor				
0	6	3	1	
1	1	6	1	
2	10	12	5	0.4801
3	14	17	10	
Unknown	0	1	0	
Metastasis present/absent				
Lung	25/6	34/5	0/17	0.0001
Bone	11/20	33/6	17/0	0.0001
Brain	29/2	24/15	17/0	0.0001
Liver	20/11	34/5	16/1	0.0170
Adrenal gland	28/3	23/16	13/4	0.0122
Lymph nodes	31/0	17/22	14/3	0.0001
Other sites	21/10	33/6	17/0	0.0180

Discussion

Approximately half of all patients with lung cancer will have distant metastases at diagnosis (4, 5). Many studies have examined distant metastases from lung cancer (6-9), and these studies have shown that the most common sites of metastasis in pretreatment evaluations are lung, bone, brain, liver and adrenal gland (6-9), and many patients have multiple organ metastases, not single organ metastasis (6-9, 13, 22-26). Only a few studies, however, have reported on multiple organ metastases (10-13, 25, 26), due to the difficulty in statistically analyzing multi-site data. In addition, standard statistical methods to analyze multiple organ metastases have not been established. To the best of our knowledge, no studies have elucidated the 'metastatic pattern' of lung cancer patients using antemortem clinical information.

We have previously studied distant metastases in lung cancer (13, 22-26). Using an analysis based on the theory of

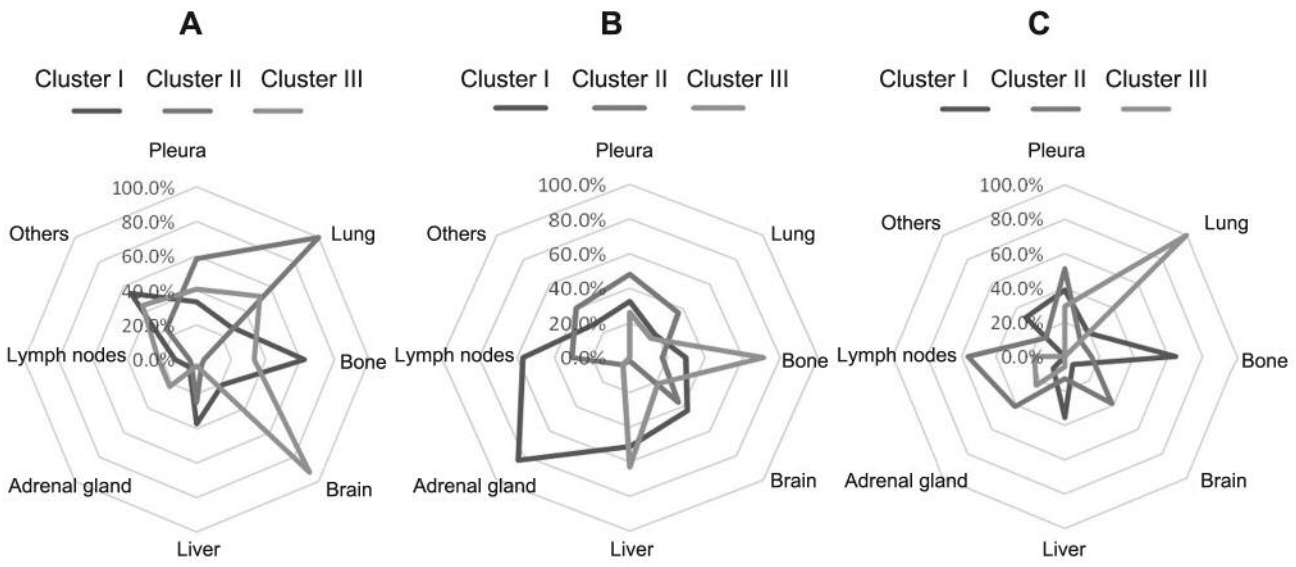

Figure 2. *Probability of having a metastasis within each cluster. (A) Lung adenocarcinoma patients with an epidermal growth factor receptor (EGFR) mutation, (B) Small cell lung cancer (SCLC) patients, and (C) squamous cell lung cancer patients.*

condition probability, we found that 'metastases do not occur randomly' (13). A large scale study of metastatic patterns examined 4,728 patients with adenocarcinoma from various organs, however, the study dealt only with autopsy records (27). Autopsy data may reflect metastatic lesions that are not readily detectable clinically, and therefore not relevant in terms of patient management (28).

Collection of data on 'clinically' meaningful metastases, such as through currently available images, and clinical symptoms (current or future symptoms) is important. Individual variation among patients adds further complexity to the study of metastatic behavior in malignant tumors. Yet, a robust understanding of metastatic patterns is of vital use to clinical case management and is significant for the field of cancer biology (14). Knowledge of metastatic patterns will improve efficiency in detecting metastases through imaging, and will enable effective treatment of metastatic sites, making better use of medical resources and reducing medical costs. It would also allow lung cancer patients to prepare for metastatic disease that may lower the quality of life, such as brain and bone metastasis.

Cluster analysis is one of the most useful mathematical methods, but a relatively novel analytical method in clinical oncology (29, 30). We used cluster analysis to evaluate the metastatic patterns of three distinctive groups of lung cancer patients and had three significant findings. Firstly, we found that metastatic patterns existed in each of the three target lung cancer groups. In light of these results, we must next evaluate 'metastatic patterns' in other, non-*EGFR* mutant adenocarcinomas, such as those with an anaplastic lymphoma kinase (ALK) fusion or lung adenocarcinomas of unknown driver mutation. Secondly, we found that different metastatic patterns did not generally associate with any difference in clinicopathological features. The only two exceptions were maximum diameter of the primary tumor in patients with SCLC and age at diagnosis in patients with squamous cell lung cancer. These results suggest that cluster analysis of metastatic patterns may provide new information independent to the established clinicopathological variables and may also contribute to personalized medicine. Thirdly, we clearly showed the statistical difference in metastatic patterns of the three lung cancer groups. The results of this study suggest that cluster analysis will be possible in other lung cancer patient groups and cancers other than lung cancer, advancing our current knowledge of distant metastases.

Although this study has provided novel information, there were certain limitations. Firstly, there was no pathological confirmation of distant metastases determined from diagnostic imaging methods. Secondly, although our study used consecutive patients with pathologically proven lung cancer, our small-scale patient base might not reflect the overall patient population in the community. Thirdly, this was a retrospective analysis of metastatic data available at the time of initial diagnosis. There was no evaluation of metastases that developed during the clinical course of the disease. We hypothesized that the results may differ if we also considered metastases that developed during the clinical course. Furthermore, the type of diagnostic imaging techniques performed and the intervals at which they

occurred may have generated caveats to our analysis. This study was not focused on the biological mechanism or microscopic evaluation of distant metastasis. Rather, we studied 'clinically' meaningful metastases, those found in currently available images, in patients with clinical symptoms (known at the time or subsequently). Data obtained from research approaches such as this will feed back to molecular biological studies on metastatic patterns.

Distant metastasis may occur non-randomly and we showed that there are specific patterns of distant metastasis in three subsets of lung cancer patients. These metastatic patterns, revealed by statistical analysis, suggest the progression of distant metastases involves more than the 'mechanical theory' (1) and 'soil-seed hypothesis' (3). Increased knowledge of specific metastasis patterns will improve individualized treatment.

Conflicts of Interest

The Authors have no conflicts of interest to declare regarding this study.

Authors' Contributions

HW, HS, and NH designed the study. HW, SO, HY, SS, KM, and TK collected the data. HW, SO and HS analyzed the data and prepared the manuscript. All Authors approved the final version of the article.

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