rapidly and then increased and became stable after the trainee had 12 puncture experiences. In contrast, the diagnostic rate in the post-threshold data increased until the trainee had 12 puncture experiences and then became stable (Fig. 3B). The accumulated histological sampling rates in the prethreshold and post-threshold periods for each threshold point are depicted as a curve in Fig. 3C and D. The curve of the prethreshold data (Fig. 3C) shows a gradual decline, whereas the curve of the post-threshold data appears to be almost even throughout the entire period (Fig. 3D). When the analysis is focused on the threshold point of 12 experiences (Table 4), the pooled diagnostic rates in the prethreshold and post-threshold periods were 85.4% and 93.9%, respectively ($p=0.027$), whereas pooled sampling success rates in the prethreshold and post-threshold periods were 89.6% and 83.5%, respectively ($p=0.22$). The average node sizes (short axis) in the prethreshold and post-threshold periods were 14.9 and 12.6 mm, respectively ($p=0.0024$).

Table 3 – Trainee success rate for each station (threshold=12).

Node station	N	Dx (+)	Hx (+)	Failure
Total				
Mediastinum				
#2R	8	100%	87.5%	0%
#4R	72	91.7%	81.9%	2.7%
#4L	30	73.3%	76.7%	16.7%
#7	97	91.8%	87.6%	2.1%
Hilar and peripheral				
#10L	3	100%	66.7%	0%
#11L	36	97.2%	86.1%	2.8%
#11s	35	94.3%	88.6%	5.7%
#11i	22	95.5%	90.9%	4.5%
#12	5	80%	100%	0%
Until 12 TBNA experiences				
Mediastinum				
#2R	2	100%	100%	0%
#4R	20	85%	85%	5%
#4L	5	80%	80%	20%
#7	44	81.8%	88.6%	4.5%
Hilar and peripheral				
#10L	0	0%	0%	0%
#11L	9	100%	100%	0%
#11s	10	100%	100%	0%
#11i	4	75%	75%	25%
#12	2	50%	100%	0%
After 12 TBNA experiences				
Mediastinum				
#2R	6	100%	83.3%	0%
#4R	52	94.2%	80.8%	1.9%
#4L	25	72%	76%	16%
#7	53	100%	86.8%	0%
Hilar and peripheral				
#10L	3	100%	66.7%	0%
#11L	27	96.3%	81.5%	3.7%
#11s	25	92%	84%	8%
#11i	18	100%	94.4%	0%
#12	3	100%	100%	0%

Dx (+): success of diagnosis, Hx (+): success of histological sampling.Failure: failure to perform TBNA (neither diagnosis nor histological sampling).

4. Discussion

4.1. *Cytological diagnosis*

In the diagnostic rate prethreshold and post-threshold curves (Fig. 3A and B), 12 experiences appeared to be a key value. The shape of the prethreshold curve changed from decreasing to increasing at this point, and the shape of the post-threshold curve nearly achieved a plateau beginning at this point. The threshold curve depicts all pooled trainee data; therefore, it represents an approximate generalized learning curve. In addition, we assume that the prethreshold curve represents accumulated progression of TBNA technique (Fig. 1), and the decreasing trend of this curve indicates that a fair percentage of trainees still fail to make proper diagnoses. Furthermore, the post-threshold curve represents a prediction of the future diagnostic rate according to our learning system (Fig. 1), and the increasing trend of this curve suggests that trainee skills can continue to improve. With these data in mind, we propose that 12 experiences appears to be (1) an inflection point prethreshold, where after this point, trainees seldom failed to make a correct diagnosis and could still improve their own diagnostic rates through further personal clinical experience, and (2) a changing point post-threshold period, where after this point, the curve become nearly constant, as does the trainees' development.

In our study, the diagnostic rates were unexpectedly high, even during the early training period. Based on these results, we speculate that target nodes were properly selected by the supervisors according to the acquisition level of each trainee. When the relationship between target node size and each prethreshold period was analyzed (Fig. 4), the nodal size tended to decrease as the number of experiences increased. Mean nodal size were 17.4 mm until 3 punctures and 13.7 mm until 42 punctures. As indicated in Fig. 4, for trainees with 12 or fewer experiences, relatively large nodes were punctured, which may have made diagnosis easier for the relative beginners. This bias of supervisor choice led to a more positive experience for each trainee in terms of providing an appropriate node suited for each step of the learning curve, and this bias was necessary to avoid excess pain in the patient and to adequately follow ethical guidelines. Thus, the number of EBUS-TBNA experiences affected the diagnostic rate.

4.2. *Histological sampling*

In contrast, the histological sampling rate (Fig. 3C and D) did not show that any particular trend was associated with the number of experiences. Even at the threshold point of 12 experiences, which was considered to be the threshold point for acquisition of satisfactory skill for diagnosis, the pooled histological sampling rates in the prethreshold and post-threshold periods were almost equal. Such results may have been due to nodal selection, since it is clear that small nodal size was a limiting factor for histological sampling. The average nodal sizes for which trainees could and could not obtain a histological core were significantly different (Table 2), and the average node short axis was significantly

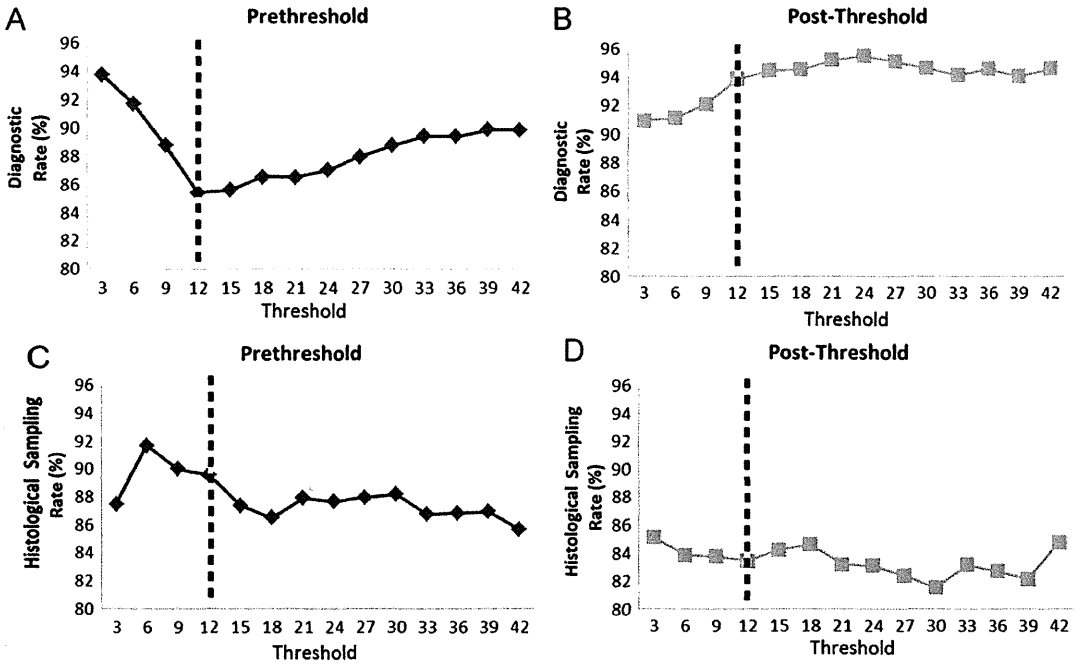

Fig. 3 – Diagnostic rate and histological sampling rate in the prethreshold and post-threshold periods. The diagnostic rates accumulated until the threshold point (prethreshold period; A), and those after the threshold point (post-threshold period; B) for each threshold value were plotted. When the threshold was set as 12 (dotted line), the prethreshold pooled data showed an accumulated rate that consisted of the results from the first puncture to the 12th puncture for all trainees, calculated from outcomes in the pooled database. The post-threshold pooled data then showed an accumulated rate consisting of the rest of the results, from the 13th puncture in the pooled database. The histological sampling rates accumulated until the threshold point (prethreshold period; C), and those after the threshold point (post-threshold period; D) for each threshold value were plotted.

Table 4 – Comparison of outcomes among training phases until and after 12 TBNA experiences.

	Until 12 experiences	After 12 experiences	p value
Pooled diagnostic rate	85.4%	93.9%	0.027
Pooled histological sampling rate	89.6%	83.5%	0.22
Average nodal diameter	14.9 mm	12.6 mm	0.0024

larger before the threshold point of 12 experiences (Table 4). Considering the larger node selection during the early phases of training, the consistency of the high histological sampling rate during the entire training period could not be negative in this training system.

4.3. *Evaluation of our method*

Kemp et al. established a TBNA learning curve using cusum analysis and reported a pooled sensitivity of 67.4% for 5 operators [15]. Almost all of the target nodes were 10 mm or more in their report, which is different from the node size in the present analysis. Small size (less than 10 mm) was a negative predictive factor; however, 10.3–20% of nodes less than 10 mm in size have been reported to contain malignant cells [16,17].

Steinfort et al. reported that diagnostic performance continued to improve even after 50 cases were performed [11]. This outcome differs from that of the present study, in which the learning curve peaked at 12 TBNA experiences. Shown et al. concluded that approximately 10 procedures were required for thoracic surgeons [10] and reported an overall 95.6% accurate diagnostic rate after 10 procedures. However, these data were calculated for only 1 board-certified thoracic surgeon. The pooled database in the present study consisted of the results from 11 EBUS-TBNA beginners, including a resident; therefore, these results are likely representative of a more general physician population.

4.4. *The issue for the #4L node*

The unsatisfactory puncture acquisition rate for the #4L node was due to its anatomical location, between the pulmonary artery and aortic arch, which often makes it difficult to determine an appropriate puncture route and can cause anxiety in the physician during the procedure. In addition,

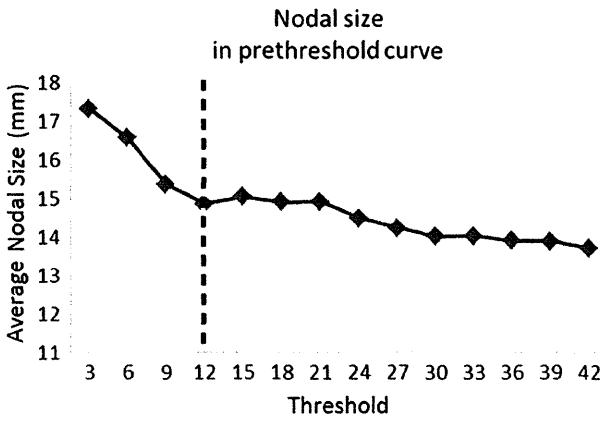

Fig. 4 – Change in target nodal size during the training period. Mean sizes of the target nodes during each prethreshold period.

this node was sometimes difficult to find with a dedicated needle inserted with a convex probe, even if the node could be viewed before insertion of the needle. We believe that both understanding the complete anatomical relationship between the node and surrounding structures, such as great vessels, and being able to convert these structures into a stereoscopic view from the airway are required to successfully puncture such "invisible" nodes. This requirement may make the #4L node a hurdle for beginners, but was relatively easy for thoracic surgeons since they have experience matching radiologic images and actual anatomy during surgery.

4.5. *Study limitations*

We recognize that the present study has some limitations. First, the results are based on only 1 puncture for each trainee. In general, 3 punctures per target node are recommended [18]. Performing multiple punctures using our learning system may have increased the diagnostic/histological sampling rate.

Second, to implement this system, at least 1 EBUS-TBNA expert is needed at the institution. In institutions that are just starting to perform EBUS-TBNA, the trainees must visit another institution that employs skilled EBUS-TBNA operators in order to master the 5 steps. This system can only guarantee the quality of each trainee according to the expertise of the supervisors and is therefore similar to an apprenticeship system or internship for EBUS-TBNA.

Third, this study was performed at a single institution. It is unclear whether the calculated threshold (12 experiences) could be generalized for other supervisors at other institutions. The quality and talent of supervisors are also important factors in this learning system.

Fourth, we did not implement a simulator. Stather et al. reported that using an EBUS simulator led to more rapid acquisition of skill when performing EBUS [12]. Such a simulator may reduce the length of steps 1–4 in our learning system. In Japan, local training courses (wet and dry lab) are held, and these EBUS-TBNA learning opportunities may also be useful to reduce the learning curve.

5. Conclusions

Our EBUS-TBNA learning system was nearly satisfactory with respect to diagnosis rates. Histological sampling from smaller nodes or from the #4L node remains an issue for beginners. Nevertheless, we believe that accessibility of EBUS-TBNA will improve through implementation of our learning system.

Conflict of interest

Authors have no potential conflict of interest.

Acknowledgments

All authors have read and approved the final version of this manuscript. Statistical analyses were revised by Dr. Yasunori Sato (Chiba University Hospital Clinical Research Center).

Other contributions

We thank Dr. Kazuhiro Yasufuku (Toronto General Hospital), Dr. Takahiro Nakajima (Toronto General Hospital), and Dr Taiki Fujiwara (Kimitsu Chuo Hospital) for contributions that helped to establish the previous EBUS-TBNA learning system at Chiba University Hospital.

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Official Journal of the Asian Pacific Society of Respirology
Respirology

ORIGINAL ARTICLE

Desquamative interstitial pneumonia may progress to lung fibrosis as characterized radiologically

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ABSTRACT

Background and objective: **In some patients, desquamative interstitial pneumonia may progress to lung fibrosis. The aim of this study was to assess the long-term radiological follow-up results in patients with desquamative interstitial pneumonia.**

Methods: **Among 75 patients suspected of having desquamative interstitial pneumonia, 31 who fulfilled the criteria were included in this study. Clinical characteristics at presentation, responses to treatment and long-term follow-up were evaluated.**

Results: **The 31 patients were predominantly males (94%), and the mean age was 55 years; 93% (28/30) had a history of smoking. The clinical findings included high serum levels of lactate dehydrogenase and immunoglobulin G. Bronchoalveolar lavage (26 patients, 84% of cases) frequently showed an increased percentage of eosinophils (mean 17%). Computed tomography (CT) or high resolution (HR) CT at presentation showed ground glass opacities and/or consolidation in all patients, with one third of patients also showing thin-walled cysts within the ground glass opacities. There was no honeycombing on CT or HRCT scans at presentation. Corticosteroid therapy was effective early in the course of the disease; long-term follow-up (mean 99 months) of 31 patients showed only one death due to progression of the disease, but long-term follow-up of 14 patients (mean 125 months) by HRCT showed the development of new thin-walled cysts and honeycombing in five and lung cancer in four patients, respectively.**

Conclusions: **In a proportion of patients, desquamative interstitial pneumonia may progress to lung fibrosis with honeycombing on HRCT, despite therapy.**

SUMMARY AT A GLANCE

A clinical study of patients with desquamative interstitial pneumonia was performed to assess the outcomes of long-term follow-up by high resolution computed tomography (HRCT). Of the 31 patients studied, 14 were followed-up for more than 60 months (mean 122 months). Five patients developed honeycombing on HRCT.

Key words: desquamative interstitial pneumonia, honeycombing, interstitial pneumonia, surgical lung biopsy.

INTRODUCTION

Desquamative interstitial pneumonia (DIP) was first reported by Liebow *et al.* in 1965.[1] In 1978, Carrington *et al.* compared usual interstitial pneumonia (UIP) with DIP, in a classic paper and showed that in contrast to UIP, DIP responded relatively well to corticosteroid treatment, which resulted in a good prognosis.[2] DIP is currently considered to be a different entity from UIP;[3] the latter is encountered in idiopathic pulmonary fibrosis (IPF), as defined according to the criteria of the international multidisciplinary consensus classification of idiopathic interstitial pneumonia (international classification)[3] and the more recent IPF

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Received 2 February 2012; invited to revise 17 March 2012, 4 May 2012; revised 9 April 2012, 8 May 2012; accepted 9 May 2012 (Associate Editor: Toby Maher).

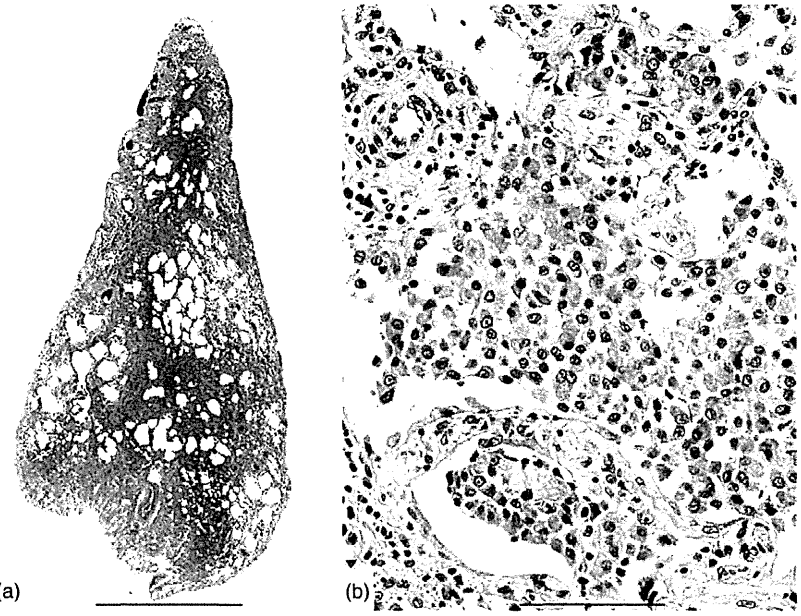

Figure 1 Histology of desquamative interstitial pneumonia (DIP). (a) Definitive DIP. The specimen shows diffuse, homogeneous involvement. Panoramic view; scale bar 5 mm; haematoxylin and eosin (HE) staining. (b) Large numbers of large eosinophilic macrophages in the alveoli with mild interstitial inflammation. ×300 magnification; scale bar 100 μm; HE staining.

guidelines.[4] There are relatively few long-term follow-up studies of DIP, and none of these evaluated the high-resolution computed tomography (HRCT) findings late in the disease. We have identified and analyzed the data for a relatively large number of patients with DIP in order to clarify some of these issues.

METHODS

This was a multi-institutional, retrospective study that was approved by the ethics committee of Saitama Prefectural Cardiovascular and Respiratory Center (number 2011008) where this work was performed. The committee did not require patient approval or informed consent for the retrospective review of medical records, pathology results and images. The study was also approved by the ethics committees at the other authors' and contributors' institutions, at which patients were treated. One of the authors (YK) identified 75 cases (48 from consultations and 27 newly diagnosed cases throughout Japan over 30 years) of suspected DIP that had been diagnosed by surgical lung biopsy together with clinical radiological and pathological correlation, although not all cases were diagnosed according to the most recent criteria.[3] Twenty-four cases have been reported previously;[5–11] most of those previous reports did not include the long-term clinical and radiological follow-up that was evaluated in this study.

Three pulmonary pathologists (YK, TT, AH), who were totally blinded to the clinical information, independently re-reviewed the pathology results for all 75 surgical lung biopsies, and used the following pathological criteria for a definitive diagnosis of DIP:[3] (i) homogeneous distribution, (ii) prominent accumulation of alveolar macrophages, (iii) mild to moderate fibrosis of alveolar septa, and (iv) mild interstitial chronic inflammation (including lymphoid aggregates) (Fig. 1). For patients in whom more than one site/lobe was biopsied, the diagnostic features were required to be present in both biopsies for the diagnosis to be definitive. When the diagnoses made by the three observers were in conflict, agreement between two of the three pathologists was used to obtain a consensus diagnosis. These criteria were fulfilled for 31 patients. Cases that did not meet one or two of these criteria were diagnosed as probable DIP (31 cases) and possible DIP (13 cases), respectively and were excluded from the analysis. A fourth pathologist (TVC) confirmed the diagnosis in all cases.

Histological evaluation of the surgical lung biopsies included assessment of the degree of interstitial fibrosis, which was estimated on the basis of the most severely affected lobule. Three grades of fibrosis were identified: mild with preserved lung structure; moderate with unclear lung structure due to fibrosis and/or formation of microscopic cysts (Fig. 2); and severe with dense fibrosis, smooth muscle proliferation and/or microscopic honeycombing.

The clinical information that was collected from the medical records included gender, age, smoking index (packs/day × years), laboratory data, bronchoalveolar lavage findings, therapy, effects of therapy and follow-up, including radiological follow-up. Four patients with other immunological disorders (see Table 1) were included, as were three patients with known collagen vascular diseases, because there were no histological differences between these and the other cases. One patient developed rheumatoid arthritis 4 years after the initial pathological diagnosis; this patient was previously reported as having idiopathic DIP.[5]

Chest radiographs, CT or HRCT scans of the lungs of these patients were independently assessed by two

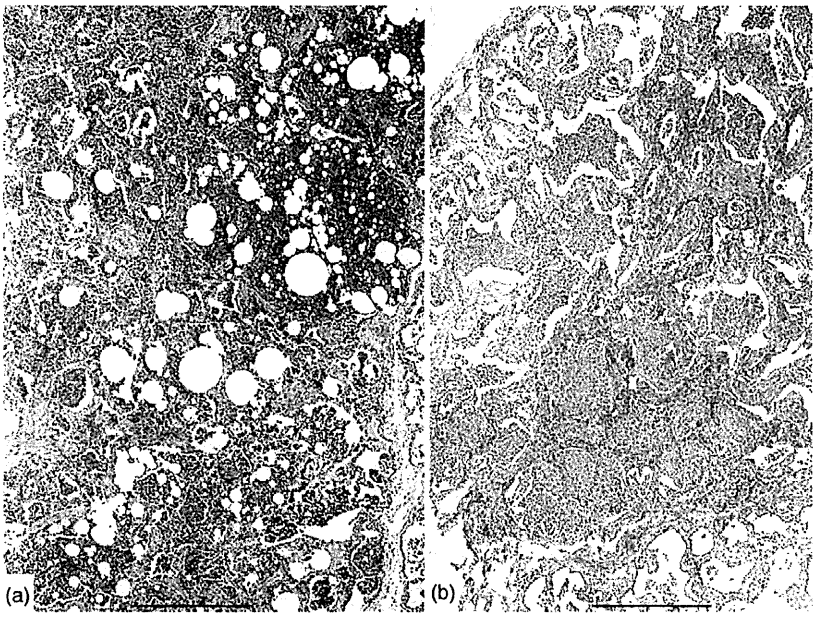

Figure 2 Histological grading of interstitial fibrosis. (a) Mild interstitial fibrosis in patients with definitive desquamative interstitial pneumonia (DIP). The alveoli were filled with eosinophilic macrophages and the degree of interstitial fibrosis was mild.×40 magnification; scale bar 1 mm; haematoxylin and eosin (HE) staining. (b) Moderate interstitial fibrosis in patients with definitive DIP. The alveoli were filled with eosinophilic macrophages and interstitial fibrosis was moderate, with unclear structure and mild cystic changes. ×40 magnification; scale bar 1 mm; HE staining.

Table 1 Clinical data at admission for the patients with desquamative interstitial pneumonia (DIP)

	Patients with DIP
Number (males : females)	31 (29:2)
Age range, mean ± SD	27–70, 55 ± 13
Associated CVD, number (type)	3 (SLE, SJS, RA)
Other organ specific immunological disorders identified	ITP, chronic thyroiditis, IgA nephropathy
Smoking, ratio (SI), mean ± SD	28/30 (93%), 52 ± 41
Laboratory data	
ESR mm/h, *n* tested, mean ± SD	22, 53 ± 42
High LDH levels, *n* tested, %	31, 52%
IgG, *n* tested, mean ± SD, % > 18000 mg/L	23, 21450 ± 6600, 74%
High IgE levels, *n* tested, % > 250 IU/mL	16, 44%
High KL-6 levels, *n* tested, % > 500 U/mL	14, 93%
BAL, *n* tested	26
Eosinophils, range, mean, median	0–62%, 17%, 11%
Neutrophils, range, mean, median	0–62%, 14%, 9%
Lung function and blood gas analysis	
%VC, *n* tested, mean ± SD	30, 84 ± 23
PaO_2, mm Hg, *n* tested, mean ± SD	31, 79 ± 11
X-ray and HRCT	
X-ray, *n* tested, % with predominant middle and lower lobe disease	30, 77%
HRCT, *n* tested, % with GGO	26, 88%
HRCT, % with cysts, % with honeycombing	38%, 0%
Degree of interstitial fibrosis by histology mild/moderate/severe, %	35/65/0

BAL, bronchoalveolar lavage; CVD, collagen vascular disease; ESR, erythrocyte sedimentation rate; GGO, ground glass opacity; HRCT, high resolution computed tomography; Ig, immunoglobulin; ITP, idiopathic thrombocytopenic purpura; LDH, lactate dehydrogenase; PaO_2, partial pressure of arterial oxygen; RA, rheumatoid arthritis; SI, smoking index; SJS, Sjogren's syndrome; SLE, systemic lupus erythematosus; VC, vital capacity.

pulmonary radiologists (FS, TK) and evaluated according to criteria reported previously;[12,13] the criteria for honeycombing were also based on a previous report.[13] Agreement between the two pulmonary radiologists was required.

When a patient showed significant radiological clearing of pulmonary opacities (by CT or HRCT) and/or an increase in partial pressure of arterial oxygen (PaO_2) of >10 mm Hg following therapy, this was defined as clinical improvement.[5]

Patients followed up for more than 60 months, either by lung function assessment and blood gas determinations or by CT/HRCT, were assessed differently because each subgroup was different.

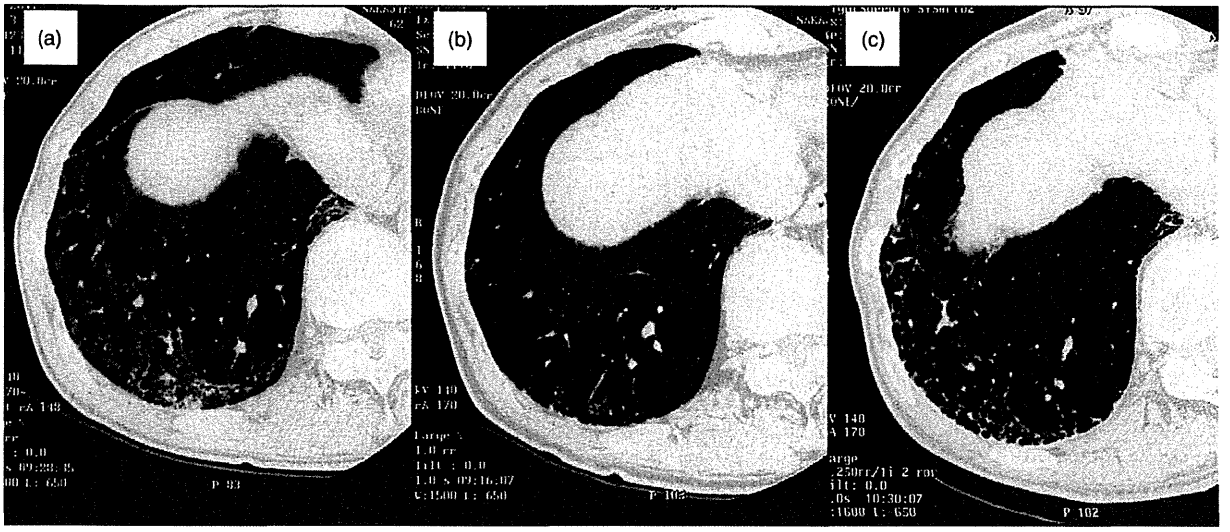

Figure 3 Follow-up high resolution computed tomography. (a) At admission, extensive ground-glass opacities (GGO) with thin-walled cysts were seen in the peripheral area of the right lower lobe. (b) Two years later, there was a marked decrease in GGO, but the presence of thin-walled cysts was suspected. (c) Eight years later, honeycombing shadows had newly appeared in the peripheral region.

RESULTS

Clinical and pathological data before therapy

Thirty-one cases were selected; 20 of these have been reported previously,[5,6,8] but long-term data were not included in the previous reports. The patients were predominately males (29:2) and middle aged (mean age 55 years) (Table 1). The proportion of patients who smoked was high (28/30, 93%), and the smoking index was also high (mean 52). Many patients had moderately increased erythrocyte sedimentation rates (53 mm/h), lactate dehydrogenase (LDH) levels and IgG levels (mean 21 400 mg/L). KL-6 levels were also increased. Analysis of bronchoalveolar lavage showed increases in the percentages of eosinophils (range 0–62%, mean 17%) and neutrophils (range 0–62%, mean 14%). These data are similar to those reported previously.[5]

Chest radiographs at diagnosis showed bilateral ground glass opacities (GGO), with or without consolidation, and with predominant involvement of the middle and lower lung fields (77%). CT and HRCT images at diagnosis showed a high frequency of GGO with or without consolidation (88%), and the percentage mainly with consolidation was 12%. The frequency of tiny (2 to 4 mm in diameter), thin-walled cysts that formed within the regions of GGO was 10/26 (38%). No honeycomb changes were identified. These data are also similar to those reported previously.[5] Chest radiograph and CT/HRCT features at presentation were interpreted as being typical of DIP.

Histological analysis showed that surgical lung biopsies taken at multiple sites frequently showed differences in the degree of interstitial fibrosis and variation in the degree of fibrosis from lobule to lobule. In general, the subpleural regions of lower lobe biopsies tended to show the greatest fibrosis of alveolar walls which, overall, was mild in 35% of patients and moderate in 65%. No histological honeycombing was seen. Thin-walled cysts, as noted radiologically, were observed in one (4%) of the patients with mild interstitial fibrosis, as assessed histologically, and in nine (34%) of the patients with moderate interstitial fibrosis, as assessed histologically.

Effects of therapy and follow-up

Pulse corticosteroid and/or oral corticosteroid therapy (15 to 80 mg, mean 38 ± 19 mg, initial dose for 2 to 4 weeks with a gradual decrease in dose) were effective in 90% of cases and lead to clinical improvement as defined previously (Fig. 3a,b) (Table 2). Only six patients developed new shadows, and one of these showed repeated new shadows despite corticosteroid therapy. The changes in % vital capacity (VC) among the three patients who could be examined were 44 to 65%, 102 to 88% and 58 to 102%. Seventeen patients stopped smoking before or after therapy. The average follow-up period was 99 months from diagnosis. During the follow-up period, one patient died due to progression of DIP, one patient died due to lung cancer, one patient died due to fulminant lung disease (post lobectomy), with diffuse alveolar damage, histologically and four patients died due to unrelated conditions. Three patients had undergone lobectomies for lung cancer; two are currently alive, and one patient died due to postoperative acute respiratory failure as stated previously. A Kaplan–Meier survival curve indicated that the 10-year survival rate was 78% (Fig. 4).

Table 2 Effects of therapy and follow-up data for patients with desquamative interstitial pneumonia (DIP)

	Patients with DIP
Favourable effects of corticosteroid therapy, *n*, %	30, 97%
Duration of follow-up after diagnosis, *n*, months	31, 33–165
Outcome, DOD, DORD, DOOD	1, 2 (lung cancer, DAD), 4
Occurrence of lung cancer, *n*	4 (3 lobectomy, one death)

DAD, diffuse alveolar damage; DOD, died due to disease; DOOD, died due to other disease; DORD, died due to related disease.

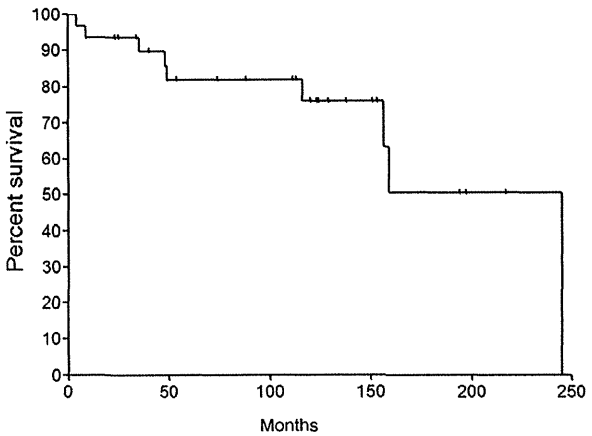

Figure 4 Kaplan–Meier survival curve for patients with desquamative interstitial pneumonia. The 10-year survival rate was 78%.

Long-term follow-up data on lung function and CT and HRCT

The pulmonary function test results and blood gas determinations at long-term follow-up are shown in Table 3. The mean %VC was mildly increased from 82 to 94%; there were no changes in $\%FEV_1$ or partial pressure of arterial oxygen (PaO_2).

Fourteen patients had follow-up CT or HRCT assessments at a mean of 125 months. Four patients had thin-walled cysts at presentation and new thin-walled cysts developed in an additional five patients, giving a total of nine of 14 patients (65%) with thin-walled cysts at the last evaluation. Honeycombing (Fig. 3c) developed in five patients (without extension of lung shadows in three patients and no elevation of the diaphragm in four patients); two of these patients had thin-walled cysts at presentation, and four of these patients were among those with thin-walled cysts at the last follow-up. New thin-walled cysts and honeycombing appeared in patients with both grade 1 and grade 2 interstitial fibrosis, as assessed by histology of the initial diagnostic biopsies. Long-term follow-up changes in %VC among the three patients who developed honeycombing were: 58 to 102%, 109 to 125% and 92 to 82%.

There were no differences among those patients with collagen vascular disease, those with immunological disorders, and the other patients, with respect to clinical features (the former had slightly high erythrocyte sedimentation rates and IgG levels), the effect of therapy or long-term follow-up data.

DISCUSSION

This study has shown that long-term HRCT follow-up of 31 patients with DIP identified an increase in the frequency of tiny, thin-walled cysts in GGO, and the new appearance of honeycombing in five of 14 cases. These findings suggest progression of lung fibrosis in some patients, but interestingly this finding was not accompanied by a decrease in %VC. Long-term follow-up of lung function and blood gas measurements in limited numbers of patients indicated no deterioration. Four of the patients who were followed-up long-term developed lung cancer.

The clinical features of the patients in this series included many of those identified in a previous report,[5] and were characterized by inflammatory reactions such as increased erythrocyte sedimentation rates, high IgG, LDH and KL-6 levels and increased percentages of eosinophils (mean 17%) and neutrophils in bronchoalveolar lavage. Corticosteroid therapy was effective, with 90% of patients showing a response.

The frequency of thin-walled cysts on CT or HRCT at presentation was 10/26 cases (38%, Table 1); thin-walled cysts were identified at presentation in four of 14 cases and at last follow-up in nine of 14 cases (65%) (five new appearance cases) (Table 3). The presence of thin-walled cysts is characteristic, if not specific, for DIP.[5,12,14]

Honeycombing that was not observed at presentation, was identified at follow-up in five patients (36%), three of whom had thin-walled cysts at presentation. This study showed that DIP can progress to radiological fibrosis in one third of patients. Carrington *et al.* suggested that fibrosis and honeycombing were non-specific features of both UIP and DIP.[2] Previous reports also suggested that DIP might progress to diffuse pulmonary fibrosis, honeycombing or UIP,[15–17] although the definitions of UIP and DIP used in these reports differed from that of the international classification,[3] and there was no concept of non-specific interstitial pneumonia[3] at that time. Several studies, performed both before and after the international classification[3] was formulated, have confirmed the appearance of honeycombing during follow-up of patients with DIP.[18,19] This study has included the longest follow-up, to date, to assess the new appearance of honeycombing in patients with DIP, and this was identified in five of 14 patients (36%). Further studies are necessary to determine whether honeycombing in this setting carries the same prognostic implications (e.g. more aggressive disease) that it does in the setting of IPF.

Table 3 Follow-up data for lung function, PaO_2 and HRCT features, including presence and new appearance of thin-walled cysts and honeycombing at more than 60 months

Lung function and PaO_2				
	Duration of follow-up, range, mean (months)	Number of patients	At diagnosis	At last follow-up
%VC	60–209, 94	11	82%	94%
$\%FEV_1$	60–209, 116	10	78%	77%
PaO_2	60–209, 110	9	82 mm Hg	85 mm Hg
HRCT features				
	Duration of follow-up, range, mean (months)	Number of cases	At diagnosis	At last follow-up
Thin-walled cysts	66–187, 125	14	4/14 (29%)	9/14 (65%)
Interstitial fibrosis grade 1/grade 2			0/4	3/2
Honeycombing	66–187, 125	14	0	5/14 (36%)
Interstitial fibrosis grade 1/grade 2				2/3

FEV_1, forced expiratory volume in 1 s; HRCT, high resolution computed tomography; PaO_2, partial pressure of arterial oxygen; VC, vital capacity.

Limitations of this study were the absence of follow-up of pathological findings and lack of continued monitoring of lung function.

Based on the present findings, we propose a sequence of radiological changes that may take place over time in patients with DIP (Fig. 5). Radiologically, GGO or consolidation may gradually progress to GGO with thin-walled cysts, and some of these may progress to honeycombing (end-stage fibrosis). Although the number of patients was limited, this study suggests that progression to radiological fibrosis appears to correlate with the degree of histological fibrosis at presentation.

In these patients, %VC increased slightly during long-term follow-up, and this was noted even in two of three patients with honeycombing. The apparent discrepancy between improvement in %VC and progression of radiological lung fibrosis (honeycomb formation) could not be explained, but is intriguing, even though the number of patients was very small. This finding contrasts with the natural history of IPF and may be another distinct feature of DIP that warrants further study. Four patients developed lung cancer. The presence of both lung fibrosis and a history of smoking may increase the risk of this occurrence.[20]

Most investigators report that the prognosis for patients with DIP is relatively favourable, particularly in comparisons to patients with UIP. The data from several studies is shown in Table 4.[2,19,21] The 10-year survival rate was 78% in the present study. The relatively high incidence of lung cancer in this small series could reflect the long period of follow-up.

Honeycombing in patients with DIP was not associated with extension of lung shadows in three patients or elevation of the diaphragm in four patients, as assessed by chest X-ray, and did not correlate with a decrease in %VC in two of three patients. Honeycombing in DIP may have a different implication from that in IPF, but the number of patients in the present study was too small to reach any definitive conclusions.

In conclusion, DIP has a chronic course, and generally shows a favourable response to corticosteroid therapy. Over time, some patients progress to fibrosis with honeycomb changes on CT.

ACKNOWLEDGEMENTS

The submission of cases including follow-up data by the following Japanese clinicians is acknowledged: Dr H. Ukita (KKR Sapporo Medical Center), Dr Y. Fukusima (Dokkyou Medical University), Dr R. Saito (AYNHO Nishi-Gunma Hospital), Dr S. Tsuchiya (Tsuchiya Clinic), Dr S. Koyama (Jichi Medical University), Dr N. Hata (Kawaguchi Municipal Medical Center), Dr T. Endo (NHO Mito Medical Center), Dr T. Saito (NHO Ibarakihigashi National Hospital), Dr Y. Horie (Matudo Municipal Hospital), Dr M. Yatomi (Public Chousei Hospital), Dr K. Asano (Keio University Hospital), Dr T. Uekusa and Dr T.Nagaoka (Juntendo University School of Medicine), Dr Y. Terasaki (Nihon Medical School), Dr K. Nomura (Tokyou Jikeikai Medical School Third Hospital), Dr N. Kobayashi (National Centre for Global Health and Medicine), Dr T., Ando (Japanese Red Cross Medical Centre), Dr T. Yamaguchi and H Amano (Japan Railway Tokyo General Hospital), Dr H. Tokuda (Shakai Hoken Central Hospital), Dr S. Akagawa (NHO -Tokyo Hospital), Dr T. Uchiyama (Double Red Cross Hospital), Dr S. Yoshikawa (NHO-Chusin-Matumoto Hospital), Dr T. Ishizaki (Fukui University Hospital), Dr Y. Yoshida (Numazu Municipal Hospital), Dr Y. Noda (Municipal Toyohashi Hospital), Dr Y. Hasegawa (Nagoya University School of Medicine), Dr Y. Taguchi (Tenri Hospital), Dr T. Kawamura (NHO-Himeji Medical Center), Dr Y. Tomioka (Kobe City Medical Centre West Hospital), Dr M. Kataoka and Y. Tanimoto (Okayama University Medical School), Dr T. Kishimoto (Okayama Rousai Hospital), Dr Nakanishi and Dr Y. Ohtsuki, (Ehime Prefectural Central Hospital), Dr T. Ogino (Takamatu Red Cross Hospital), Dr

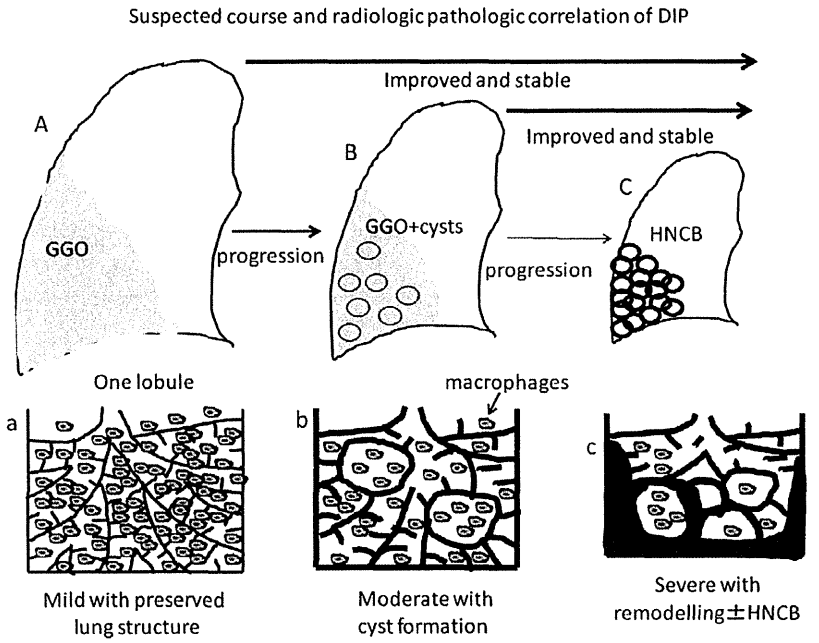

Figure 5 Suspected course of desquamative interstitial pneumonia (DIP) after treatment and radiological pathological correlations in DIP. (A) Patients with early stage DIP have ground-glass opacities (GGO) or radiological consolidation and most show improvement and become stable with corticosteroid therapy. Untreated DIP progresses. (B) Patients with the next stage of DIP have thin-walled cysts in GGO or consolidation. Most show improvement and become stable with corticosteroid therapy. Untreated DIP progresses. (C) Limited DIP progresses to end-stage fibrosis, with or without honeycombing (HNCB). Histologically, (A) corresponds to mild interstitial fibrosis with preserved lung structure and numerous macrophages (a). The next stage, corresponding to (B), is moderate interstitial fibrosis with frequent cystic changes (b). The final stage, corresponding to (C), is severe fibrosis with structural remodelling, with or without HNCB and decreased numbers of macrophages.

Table 4 Comparative data on survival of patients with desquamative interstitial pneumonia (DIP)

Publication	Number of patients	Duration of follow-up, years	Survival rate
Carrington *et al.*[2]	40	Died, mean 12.2 Survivors, mean 9.1	10-year: 72.5%
Craig *et al.*[19]	Smokers 8	Mean 8.8 (range 1.5–19)	100%
	Non-smokers 7	Mean 7.0 (range 1.5–17)	100%
Travis *et al.*[21]	16	Not stated for patients with DIP	100%
Present study	31	Mean 8.2	10-year: 78%

Y. Ishimatu (Nagasaki University Hospital), Dr K. Wakamatsu and M. Kawasaki (NHO-Ohmuta Hospital), Dr H. Ishii (Oita University Hospital), Dr S. Mizunoe (Oita Prefectural Hospital), Dr H. Ichiyasu (Kumamoto University Hospital), and Dr H. Iwagoe (Ohmuta-tenryou Hospital). The authors also thank T. Shimizu, MD, for help in endorsing data and conclusions, and K. Kurashima, MD, for providing the Kaplan–Meier survival curve.

The Desquamative Interstitial Pneumonia Study Group includes Kingo Chida, MD, PhD (Second Division, Department of Internal Medicine, Hamamatsu University School of Medicine, Hamamatsu, Shizuoka); Akihiko Yoshizawa, MD, PhD (Department of Laboratory Medicine, Shinshu University Hospital, Matsumoto, Nagano); Koichiro Tatsumi, MD, PhD (Department of Respirology, Graduate School of Medicine, Chiba University, Chiba) and Ichiro Yamadori, MD, PhD (Department of Laboratory Medicine, NHO Okayama Medical Center, Okayama), who submitted also four cases and reviewed this article.

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Molecular mechanisms of lung-specific toxicity induced by epidermal growth factor receptor tyrosine kinase inhibitors (Review)

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Received May 17, 2012; Accepted August 14, 2012

DOI: 10.3892/ol.2012.872

Abstract. Lung-specific toxicity induced by epidermal growth factor receptor (EGFR) tyrosine kinase inhibitors (TKIs) for the treatment of non-small cell lung cancer (NSCLC) has emerged as a critical side-effect. Although the clinical features of the pulmonary side-effects of TKIs have been characterized, the details of the molecular mechanisms in the development of this lung-specific toxicity remain to be elucidated. EGFR-dependent epithelial regeneration and restoration plays an important role in the recovery process from lung injury. The lung comprises a unique environment where epithelial cells are exposed to internal agents in the systemic circulation and to airborne particles through the mouth and nose. This unique environment may also be associated with the development of lung-specific toxicity induced by EGFR-TKIs. Therefore, the aim of this review was to provide further insight into the molecular mechanisms of lung-specific toxicity in the context of treatment with EGFR-TKIs.

Contents

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Key words: epidermal growth factor receptor, tyrosine kinase inhibitors, lung fibrosis

1. Introduction

The lung-specific toxicity induced by epidermal growth factor receptor (EGFR) tyrosine kinase inhibitors (TKIs) for the treatment of non-small cell lung cancer (NSCLC) has emerged as a critical side-effect (1-7). Although the clinical features of the pulmonary side-effects of TKIs have been characterized (8-10), the details of the molecular mechanisms in the development of this lung-specific toxicity remain to be elucidated. The aim of this review was to provide further insight into the molecular mechanisms of lung-specific toxicity in the context of treatment with EGFR-TKIs.

2. Tyrosine kinase inhibitors (TKIs)

Recent advances in molecular targeted therapy have demonstrated improved response rates and progression free survival, particularly with TKIs which may act on the EGFR, in NSCLC patients (11,12). Tyrosine kinases are enzymes that transfer the terminal phosphate from ATP to tyrosine residues in order to activate them, and the signaling pathways through receptor tyrosine kinases (RTKs) have been demonstrated to play a role in cancer development. Therefore, different types of TKIs have been developed and used in the treatment of cancer. EGFR-TKIs were adapted for lung cancer treatment since EGFR is frequently overexpressed and occasionally mutated in NSCLC cells (13-15).

There are two types of TKIs: non-receptor TKIs and receptor TKIs. The former TKIs bind to the active site of a non-receptor tyrosine kinase to prevent phosphorylation. Imatinib is a non-receptor TKI, which is used for the treatment of chronic myelogenous leukemia (CML), gastrointestinal stromal tumors (GISTs) and other diseases (16). The latter TKIs are able to bind to the active site of a RTK and some may exhibit a selective inhibitory effect on a certain RTK. Erlotinib and gefitinib are selective inhibitors of the EGFR tyrosine kinase domain. Erlotinib is used for the treatment of pancreatic carcinomas and NSCLC, while gefitinib is used for the treatment of NSCLC (9). A number of TKIs targeting several cancers have been approved for clinical use, and numerous newly developed TKIs are currently undergoing clinical trials (17).

Lung-specific toxicities induced by treatment with TKIs are rare (8). However, lung fibrosis associated with

EGFR-TKIs is the most prominent in specific toxicity (9). The incidence of lung fibrosis ranges from 0.2 to 1.1% in erlotinib-treated patients and from 0.38 to 2.0% in gefitinib-treated patients (9). Additionally, a higher incidence of gefitinib-induced lung fibrosis is observed in Japanese patients (1). The predictive risk factors for the development of lung fibrosis include male gender, smoking history and antecedent lung fibrosis (4).

Randomized controlled trials (RCTs) of a new TKIs (originally BIBF 1120) assessing lung fibrosis were recently launched (18). This TKI is a per os active fibroblast growth factor receptor (FGFR), vascular endothelial growth factor receptor (VEGFR) and platelet-derived growth factor receptors (PDGFR)-TKI that is supposed to be effective against lung fibrosis since certain tyrosine kinase pathways have been demonstrated to be included in the development of lung fibrotic lesions (18). However, lung fibrosis is one of the adverse effects induced by an active selective EGFR-TKI for the treatment of NSCLC (8-10). These results indicate that only the EGFR pathway in the tyrosine kinase activities may account for the lung fibrosis in the patients treated with these drugs. Additionally, the EGFR-dependent pathway may be essential for the maintenance of the lung parenchyma consisting of the alveolar epithelium.

3. The pathogenesis of lung fibrosis and epidermal growth factor receptor (EGFR)

A unifying mechanism that is able to completely explain all fibrotic lung diseases remains to be elucidated. The original hypothesis regarding the pathogenesis of lung fibrosis indicated that a chronic inflammatory process may activate the fibrotic response through an anti-inflammatory mechanism disorder and a persisting exposure to an injurious antigen, thus resulting in the migration of hyperproliferative fibroblasts and subsequent production of the extracellular matrix (19). However, current evidence suggests that the sequentially injured and abnormally activated alveolar epithelial cells (AECs) are sufficient to drive the fibrotic response. These injured AECs release certain types of mediators, including transforming growth factor-β, which are able to induce the formation of fibroblast foci through the epithelial to mesenchymal transition (EMT) as well as the proliferation and transformation of residential mesenchymal cells (20).

EGFR, a cell surface receptor, is activated by binding to ligands, including the epidermal growth factor and transforming growth factor α (TGF-α). Activated tyrosine kinase through the EGFR regulates cell growth, apoptosis and differentiation (21). EGFR is a member of the ErbB family, which includes EGFR (ErbB-1), HER2/c-neu (ErbB-2), Her 3 (ErbB-3) and Her 4 (ErbB-4) (22). Previous studies have demonstrated that EGFR and TGF-α played an important role in the development of lung fibrosis (23,24). Alveolar type II cells in fibrotic lung tissues express higher levels of EGFR in comparison to cells in normal lung tissue, thus resulting in focal hyperplasia of alveolar epithelial cells. This indicates that epithelial regeneration through the EGFR-mediated pathways may be a potential mechanism for recovery from lung injury (25,26). Therefore, EGFR inhibition induced by EGFR-TKIs is suggested to impair the ability of the type II cells to proliferate in order to regenerate the epithelial cells and augment lung fibrosis. TGF-α is a ligand that binds to EGFR and functions as a mitogen, which encourages cultured epithelial cells, fibroblasts and endothelial cells to proliferate. TGF-α has protective roles against lung injury, which include the attenuation of inflammation and the reduction of pulmonary edema (24). The blockade of EGFR-dependent phosphorylation by EGFR-TKIs inhibits EGFR-mediated signaling, resulting in the impairment of these protective roles by TGF-α and the exacerbation of lung fibrosis (27).

4. Lung metabolic function

The lung is extensively affected by exposure to internal agents in the systemic circulation as well as exposure to airborne particulates through the mouth and nose. Pulmonary vasculature receives the entire cardiac output. Similar to the liver, the lung is a metabolic organ containing chemical-metabolizing enzymatic systems, including P450 enzymes. Therefore, circulating agents, including toxicants, may be extracted from the plasma and become concentrated in the lung (28). Exposure to airborne particulates, including viruses, bacteria, vapors, fumes, dusts, gases and mists, are supposed to cause particulates-related lung diseases, e.g., lung fibrosis.

Although the average concentrations of P450 enzymes in the lung have been identified to be lower compared with that in the liver, alveolar type II epithelial cells and Clara cells appear to possess a higher P450 enzyme concentration (29). The high concentration of these enzymes in certain lung cell types appears to accelerate P450-dependent bioactivation, which subsequently generates highly toxic metabolic products. Lung exposure to these toxicants is able to induce epithelial injury, which may be associated to the development of lung-selective toxicity (29,30).

Pyrrolizidine alkaloids (i.e., monocrotaline), included in various plants belonging to the *Crotalaria* and *Sencicio* genera, cause pulmonary hypertension in animal models (31). Pyrrolizidine alkaloids have been demonstrated to transit to alkylating agents through P450-dependent bioactivation in the liver, which is transported via the systemic circulation to the lung vasculature and may induce pulmonary arterial hypertension as well as pulmonary vascular remodeling (32). Similar to pyrrolizidine alkaloids, EGFR-TKIs are metabolized by P450 3A4 enzymes, which are predominantly present in the liver (33). Therefore, it is possible that toxic metabolic products from these TKIs, through P450-dependent bioactivation in the liver, may be transported to the lung and induce direct lung epithelial injury. Gefitinib and erlotinib are EGFR-TKIs and have similar chemical backbone structures. However, patients treated with gefitinib have a higher incidence of TKI-induced lung fibrosis compared with those treated with erlotinib (9). Gefitinib has been demonstrated to be more susceptible to P450-mediated metabolism in liver compared with erlotinib (34), which may contribute to the higher concentration of toxic metabolic products from gefitinib than erlotinib in the lung. The different susceptibilities between both TKIs to the liver metabolizing enzymes may therefore explain the different incidence of TKI-induced lung toxicity in the patients treated with gefitinib and erlotinib.

5. Conclusion

Patients with any coincidental interstitial pneumonia, epithelial regeneration and restoration of the barrier function through EGFR-dependent epithelial cell proliferation may be stimulated by continuous unknown epithelial injury. Patients with predictive risk factors for the development of lung fibrosis (4) may have a reduced ability to regenerate and restore epithelial cells due to a poor functional status from cancer development. A decrease in pulmonary epithelial cell regeneration through the blockade of EGFR-dependent phosphorylation may play an important role in the development of EGFR-TKIs lung-selective toxicity.

The unique environment of the lung, where epithelial cells are exposed to internal agents and airborne particulates, may induce and/or accelerate the lung-specific toxicity induced by EGFR-TKIs.

Acknowledgements

This study was supported by research grants from the Respiratory Failure Research Group and the Cardiovascular Diseases Research Group (9-19,22-33) from the Ministry of Health, Labor and Welfare (Japan), and a Grant-in-Aid for Scientific Research (Category C; Grant No. 22590851) from the Japanese Ministry of Education and Science, and the Takeda Science Foundation.

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INTERNAL MEDICINE

□ PICTURES IN CLINICAL MEDICINE □

Metastatic Gastric Cancer from Squamous Cell Lung Carcinoma

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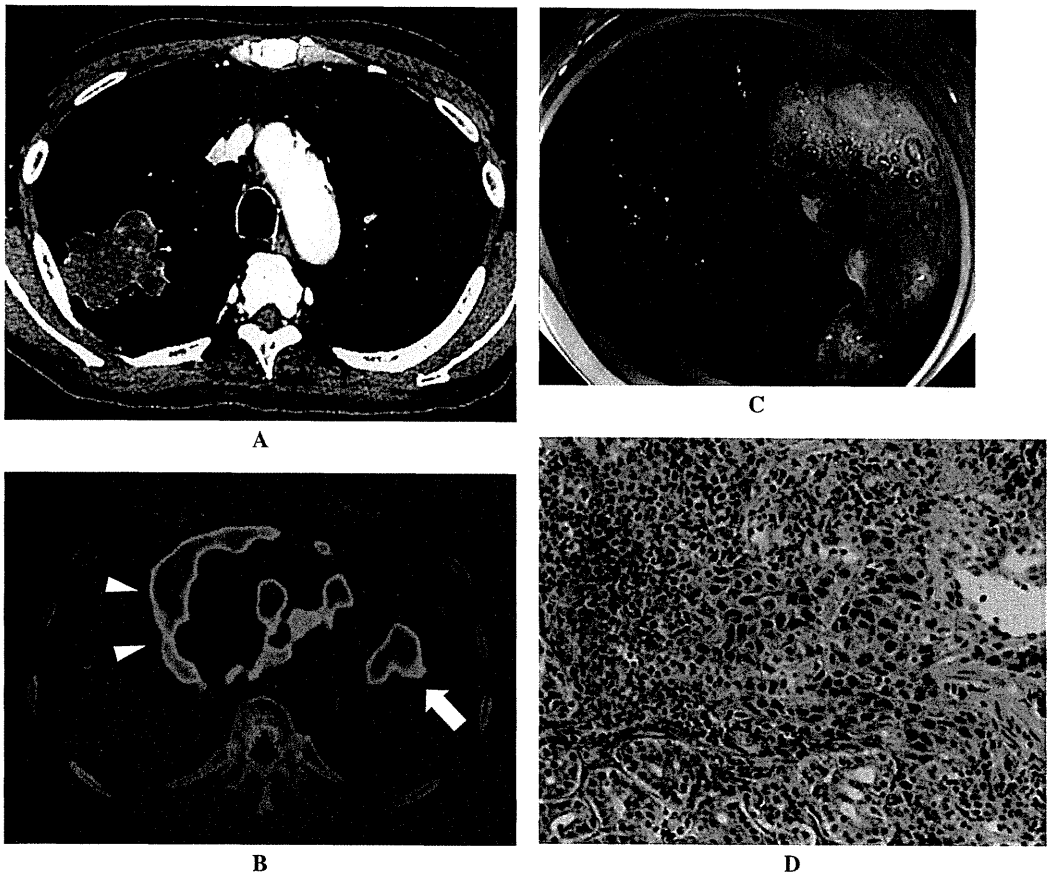
Key words: gastric metastasis, lung cancer, squamous cell carcinoma, Fluoro-2-deoxyglucose positron emission tomography (FDG-PET)/CT scanning

(Intern Med 51: 1947-1948, 2012)
(DOI: 10.2169/internalmedicine.51.7597)



Picture

The patient was a 73-year-old man who had primary lung carcinoma with multiple metastases. Chest computed tomography (CT) showed a solitary mass in the right upper lobe (Picture A), enlarged lymph nodes in the mediastinum and a liver tumor. Fluoro-2-deoxyglucose positron emission tomography (FDG-PET)/CT scanning demonstrated FDG uptake in the stomach (Picture B, arrow) as well as in the lung tumor on the right upper lobe, and in mediastinal

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Received for publication February 24, 2012; Accepted for publication April 24, 2012
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lymph nodes, the liver tumor (Picture B, arrow head) and ilium bone. Pathological examination of the bronchoscopic specimen showed squamous cell carcinoma. Gastroscopy showed a single large ulcer at the anterior wall of the gastric corpus (Picture C). Pathologic examination revealed squamous cell carcinoma suggestive of metastatic lung cancer (Picture D).

Gastric metastasis from lung carcinoma is rare (1). Metastases of other organs often coexisted at the time of discovery of gastric metastasis, therefore the prognosis is poor (1). This case suggests that FDG-PET may be a useful diagnostic tool for metastatic gastric cancer (2).

The authors state that they have no Conflict of Interest (COI).

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医学と医療の最前線

混合性結合組織病の診断と治療の進歩

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日本内科学会雑誌　第101巻　第5号別刷

2012年5月10日

などからその疾患独立性が再認識され，近年では英語文献にもしばしばこの疾患名が見られるようになった．我が国では1982年に厚生省の特定疾患に指定され調査研究班が結成された．さらに1993年には特定疾患治療研究対象疾患に指定され，医療費公費負担の対象疾患となった．このため第一次医療機関や患者間でMCTDの病名は広く知られ定着してきた．さらに学会などでも関心が高く，その自然経過などに関する成績も集積されてきた．このため我が国ではMCTDが独立疾患であることが以前から認識されている．

2. MCTDの疫学と診断の手引き

MCTDは前述のごとく特定疾患の一つに認定されており，その個人調査票を基準とした調査では平成22年におよそ9,000名の登録が確認されている．女/男比はおよそ15/1と圧倒的に女性に多い疾患である．年齢分布は40歳代が最も高頻度で，平均年齢は45歳である．また推定発症年齢はこれより10年若く平均36歳で，30歳代の発症の頻度が最も高い．診断については，いくつかの診断基準が提唱されている．その中で我が国の厚生省研究班により1988年に作成された「疫学調査のための診断の手引き」は国際的にも評価されていて最も普遍的である．1996年に抗U1-RNP抗体以外の自己抗体についてより明確化するよう改訂された．さらに2004年に改訂され，共通所見にあげられている2所見(Raynaud現象，指ないし手背の腫脹)に肺高血圧症が加えられ，この3所見のうち1所見以上が陽性であることを診断の必須条件としている[1](表)．

3. 合併症としての肺高血圧症について

1) 膠原病と肺高血圧症(pulmonary hypertension：PH)の関係

膠原病の分野でPHが注目されたのは，MCTDにおける死因の分析による．つまり従来予後良好と考えられていたMCTDの第一の死因がPHであったことによる．このため膠原病全般におけるPHの頻度について検討がなされた．

厚生省のMCTD調査研究班が1998年に世界に先がけて全国疫学調査を行った[2]．それによると，MCTD1,651例中PH合併例は83例(5.02%)，SLEで9,015例中82例(0.90%)，SScで3,778例中100例(2.64%)，PM/DMで3,349例中19例(0.56%)にみられた．特発性肺動脈性肺高血圧症（idiopathic pulmonary arterial hypertension：IPAH)の一般人口における有病率が100万人あたり1～5人であることを考えると，この膠原病四疾患のPH合併率は著しく高いことがわかる．これらの数字は主治医がPHと診断した例，つまり臨床所見の見られた症例である．これ以外に臨床徴候のないPHが隠れている可能性も考えられたため，2003年に厚労省の別の班で，症状の有無にかかわらず無作為に抽出した膠原病患者にPHの検索を行った[3]．するとMCTDで16.0%，SLEで9.3%，SScで11.4%にPHがみられ，臨床徴候のあるPHと同数かそれ以上の無症状のPHの症例がみられた．これはPHを疑う徴候の見られない膠原病患者でもその検索の必要なことを示唆している．我が国のこれらの成績は，北米における同様の調査[4]でも裏付けられた．すなわち北米50施設の検討によると，MCTDでは主治医診断で11.7%，心エコー検査では19.1%，SScでも主治医診断で18.9%，心エコー検査で27.7%にPHがみられた．これら日本と北米の成績はいずれも心エコー検査による推定肺動脈圧に基づくものであり，その不正確性は常に批判の対象

表. MCTD診断基準（2004年度再改訂版）（文献1による）

混合性結合組織病の概念：
全身性エリテマトーデス，強皮症，多発性筋炎などに見られる症状や所見が混在し，血清中に抗U1-RNP抗体が見られる疾患である．

Ⅰ 共通所見
1．Raynaud現象
2．指ないし手背の腫脹
3．肺高血圧症

Ⅱ 免疫学的所見
抗U1-RNP抗体陽性

Ⅲ 混合所見
A．全身性エリテマトーデス様所見
1．多発関節炎
2．リンパ節腫脹
3．顔面紅斑
4．心膜炎または胸膜炎
5．白血球減少（4,000/μl以下）または血小板減少（100,000/μl以下）
B．強皮症様所見
1．手指に限局した皮膚硬化
2．肺線維症，肺拘束性換気障害（%VC＝80%以下）または肺拡散能低下（%DLCO＝70%以下）
3．食道蠕動運動低下または拡張
C．多発性筋炎様所見
1．筋力低下
2．筋原性酵素（CK）上昇
3．筋電図における筋原性異常所見

診断：
1．Ⅰの1所見以上が陽性
2．Ⅱの所見が陽性
3．ⅢのABC項のうち，2項目以上につき，それぞれ1所見以上が陽性以上の3項目を満たす場合を混合性結合組織病と診断する．

付記
1．抗U1-RNP抗体の検出は，二重免疫拡散法あるいは酵素免疫測定法（ELISA）のいずれでもよい．ただし免疫拡散法が陽性でELISAの結果と一致しない場合には，二重免疫拡散法を優先する．

となっていた．しかしながら最近，イタリアとフランスのSSc症例において，ハイリスクの患者にprospectiveに右心カテーテル検査を行い，心カテによる直接的な肺動脈圧を測定した．その結果，やはり5～6%の強皮症患者にPHのあることが確認され[5]，SScでのPH合併率が著しく高値であることが最終的に確定した．PHに関するWHO（世界保健機関）のシンポジウムでも，SSc関連疾患（つまりSScとMCTD）では，症状の有無や変化に関わらず毎年心エコー検査をすべきであるとしている．

2）膠原病に伴うPHの病態，病型分類

肺動脈圧が高くなる原因として肺毛細血管より前に原因あるものと後にあるものがあり，前者を前毛細血管性PH，後者を後毛細血管性PHと呼んでいる．PAH（pulmonary arterial hypertension）は前毛細血管性PHに含まれる．最新の分類である2008年のDana Point分類[6]によると，膠原病によるものは，PAHの中に分類されている．しかしPAH以外のものもみられ，現在，病