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The efficacy and safety of low-dose sirolimus for treatment of lymphangioleiomyomatosis

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ABSTRACT

Background: Lymphangioleiomyomatosis (LAM) is a rare disease caused by dysregulated activation of the mammalian target of rapamycin (mTOR). Sirolimus, an inhibitor of mTOR, has been reported to decrease the size of angiomyolipomas and stabilize pulmonary function in patients with LAM. However, the optimal dose for the treatment of LAM remains unclear.

Methods: We conducted a retrospective, observational study of 15 patients with LAM who underwent sirolimus therapy for more than 6 months. The efficacy was evaluated by reviewing the patients' clinical courses, pulmonary function and chest radiologic findings before and after the initiation of sirolimus treatment.

Results: All patients had blood trough levels of sirolimus lower than 5 ng/mL. Sirolimus treatment improved the annual rates of change in FVC and FEV_1 in the 9 patients who were free from chylous effusion (FVC, −101.0 vs. +190.0 mL/y, $p=0.046$ and FEV_1, −115.4 vs. +127.8 mL/y, $p=0.015$). The remaining 7 patients had chylous effusion at the start of sirolimus treatment; the chylothorax resolved completely within 1–5 months of treatment in 6 of these cases. These results resembled those of previous studies in which blood trough levels of sirolimus ranged from 5 to 15 ng/mL.

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Conclusions: Low-dose sirolimus (trough level, 5 ng/mL or less) performed as well as the higher doses used previously for improving pulmonary function and decreasing chylous effusion in patients with LAM.

1. Introduction

Lymphangioleiomyomatosis (LAM), a rare disease seen primarily in women of childbearing age, is characterized by the proliferation of abnormal smooth muscle-like cells (LAM cells), which lead to cystic destruction of the lungs, chylous effusions, lymphangioleiomyomas, and, frequently, renal angiomyolipoma (AML) [1,2]. This condition can occur as a sporadic disease (sporadic LAM) or as a pulmonary manifestation of tuberous sclerosis complex (TSC) (TSC-associated LAM) [3–5]. The apparent cause of TSC-associated LAM is mutation of either of the tumor suppressor genes TSC1 and TSC2, whereas sporadic LAM results mainly from mutation of TSC2 [6–8]. Loss of TSC gene function allows constitutive activation of the mammalian target of rapamycin (mTOR) signaling pathway, which regulates multiple cellular functions such as growth, motility, and survival [9].

The mTOR inhibitors such as sirolimus and everolimus block mTOR-mediated activation of downstream kinases and restore homeostasis in cells with defective TSC gene function [9]. Administration of mTOR inhibitors has previously been shown to decrease the size of AML and stabilize lung function in patients with LAM [10,11]. The CAST (Cincinnati Angiomyolipoma Sirolimus Trial) was a phase 1–2 trial comprising 20 patients with TSC or sporadic LAM, and sirolimus reduced the size of renal AML in both groups [10]. In that study, the optimal dose for the reduction of AML was determined by first administering sirolimus at 0.25 mg per square meter of body-surface area to achieve a blood trough level between 1 and 5 ng/mL (1–5 ng/mL). If this did not decrease the size of the target AML by 10% of the baseline value within 2 months, the dose was increased to achieve a trough level of 5–10 ng/mL. Finally, if the lesion did not decrease in size by 10% of the baseline value within 4 months, the dose was increased to achieve a trough level of 10–15 ng/mL, and that dose was continued throughout the next 12 months. Under this regimen, 1 of the 20 patients remained at the initial target trough level of 1–5 ng/mL, whereas the other 19 patients were dosed to reach the highest target level (10–15 ng/mL).

The MILES (Multicenter International LAM Efficacy of Sirolimus) Trial was a double-blind, placebo-controlled trial in which sirolimus was shown to stabilize lung function and improve the quality of life in patients with LAM [11]. In that trial, sirolimus was administered to maintain blood trough level of 5–15 ng/mL, which was chosen on the basis of results of the CAST. However, it remains unclear whether this is the optimal level for the treatment of LAM, especially as other recent case reports have shown that a lower dose was also effective [12,13]. Therefore, to determine the efficacy and safety of sirolimus at a concentration lower than the currently recommended trough level, we conducted an observational study of Japanese patients with LAM who took sirolimus at doses that maintained blood trough levels lower than the currently recommended target of 5–15 ng/mL.

2. Materials and methods

2.1. Study population

Records from 21 patients with LAM who were treated with sirolimus in the Department of Respiratory Medicine at Juntendo University Hospital between November 2009 and January 2012 were reviewed for this study. We excluded 6 patients for the following reasons: 2 patients discontinued sirolimus treatment within 2 months due to adverse events (drug-induced lung injury and skin rash/chest discomfort, respectively), 1 patient lacked serial data, 2 patients received sirolimus for too short a period to support analysis, and 1 patient underwent lung transplantation. The remaining 15 patients with LAM underwent sirolimus treatment for more than 6 months and had serial data available for retrospective analysis. To determine the efficacy of sirolimus, we retrospectively reviewed the patients' clinical courses, including adverse events, as documented in their medical records, and compared chest radiologic findings and pulmonary function before and after the initiation of sirolimus treatment. Patients' data were used under the comprehensive consent from the patients of its use and the approval of IRB (31 July 2007).

All patients were female, and their ages at the initiation of sirolimus therapy ranged from 27 to 56 years (mean, 40 y) (Table 1). Fourteen of the patients had been diagnosed with sporadic LAM and one with TSC-associated LAM. Seven patients had chylous effusion; all had chylothorax, and 3 of the 7 had chylous ascites. One patient had renal angiomyolipomas, 2 had pulmonary lymphedema, 2 had abdominal lymphadenopathy, 1 had pelvic lymphadenopathy, and 7 had both abdominal and pelvic lymphadenopathy. The diagnosis of LAM was established by histopathological examination ($n=13$), by cytological examination of chylous fluid [14] ($n=1$), or from a combination of characteristic high resolution computed tomography (HRCT) findings and an elevated serum VEGF-D level ($n=1$). Serum VEGF-D was measured using a commercially available enzyme-linked immunosorbent assay (ELISA) kit according to the manufacturer's instructions (R&D Systems, Inc., Minneapolis, MN, USA) [15].

Fourteen patients began the regimen with a dose of sirolimus of 1 mg/day. The dose was increased to 2 mg/day to improve the response in 4 cases (JUL46, JUL91, JUL248, and JUL316). Patient JUL97 had been treated for 1 year with everolimus, a similar mTOR inhibitor, at a dose of 1 mg/day before starting treatment with sirolimus. The trough level of sirolimus was measured within 1 month after the initiation of treatment or the increase in the dose. Blood sirolimus

Table 1 – Patients' characteristics at the initiation of sirolimus therapy.

	LAM (n=15)
Age—yr (range)	39.9±8.0 (27–56)
Sporadic/TSC-associated LAM	14/1
Clinical features–no. (%)	
Chylous effusion	7 (46.7)
Chylothorax only	4 (26.7)
Chylothorax with ascites	3 (20.0)
Pulmonary lymphedema	2 (13.3)
Angiomyolipoma	1 (6.7)
Abdominal lymphadenopathy	9 (60.0)
Pelvic lymphadenopathy	8 (53.3)
Home oxygen therapy	8 (53.3)
Diagnostic test–no. (%)	
TBLB	2 (13.3)
VATS	7 (46.7)
Abdominal lymph node biopsy	2 (13.3)
Pelvic tumor resection	2 (13.3)
Cytology of chylous fluid	1 (6.7)
Clinical diagnosis[a]	1 (6.7)
Serum VEGF-D (pg/mL) (range)	4074±1927 (1346–8281)

Plus–minus values are means±SD.
Abbreviations: TBLB, transbronchial lung biopsy; TSC, tuberous sclerosis complex and VATS, video-assisted thoracic surgery.
[a] Diagnosed on the basis of the combination of characteristic high resolution computed tomography (HRCT) findings and an elevated serum VEGF-D level.

levels were assayed by Towa Environment Science Co., Ltd., (Osaka, Japan) using the method described by McCormack, et al. [11].

2.2. *The effect of sirolimus on LAM*

Our evaluation of sirolimus in patients with LAM included assessment of the changes in various parameters within the groups of patients with and without chylous effusion. In the 8 LAM patients without chylous effusion (Table 2), we used the Wilcoxon signed-rank test to compare the serial values of pulmonary function, forced vital capacity (FVC), and forced expiratory volume in 1 s (FEV_1) before and after the administration of sirolimus. The statistical analysis was conducted using SPSS version 20. For all statistical analyses, a p value less than 0.05 was considered significant.

For the 7 patients with chylous effusions, we reviewed the size or amount of effusion as assessed by radiologic or ultrasonographic examinations. We defined complete resolution (CR) as the absence of pleural effusion detectable by radiologic examination and a "stabilized" condition as effusion that persisted but in a smaller quantity than present at baseline. For chylous ascites, we defined "stabilized" as ascites that persisted but was no worse than that previously visible and "improved" as a quantity of ascites greater than physiologically normal but less than present at baseline. The patients' medical records indicated whether the radiologic findings accompanied changes in other parameters related to effusions, such as symptoms (dyspnea, heaviness in the chest, or a feeling of abdominal distention), abdominal circumference, and body weight.

3. Results

3.1. *Trough sirolimus levels in the blood*

Of the 15 patients treated with sirolimus who are reviewed here, all had trough blood levels lower than 5 ng/mL (mean, 2.16 ng/mL; range, 0.8–4.3 ng/mL). Accordingly, we arbitrarily designated the treatment regime "low-dose sirolimus treatment" because the trough levels of all patients were lower than those in previous studies [10,11,16,17].

3.2. *Effect of sirolimus on pulmonary function*

The clinical characteristics of the 8 patients without chylous effusion are shown in Table 2. All of these patients had sporadic LAM, and their ages at the start of sirolimus treatment ranged from 33 to 56 years. The median follow-up time as of August 2012 was 17.5 months (SD, 5.9 months). The most frequent presenting feature was exertional dyspnea, which was noted in 6 of 8 patients (75.0%). Pneumothorax and retroperitoneal lymphadenopathy were each present in 1 patient. Two patients had pulmonary lymphedema.

Their mean (SE) annual changes in FVC and FEV_1 before sirolimus treatment were −101.0 mL (314.2 mL) and −115.4 mL (86.2 mL), respectively (Table 2 and Fig. 1). The annual change improved after the start of sirolimus treatment for both FVC and FEV_1 [FVC, +190.0 mL (246.1 mL), p=0.046 and FEV_1, +127.8 mL (289.6 mL), p=0.015]. Six of 8 patients had FEV_1 values at or above their baseline values after 1 year, whereas the remaining 2 patients showed slight decreases from baseline in FEV_1 [1.69–1.62 L (JUL123) and 0.85–0.78 L (JUL248)]. These results were similar to those of previous studies in which the blood trough level of sirolimus was maintained at 5–15 ng/mL [11,16,17].

We will briefly describe a clinical course representative of the patients without chylous effusion to depict the typical response to mTOR inhibition. JUL97 presented at 27 years of age with hemosputum and Polycystic Ovarian Syndrome (PCOS) (Fig. 2). Imaging studies demonstrated lymphatic congestion and edema (predominantly in the right lung). Cystic parenchymal destruction was evident as coalescing, irregular, bizarrely shaped, cavity-like, airspaces in the right upper lobe. Everolimus therapy was initiated 5 years later while she was on a waiting list for bilateral lung transplantation. She was receiving supplemental oxygen at 5 L/min for treatment of respiratory failure and her condition was complicated by bloody chylous sputum. Treatment with everolimus for 1 year resolved the bloody chylous sputum, substantially eased the pulmonary lymphedema, and counteracted the airflow limitation. The mTOR inhibitor was then changed from everolimus to sirolimus for financial reasons. Her condition continued to improve, and she no longer requires supplemental oxygen. Later, during the third year of mTOR inhibitor treatment, she developed an *Aspergillus* infection in her right upper lung lobe, in which severe parenchymal destruction had created a cavity.

Table 2 – Patients without chylous effusion.

Registry number[a]	Age[b] (yr)	Presenting features	Duration[c] (m)	Trough level	Clinical characteristics	FVC[d]		FEV_1[d]		Serum VEGF-D
						Pre	Post	Pre	Post	Pre
JUL46	44	Dyspnea	12	1.2	AML, abdominal lymphadenopathy	2.93	3.05	0.54	0.59	ND
JUL97	33	Dyspnea	20	1.2	Pulmonary lymphedema, abdominal and pelvic lymphadenopathy	2.07	2.54	1.56	2.13	8281
JUL117	55	Dyspnea	13	ND		2.42	2.32	0.47	0.47	3142
JUL123	37	PTX	23	1.8		2.88	3.00	1.69	1.62	1346
JUL162	38	Dyspnea	27	2.1	Pulmonary lymphedema	2.7	2.7	1.82	1.99	6696
JUL197	36	Dyspnea	27	ND		1.05	1.34	0.42	0.44	3576
JUL210	56	Retroperitoneal lymphadenopathy	15	2.4	Abdominal and pelvic lymphadenopathy	3.34	2.81	1.83	1.96	3257
JUL248	41	Dyspnea	13	2.4		2.56	2.73	0.85	0.78	1354

All patients were diagnosed with sporadic LAM.

Abbreviations: AML, angiomyolipoma; FEV_1, forced expiratory volume in 1 s; FVC, forced vital capacity; ND, not determined and PTX, pneumothorax.

[a] Registry number: The registry number of each patient with LAM at Juntendo University Hospital. We use this to make it clear when cases have been

[b] Age at initiation of sirolimus therapy (years).

[c] The follow-up period after the initiation of sirolimus therapy (months).

[d] Values of FVC (L), FEV_1 (L), and serum VEGF-D (pg/mL) at baseline (pre) and 1 year after the initiation of sirolimus therapy (post).

[e] See also Fig. 2. JUL97 developed an *Aspergillus* infection 20 months after the initiation of sirolimus therapy.

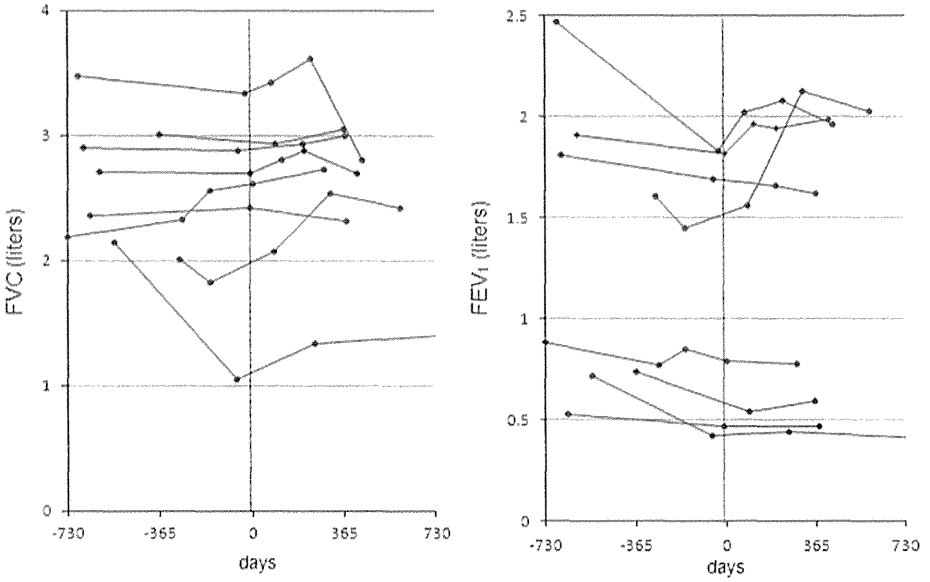

Fig. 1 – Serial changes in forced vital capacity (FVC) and forced expiratory volume in 1 s (FEV_1) before and after initiation of sirolimus treatment. Day 0 on the horizontal axis indicates the day on which sirolimus therapy was initiated; negative and positive numbers indicate days before and after the initiation of sirolimus administration, respectively.

3.3. *Effect of sirolimus on chylous effusions*

Seven patients had chylothorax, which was accompanied in 3 cases by chylous ascites (Table 3). Their median follow-up time as of August 2012 was 12.0 months (SD, 5.5). Four of the patients experienced complete resolution of chylothorax in 1 to 3 months after the initiation of sirolimus treatment. Of the 3 patients with both chylothorax and chylous ascites, 2 experienced complete resolution of the chylothorax and decreased amounts of ascites. Even the remaining patient (JUL79), in whom considerable pleural effusion and ascites persisted, had less fluid accumulation than before sirolimus therapy ("stabilized condition"). This situation may have resulted from her extremely low trough level (0.8 ng/mL).

We will describe a case TSC-associated LAM (JUL316) to illustrate how chylous effusion responded to low-dose sirolimus (Fig. 3). This 47-year-old woman had a moderate amount of right pleural effusion, dilatation of the thoracic duct, and lymphatic involvement in the mediastinum and upper abdomen (Fig. 3A and B). Lymphoscintigraphy revealed obstruction of axial lymphatic flow around the common iliac veins (Fig. 3C). She needed supplemental oxygen therapy to avoid hypoxemia. After taking sirolimus at 1 mg/day for 14 days (trough level, 2.2 ng/mL), she obtained symptomatic relief and amelioration of the lymphatic obstruction, as confirmed by lymphoscintigraphy (Fig. 3D). At her request, her dose of sirolimus was increased to 2 mg/day (trough level, 4.3 ng/mL), and her pleural effusion disappeared completely within 2 months (Fig. 3E). She eventually stopped needing supplemental oxygen.

We observed the effect of discontinuing sirolimus in 1 patient with chylous ascites (JUL137) (Fig. 4). This 43-year-old patient with sporadic LAM had received a peritoneovenous shunt 5 years prior to control intractable chylous ascites [18]. However, the small amount of chylous pleural effusion and moderate amount of chylous ascites that remained expanded her abdominal circumference to ~77–80 cm. Approximately 60 days after starting sirolimus treatment, her abdominal circumference had decreased to 72 cm, the sensation of abdominal distention disappeared, the chylothorax had completely resolved, and her body weight had decreased. However, she began to feel lower abdominal pain, probably because the lack of ascites allowed the tip of the shunt tube to irritate the inner surface of her abdomen. Accordingly, she discontinued the sirolimus therapy. Thereafter, her abdominal circumference gradually increased and returned to the baseline level within 153 days. After removal of the peritoneovenous shunt (data not shown) and reinstatement of sirolimus therapy, her chylous ascites diminished as before.

3.4. *The effect of sirolimus on the serum VEGF-D level*

Serum VEGF-D levels before and after sirolimus treatment were measured in 14 patients (all except JUL46) (Table 1). Sirolimus treatment decreased the serum VEGF-D level in all but 1 patient (JUL305) (Tables 2 and 3).

3.5. *Treatment-related adverse events*

The treatment-related adverse events are summarized in Table 4. The most common adverse events related to low-dose sirolimus were stomatitis (9 patients, 60.0%); gastrointestinal episodes (8 patients, 53.3%), including diarrhea ($n=6$) and stomach discomfort ($n=2$); and upper or lower respiratory infection (6 patients, 40.0%). Although these were not severe, i.e., usually grade 1 or 2, 1 patient (JUL316) temporarily discontinued sirolimus treatment due to grade 3 stomatitis. Hypercholesterolemia was not observed in any patient. One patient (JUL97) developed *Aspergillus* infection in her severely damaged lung parenchyma, where a cavity-like area was visible in the right upper lobe.

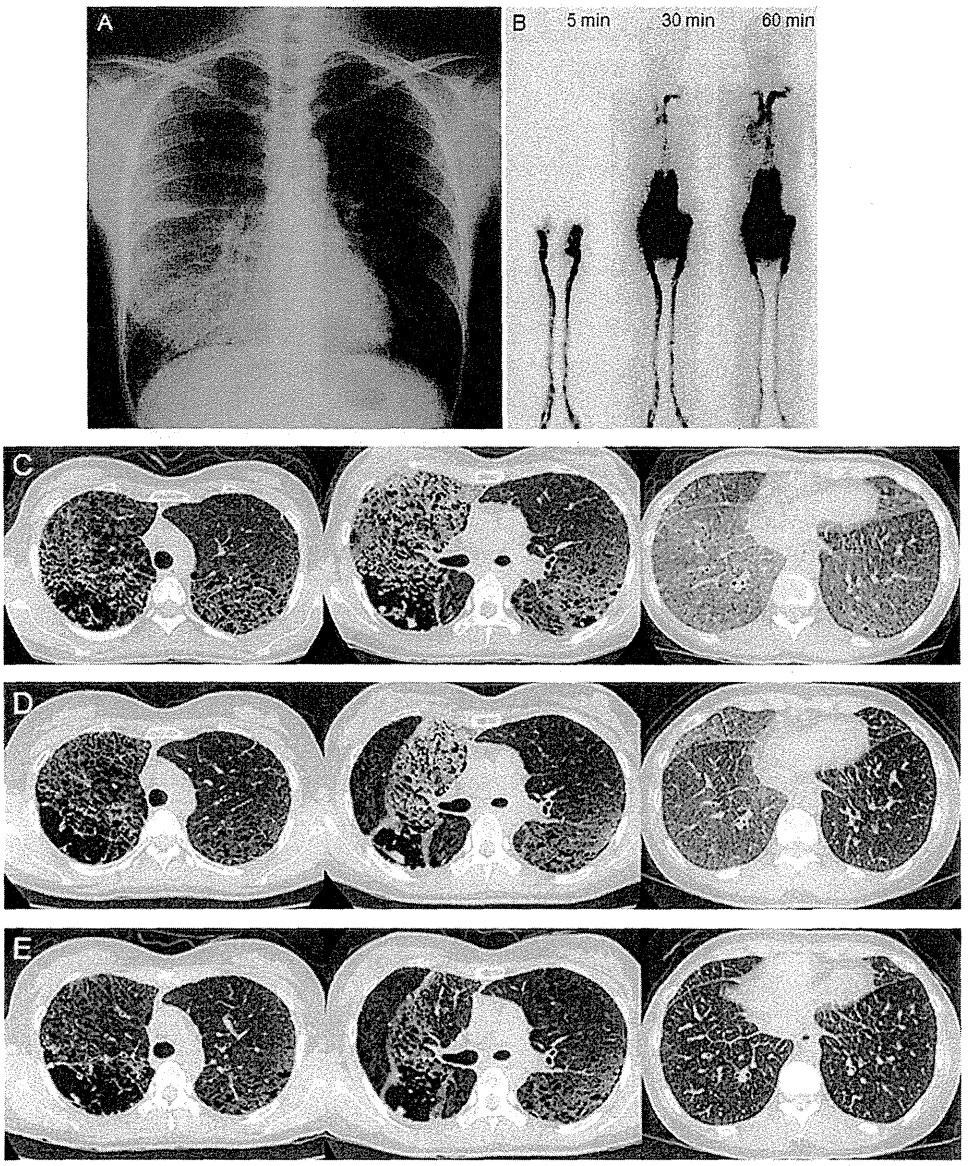

Fig. 2 – Radiologic findings for patient JUL97, whose pulmonary lymphedema resolved after administration of mTOR inhibitors. This patient's chest radiograph taken at presentation (January 2004) showed ground glass opacity in the right lower lung field (A). Neither pneumothorax nor pleural effusion was found. Lymphoscintigraphy utilizing 99mTc-labeled human serum albumin (HSA) revealed accumulation of radiolabeled HSA in the right lower lung field, as well as in the retroperitoneal and pelvic lymphangioleiomyomas, 60 min after subcutaneous injection of 99mTc-labeled HAS into the dorsal foot skin, indicating the presence of pulmonary lymphedema (B). Note the double thoracic ducts. Computed tomography (CT) images of the chest showed severe cystic destruction with irregular and bizarre shapes, especially in the right upper, right lower, and left lower lobes, as well as ground grass opacity and thickening of the interlobular septa, indicating pulmonary lymphedema (C, April 2009). Administration of everolimus at 0.5 mg/day began in April 2009, and the dose was increased to 1 mg/day from August 2009 through April 2010. CT images of the chest taken in April 2010 showed lessening of the pulmonary lymphedema (D). The mTOR inhibitor was then changed to sirolimus at 1 mg/day, and CT images of the chest in January 2011 showed almost complete resolution of the pulmonary lymphedema (E).

4. Discussion

This retrospective study documents the effectiveness of low-dose sirolimus for stabilizing pulmonary function and decreasing chylous effusion in patients with LAM. Our results resemble those of previous studies in which the blood trough level of sirolimus was maintained at 5–15 ng/mL [11,16,17]. Therefore, our data indicate that sirolimus was effective in Japanese patients even at a trough level less than 5 ng/mL. A particularly noteworthy patient was JUL97, a woman with atypical symptoms of severe lymphatic pulmonary edema and bloody chylous sputum who required continuous supplemental oxygen therapy. In contrast, Moua et al. recently described a patient with LAM in whom sirolimus therapy resolved chylous pulmonary congestion and respiratory

Table 3 – Patients with chylous effusions.

Registry number[a]	Age (yr)	Site[b]	Duration (months)	Trough level	Clinical characteristics	Response of chylothorax (time)[c]	Response of ascites	Serum VEGF-D	
								Pre	Post
Chylothorax only									
JUL235	39	R	19	2.0	Alveolar hemorrhage, abdominal lymphadenopathy	CR (1 month)		2791	976
JUL240	28	R	7	3.3	Abdominal and pelvic lymphadenopathy	CR (1 months)		5255	3024
JUL305	27	R	9	1.7	Abdominal and pelvic lymphadenopathy	CR (3 months)		2468	2570
JUL316[a]	47	R	12	4.3	Abdominal and pelvic lymphadenopathy	CR (2 months)		5143	3671
Chylothorax with ascites									
JUL79	38	L	12	0.8	Pelvic lymphadenopathy	SD	Stabilized	4931	2892
JUL91	36	B	19	2.7	Chylous vaginal discharge, abdominal and pelvic lymphadenopathy	CR (5 months)	Improved	5634	3394
JUL137	43	R	21	2.2	Peritoneovenous shunt had been placed. Abdominal and pelvic lymphadenopathy	CR (2 months)	Improved	3161	2308

Abbreviations: CR, complete response and SD, stable disease.
[a] JUL316 had TSC-associated LAM (All other patients had sporadic LAM.).
[b] Site of chylothorax: B, bilateral; L, left and R, right.
[c] Time elapsed before response (months).

failure so completely that a previously planned lung transplantation was no longer being considered [19]. We experienced a similar clinical outcome in patient JUL97 after treatment with low-dose everolimus and, subsequently, sirolimus. The response to low-dose everolimus in JUL97 clearly illustrates this regimen, like low-dose sirolimus treatment, effectively combats pulmonary lymphedema and improves lung function. We have also experienced a patient with LAM (33-year-old woman) who has been treated only with low-dose everolimus (0.5 mg/day for 42 months, blood trough level less than 2 ng/mL) and whose annual change in pulmonary function has stabilized (FVC, −110.4 vs. +84.6 mL/year and FEV_1, −171.2 vs. +14.1 mL/year). However, we excluded her from this analysis to focus on the effect of low-dose sirolimus treatment.

The clinical trials CAST and MILES demonstrated that the benefits of sirolimus treatments for 1 year disappeared after discontinuation of therapy, i.e., without the treatment, the size of AML and rate of decline in FEV_1 reverted to the baseline levels [10,11]. The effect of discontinuation on chylous effusion, however, had been unknown. Our study included 2 patients with chylous ascites who discontinued sirolimus treatment for brief periods. As represented by patient JUL137, cessation of therapy caused the abdominal circumference to revert gradually to the baseline level within 2–3 months, indicating that the beneficial effects of sirolimus on chylous effusions do not persist beyond 2 or 3 months.

The adverse events associated with sirolimus in our analysis were mostly low-grade and were consistent with the known toxicities. The incidence was similar to those previously reported despite the low-dose regimen [10,11]. There were 2 exceptions. One patient with possible sirolimus-related lung injury (2 mg/day; trough level not measured), which has never previously been reported in studies of patients with LAM, was excluded from this analysis. At baseline, this patient as receiving continuous supplemental oxygen due to respiratory failure; however, she was hospitalized and cured without specific treatment after discontinuation of sirolimus. The second was a patient with moderately limited airflow at baseline who developed mild chest tightness with slightly increased parenchymal opacity on chest radiographs after sirolimus treatment (1 mg/day; trough level, 3.7 ng/mL). These symptoms and findings resolved promptly after the discontinuation of sirolimus. *Aspergillus* infection developed in 1 patient (JUL97), indicating that particular caution is needed when prescribing mTOR inhibitors for any patient with LAM with an area of pulmonary airspace destruction. Meanwhile, hypercholesterolemia, one of the most common adverse events in earlier studies of LAM patients [10,11], was not observed in the present study. A subgroup analysis of the MILES trial recently showed that the types of adverse events differed between Japanese and American patients [20]. Accordingly, there may be racial differences in susceptibility to specific adverse events.

Our study had several limitations. First, the small number of patients included in this observational study could have biased the results. However, considering the rarity of LAM, our analysis of 15 patients is likely to be one of the larger such studies and thus warrant attention. Second, because our retrospective study was not controlled, it is possible that the pleural effusions might have resolved spontaneously. This possibility was raised in the previous study [16] but was deniable because we verified

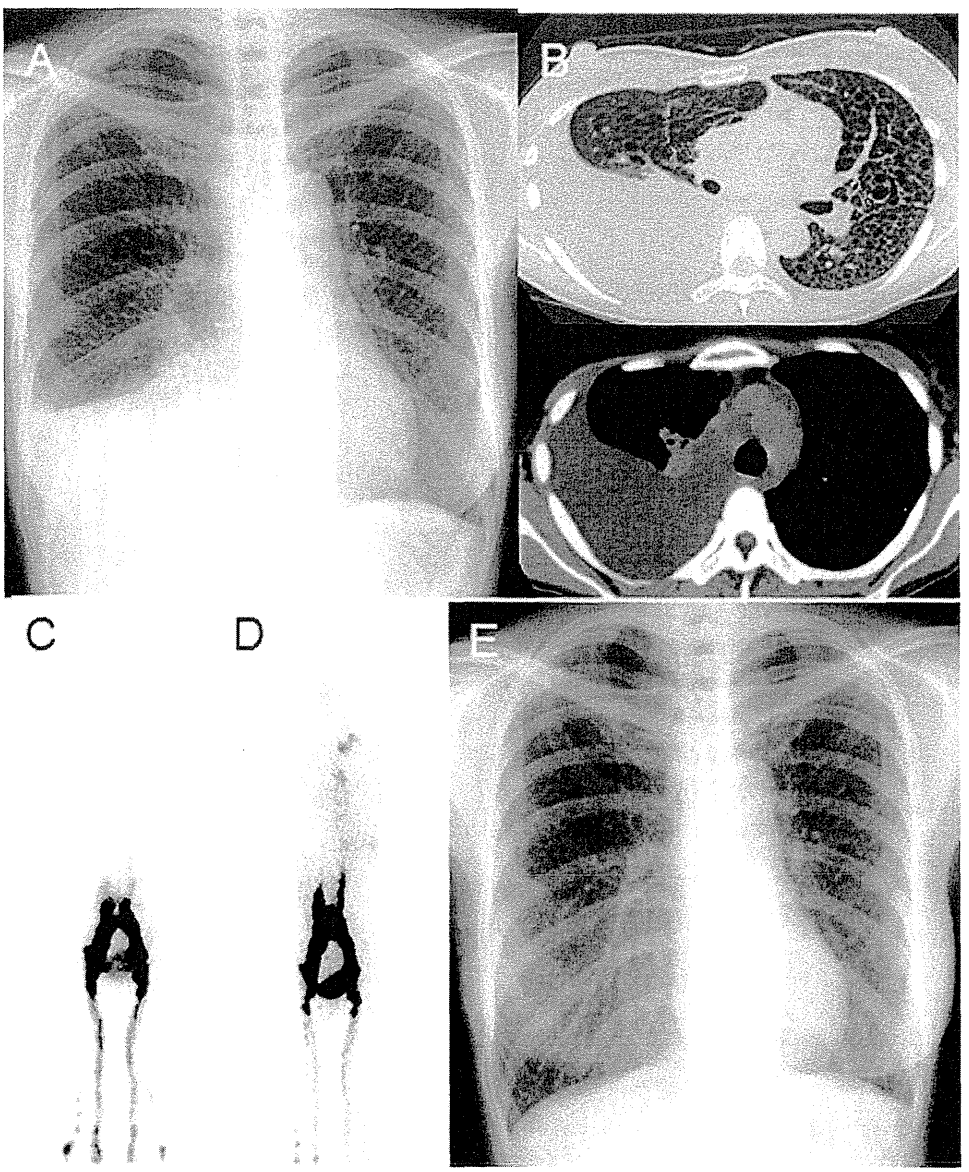

Fig. 3 – Radiologic findings for patient JUL316, whose chylothorax resolved after the administration of sirolimus. This patient's chest radiograph (A) and computed tomography (CT) images (B) at presentation showed right pleural effusion, moderate numbers of thin-walled cysts scattered throughout both lungs, and swelling of multiple mediastinal lymph nodes. Lymphoscintigraphy revealed accumulation of radiolabeled human serum albumin (HSA) in her pelvis 90 min after subcutaneous injection of 99mTc-labeled HSA into the dorsal foot skin, suggesting obstruction of axial lymphatic flow around the common iliac veins (C). Fourteen days after the initiation of sirolimus treatment, repeat lymphoscintigraphy showed the left venous angle 90 min after the injection (D), indicating the amelioration of lymphatic obstruction. Two months after the initiation of sirolimus therapy, no pleural effusion was observed (E).

that long-term GnRH therapy and a low-fat diet did not resolve the chylous effusions in our patients before initiating sirolimus treatment.

In conclusion, careful review of Japanese patients with LAM who were treated with low doses of sirolimus provided clear evidence of improved or stabilized pulmonary function and decreased chylous effusions. Even at a blood trough level of less than 5 ng/mL, sirolimus appears to be clinically beneficial in terms of effectiveness, cost, and safety. The results of our retrospective study warrant a prospective study to compare the effects of low (o 5 ng/mL) and conventional (5–15 ng/mL) doses of sirolimus on the clinical course of LAM.

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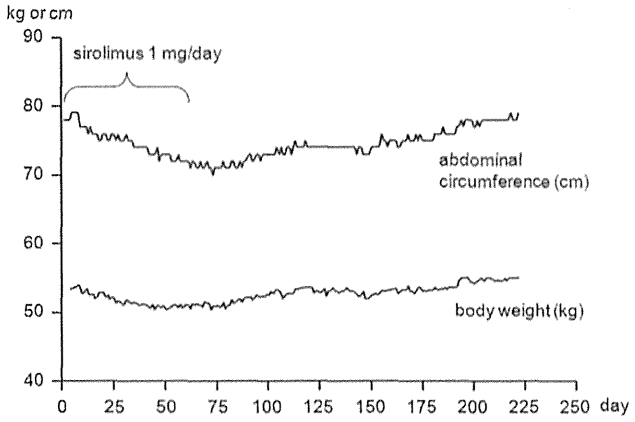

Fig. 4 – Serial changes in abdominal circumference and body weight in patient JUL137. The administration of sirolimus (1 mg/day) for 53 days decreased the patient's abdominal circumference (from 78 to 72 cm) and body weight. After discontinuation of sirolimus, the abdominal circumference gradually returned to the higher baseline level within 153 days.

Table 4 – Adverse events related to sirolimus therapy.

	Number of patients, *n* (%)		
	Total	Grade 1–2	Grade 3
Infection	6 (40.0)	5 (33.3)	1 (6.7)
Upper respiratory infection	5	5	0
Fungal (*Aspergillus*) infection	1	0	1
Hypercholesterolemia	0	0	0
Gastrointestinal event	8 (53.3)	8 (53.3)	0
Diarrhea	6	6	0
Stomach discomfort	2	2	0
Stomatitis	9 (60.0)	8 (53.3)	1 (6.7)

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Conflict of interest

The authors have reported to *Respiratory Investigation* that no potential conflicts of interest exist with any companies/organizations whose products or services may be discussed in the article.

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Case Report

Reversed Halo Sign in Tuberous Sclerosis Complex

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We describe a reversed halo sign in a teenage girl with tuberous sclerosis complex (TSC). Lung manifestations of TSC include lung cysts corresponding to lymphangioleiomyomatosis and small nodules indicating multifocal micronodular pneumocyte hyperplasia (MMPH). However, a reversed halo sign in TSC has never been reported. The lesion was microscopically consistent with MMPH. Immunohistological findings also supported the notion that the lesion is associated with TSC.

1. Introduction

Tuberous sclerosis complex (TSC) is an autosomal dominant inherited neurocutaneous syndrome characterized by various hamartomatous lesions in various organs [1]. Pulmonary manifestations of TSC include lung cysts corresponding to lymphangioleiomyomatosis (LAM) and small nodules indicating multifocal micronodular pneumocyte hyperplasia (MMPH). Pulmonary manifestations occur in 1%–2.3% of patients with TSC, but recent reports indicate that pulmonary LAM can be radiologically detected in 26%–39% of female patients with TSC [1, 2]. MMPH has been considered a rare manifestation of TSC. However, Muzykewicz et al. described that 58% of patients with TSC have pulmonary nodules that represent MMPH [3]. Typical radiological features of MMPH are multiple tiny nodules that are diffusely and randomly scattered throughout the lung [3, 4]. Kobashi et al. noted that computed tomography (CT) identified ground-glass opacity (GGO) in all of the 15 patients with MMPH [5]. On the other hand, Muzykewicz et al. described that 67% of TSC patients with multiple nodules had both solid and ground-glass nodules.

The reversed halo sign is defined as focal rounded areas of GGO surrounded by a more or less complete ring of consolidation that can be visualized by CT [6]. This sign was initially considered specific to cryptogenic organizing pneumonia [7]. However, the reversed halo sign has been associated with various infectious and noninfectious clinical entities [8].

Here, we describe our experience of a unique Japanese patient with TSC and the reversed halo sign.

2. Case Report

A teenage girl was referred to our institution with abnormal findings on chest CT and TSC diagnosed by a dermatologist based on skin lesions. She was asymptomatic upon admission, and laboratory data were normal. Chest CT showed multiple nodules in both lungs (Figures 1(a) and 1(b)) and GGO with a 30 mm diameter surrounded by dense linear consolidation in the superior segment of the right lower lobe (Figures 1(c) and 1(d)). Since pulmonary artery and vein passed the lesion showing GGO and did not correspond with surrounding linear consolidation, the lesion agreed with the reversed halo sign. No lung cysts suggested LAM, and CT revealed that she was free of liver and kidney diseases suggesting angiomyolipoma. Multiple lung nodules were diagnosed as MMPH because the patient had a background of TSC and some had GGO [2, 3, 5]. The lesion with reversed halo sign was difficult to diagnose. Differential diagnoses include cryptogenic organized pneumonia [7, 9], infectious

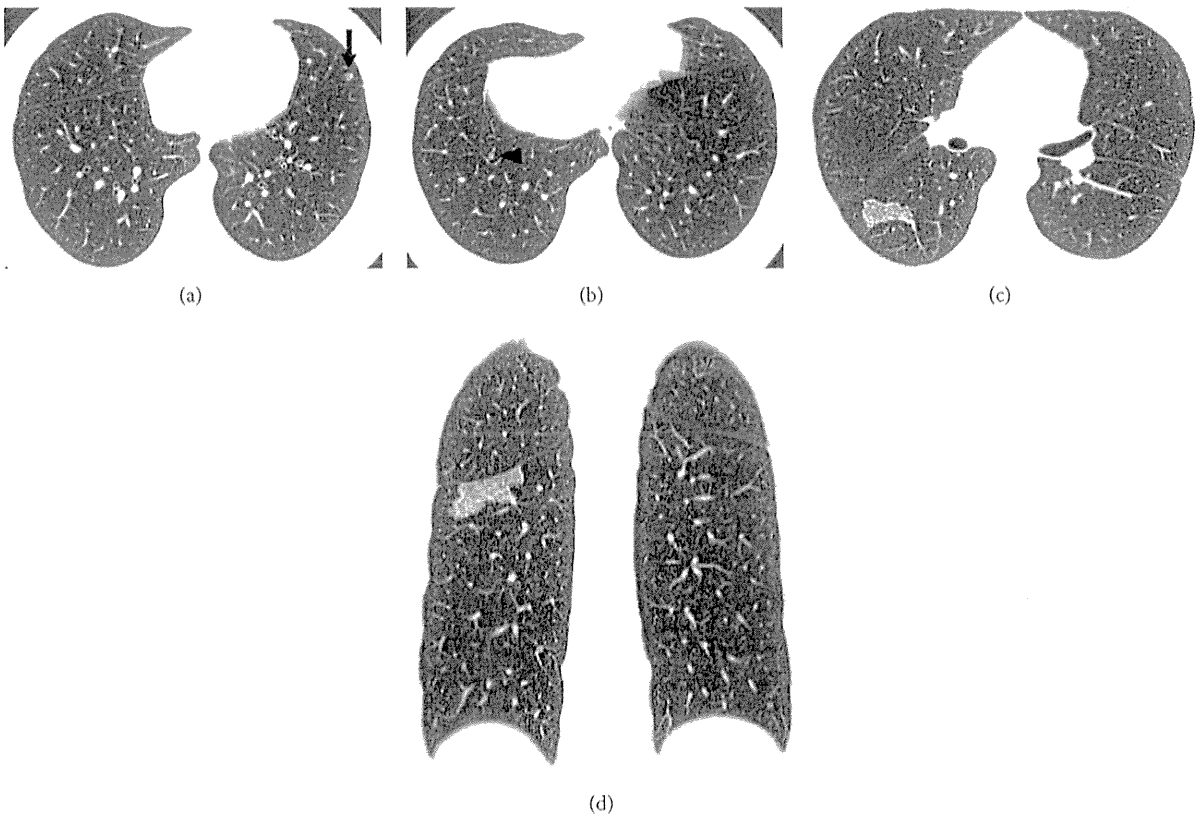

FIGURE 1: Chest CT findings. (a), (b) Axial CT images (1.5 mm thickness) of the chest show small nodules with ground-glass opacity in the lingular segment of the left upper lobe ((a), arrow) and the lateral segment of the right lower lobe ((b), arrowhead). (c) Axial CT image (1.5 mm thickness) shows ground-glass opacity with 30 mm diameter surrounded by dense linear consolidation (reversed halo sign) in the superior segment of the right lower lobe. (d) Coronal reconstructed image (3 mm thickness) also shows revered halo sign in the superior segment of the right lower lobe.

lung disease [8], and atypical MMPH. Because the shape and size of the lesion had not changed on CT images by 3 months later, a diagnosis could not be concluded based on imaging findings and the clinical course. Pathological confirmation was required to determine the optimal therapeutic strategy. The patient provided written informed consent to undergo a CT-guided transthoracic needle biopsy of the lesion, which proceeded uneventfully, and she was discharged a few days later. Histopathologically, enlarged cuboidal cells with abundant, eosinophilic cytoplasm, and large, round nuclei, lined a mildly thickened alveolar septa. The airspaces of the lesion were filled with these cells and alveolar macrophages. The alveolar septa focally comprised thickened elastic fibers and lymphocytic infiltration (Figures 2(a) and 2(b)). The enlarged cuboidal cells were immunohistochemically positive for phospho-S6 ribosomal protein (p-S6), a downstream protein of the mammalian target of rapamycin (mTOR) signaling pathway that is regulated by the *TSC* genes (Figure 2(c)). The histological and immunohistochemical findings were consistent with MMPH.

3. Discussion

We encountered MMPH with typical, tiny, and fine nodules together with the reversed halo sign on CT images of a young patient with TSC. Furthermore, the 30 mm GGO responsible for the reversed halo sign seems the largest reported as MMPH. To the best of our knowledge, a reversed halo sign has never been identified in TSC, and two different coexisting features representing MMPH is an extremely rare lung manifestation of TSC. Generally, pulmonary involvement of TSC is known as LAM and MMPH. The latter is histologically characterized by a multicentric, well-demarcated nodular growth of bland-looking type II pneumocytes along an alveolar septa that exhibits fibrous thickening, increased numbers of elastic fibers, and aggregated alveolar macrophages [10]. The radiological features of MMPH are multiple tiny nodules that are randomly and diffusely scattered throughout the lung [4]. Muzykewicz et al. recently described CT findings of either solid or ground-glass MMPH nodules with diameters ranging from 2 to 14 mm that are diffusely distributed in the lungs of patients with TSC [3]. No single nodule has presented with both solid and ground-glass features [3]. Two studies have identified MMPH nodules of <20 mm [3, 5]. An analysis of MMPH identified one Japanese patient with TSC and atypical large nodules (20 mm) and infiltrative shadows, but coexisting small lesions were not described [5]. Muzykewicz et al. also reported a target-like appearance, with ground-glass nodules of increasing density around the periphery in 5 of 42 patients with TSC [3]. However, their report includes

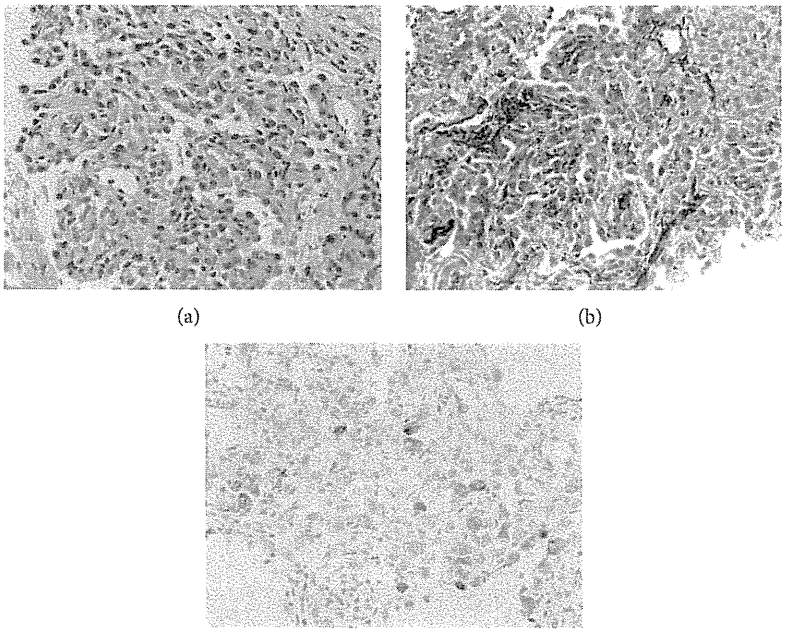

FIGURE 2: Microscopic findings. (a) Enlarged cuboidal cells have abundant, eosinophilic cytoplasm and large, round nuclei lining mildly thickened alveolar septa (hematoxylin and eosin stain). (b) Alveolar septa are focally composed of thickened elastic fibers (Elastica-Masson trichrome stain). (c) Enlarged cuboidal cells are positive for phospho-S6 ribosomal protein.

images that are not typical reversed halo signs and does not mention coexisting nodules of various sizes and opacity in these patients.

The reversed halo sign in our patient was histologically consistent with MMPH, and the immunohistochemical positivity for p-S6 protein supported a diagnosis of MMPH. The histopathological findings of the reversed halo sign in cryptogenic organizing pneumonia are considered to arise from the central GGO corresponding to an area of alveolar septal inflammation and cellular debris in the alveolar spaces, whereas ring-shaped or crescentic peripheral air-space consolidation corresponds to areas of organizing pneumonia within the distal air spaces [9]. The appearance of the lesion on CT could not be precisely correlated with the histopathological findings because the specimen obtained by needle biopsy was only part of the entire lesion. Apart from pneumocytic hyperplasia, an inflammatory process was identified in the specimen. However, we could not precisely correlate these histopathological findings with GGO and the surrounding dense linear consolidation that are two constituents of the reversed halo sign in CT.

In summary, we described a patient with the reversed halo sign in TSC that was microscopically consistent with MMPH. Two different radiological features arising from one pathological process would be quite challenging for radiologists to precisely diagnose. Nonetheless, those atypical radiological features such as large GGO and a reversed halo sign might indicate that MMPH should be borne in mind.

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ORIGINAL ARTICLE

Increased prevalence of cigarette smoking in Japanese patients with sarcoidosis

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ABSTRACT

Background and objective: Several studies have shown that individuals with sarcoidosis in Western populations are less likely to have smoked before diagnosis. Epidemiological characteristics of sarcoidosis are known to differ between Japanese and Westerners. Therefore, the relationship between cigarette smoking and sarcoidosis in a Japanese population was investigated.

Methods: Three hundred eighty-eight patients newly diagnosed with sarcoidosis between 2000 and 2008 were retrospectively identified. The results of two large surveys of smoking prevalence in Japan provided reference data. Specific clinical manifestations of sarcoidosis were compared between current smokers and never-smokers, after excluding former smokers.

Results: The prevalence of current smokers at the time of the diagnosis of sarcoidosis was 59.6% in men and 27.9% in women. With the exception of men in their 30s, the prevalence was higher in all age groups compared with the general Japanese population. The prevalence of lung parenchymal involvement tended to be higher in current smokers than in never-smokers (odds ratio = 1.33 (0.99-1.77), P = 0.054).

Conclusions: This retrospective cohort study suggests that smoking prevalence is higher in Japanese sarcoidosis patients than that reported in Western sarcoidosis patients and that there could be different relationships between smoking and the development of sarcoidosis in these populations.

Key words: epidemiology, gender, sarcoidosis, smoking, susceptibility.

SUMMARY AT A GLANCE

We evaluated the prevalence of cigarette smoking in Japanese patients with sarcoidosis. The prevalence of smoking was unexpectedly high compared with the general population. This result suggests that in Japanese subjects, as compared with Western populations, different mechanisms may influence the relationship between smoking and the development of sarcoidosis.

INTRODUCTION

Sarcoidosis is an inflammatory granulomatous multisystem disorder of unknown origin, primarily affecting the lungs and lymph nodes.[1] The epidemiological characteristics of sarcoidosis differ according to geographical distribution probably because of differences in environmental exposures and predisposing genetic factors.[2] Compared with Western countries, sarcoidosis in Japan has a low prevalence, is less severe, and has a much higher likelihood of ocular and/or cardiac involvement.[3,4]

Cigarette smoking has been demonstrated to represent an important risk factor for a number of health problems, including cardiovascular disease, chronic obstructive pulmonary disease and lung cancer. In contrast, several clinical case studies have shown that patients with sarcoidosis are less likely than control subjects or the general population in Europe or the United States to have smoked before diagnosis.[5-7] These findings suggest that smoking could influence the development of sarcoidosis. However, no reports have clarified the prevalence of smokers among patients with sarcoidosis in a Japanese population. Given the variation of clinical phenotypes for this disease in different areas and among different ethnic groups, epidemiological surveys evaluating the prevalence of smokers in this disease in each ethnic population are important.

METHODS

Study subjects

On the basis of a digital data system and medical records, we identified 388 patients newly diagnosed

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with sarcoidosis between January 2000 and December 2008 from three hospitals in Sapporo City, Hokkaido, Japan: the pulmonary clinic in the First Department of Medicine at Hokkaido University Hospital (n = 166); the Department of Respiratory Medicine at Sapporo Hospital of Hokkaido Railway Company (n = 214); and the Ohmichi Clinic of Internal and Respiratory Medicine (n = 8). The diagnosis of sarcoidosis in each patient was based on clinical findings, histological demonstration of non-caseating epithelioid cell granuloma and exclusion of other diseases capable of producing a similar histological or clinical picture, as recommended by the American Thoracic Society/European Respiratory Society/World Association of Sarcoidosis and Other Granulomatous Disorders statement.[8] All subjects showed histological evidence of non-caseating granulomas in one or more tissue samples with exclusion of any other cause of granulomatosis. The study protocol for this research was approved by the Clinical Research Ethics Committee of Hokkaido University Hospital. The Committee followed the ethics guidelines for epidemiological studies reported by two Japanese ministries: the Ministry of Education, Culture, Sports, Science and Technology; and the Ministry of Health, Labour and Welfare.

Definitions of organ involvement

Pulmonary involvement was defined when chest radiography showed abnormality according to the Scadding system: stage 0, normal chest radiographic findings; stage I, bilateral hilar lymphadenopathy alone; stage II, bilateral hilar lymphadenopathy with pulmonary infiltrates; stage III, pulmonary infiltrates without bilateral hilar lymphadenopathy; or stage IV, pulmonary fibrosis.[9] Chest radiographs were categorized independently by two respiratory physicians who were blinded to the patients' clinical information. Disagreements between physicians were resolved by consensus following review by another respiratory physician. Ocular involvement was defined as a diagnosis of uveitis by an ophthalmologist. Cutaneous involvement was defined by physician diagnosis based on skin conditions and subjective symptoms. Cardiac involvement was defined according to the Japanese Ministry of Health and Welfare guidelines for diagnosing cardiac sarcoidosis.[10]

Prevalence of smokers

Smoking status of all patients with sarcoidosis was recorded at the initial visit. Patients were then divided into three groups: current continuous smokers; former smokers who had stopped smoking for a minimum of 6 months prior to the initial visit; and lifetime never-smokers. The prevalence of smoking among patients with sarcoidosis was compared with the results of two large surveys conducted in Japan: (i) a national survey of the prevalence of asthma using the European Community Respiratory Health Survey questionnaire;[11-13] and (ii) the National Nutrition Survey (2000-2008). To evaluate the prevalence of asthma and asthma-related symptoms, a large nationwide survey using the European Community Respiratory Health Survey questionnaire was conducted in 2006 in 10 areas of Japan. In this survey, our department evaluated the prevalence of asthma in Kamishihoro town, Hokkaido.[14] The European Community Respiratory Health Survey questionnaire included questions regarding smoking status. Patients were classified into three groups according to smoking status: current smoker, former smoker and never-smoker. Data on the smoking status obtained from the National Nutrition Survey (2000-2008) conducted by Japanese Ministry of Health, Labour and Welfare was available on the site http://www.mhlw.go.jp/bunya/kenkou/kenkou_eiyou_chousa.html. This survey reports only the prevalence of current smokers.

Statistical analysis

Statistical analyses were performed using statistical program R version 2.12.1 (http://www.R-project.org/).[15] For analyses, categorical variables were compared using the c^2 test with Yates' correction, and continuous variables were compared using the Mann-Whitney U test. Interobserver variation for the radiographic stage was assessed using Cohen's kappa coefficient of agreement. To clarify the effect of smoking on clinical manifestations of sarcoidosis, we compared clinical manifestations between current and never-smokers, excluding former smokers. Multivariate logistic regression models were used to calculate adjusted odds ratios and 95% confidence intervals. For all statistical analyses, values of $P < 0.05$ were considered significant.

RESULTS

The clinical features of all patients are shown in Table 1. The male-to-female ratio was 0.64 (151 males, 237 females). Males were significantly younger than females (median age, 32 years vs 53 years, $P < 0.001$). Lung parenchymal involvement was more frequent in males than in females ($P = 0.042$), while females more frequently showed extrapulmonary involvement ($P = 0.042$), particularly in the form of ocular and cutaneous involvements, compared with males ($P = 0.004$, $P = 0.004$). Initial presentations leading to disease diagnosis were abnormal findings on chest radiography in 123 patients detected during a regular medical check-up without any subjective symptoms (32.0%) and subjective symptoms in 264 patients (68.0%) (185 patients, ocular symptoms; 13 patients, respiratory symptoms; 66 patients, other symptoms). The kappa value for diagnosis of the chest radiographic stage was 0.844 (95% confidence interval 0.816-0.872).

Smoking status at initial diagnosis of sarcoidosis for all patients (n = 388) is shown by age in Table 2. Compared with the results of the two surveys (Tables 3,4), the prevalence of current smokers in patients with sarcoidosis was not low in any age decade among either males or females except for men in their 30s. Of note, the prevalence of current smokers in their 20s was high in both male (81.5%) and female (62.2%) patients.

Table 1 Characteristics of 388 subjects with sarcoidosis

	Total (n = 388)	Male (n = 151)	Female (n = 237)	P†
Male/female‡	151 /237	151 /0	0 /237	
Age (median, range)§	49, 13–79	32, 15–78	53, 13–79	<0.001
Smoking status (never/former/current)‡	178/54/156	31/30/90	147/24/60	<0.001
Stage (0/I/II/III/IV)‡	81/190/95/22/0	24/72/47/8/0	57/118/48/14/0	0.057
Stage 0, n (%)‡	81 (20.9)	24 (15.9)	57 (24.1)	0.072
Stage I, n (%)‡	190 (49.0)	72 (47.7)	118 (49.8)	0.764
Stage II, n (%)‡	95 (24.5)	47 (31.1)	48 (20.3)	0.021
Stage III, n (%)‡	22 (5.7)	8 (5.3)	14 (5.9)	0.978
Pulmonary involvement (stage I–III), n (%)‡	307 (79.1)	127 (84.1)	180 (75.9)	0.072
Lung parenchymal involvement (stage II–III), n (%)‡	117 (30.2)	55 (36.4)	62 (26.2)	0.042
Extra pulmonary involvement, n (%)‡	315 (81.2)	112 (74.2)	203 (85.7)	0.004
Ocular involvement, n (%)‡	249 (64.2)	83 (55.0)	166 (70.0)	0.004
Cutaneous involvement, n (%)‡	87 (22.4)	21 (13.9)	66 (27.8)	0.002
Cardiac involvement, n (%)‡	23 (5.9)	11 (7.3)	12 (5.1)	0.495
Serum ACE activity (median, range)§	24.3, 0.9–66.9	23.9, 4.1–66.9	23.9, 0.9–57.8	0.434
Reason to visit hospital (symptom/health check)‡	265/123	84/67	181/56	<0.001

† P: male versus female.
‡ χ^2 test.
§ Mann–Whitney U test.
ACE, angiotensin converting enzyme.

Table 2 Prevalence of smokers by age among patients with sarcoidosis

		Age							
	Smoking status	0–19†	20–29	30–39	40–49	50–59	60–69	· 70	Total
Male	Current n (%)	1 (33.3)	53 (81.5)	9 (39.1)	8 (66.6)	9 (60.0)	10 (41.7)	0 (0)	90 (59.6)
	Former, n (%)	0 (0)	4 (6.2)	6 (26.1)	2 (16.7)	5 (33.3)	8 (33.3)	5 (55.6)	30 (19.9)
	Never, n (%)	2 (66.7)	8 (12.3)	8 (34.8)	2 (16.7)	1 (6.7)	6 (25.0)	4 (44.4)	31 (20.5)
	Total, n (%)	3 (100)	65 (100)	23 (100)	12 (100)	15 (100)	24 (100)	9 (100)	151 (100)
Female	Current n (%)	1 (25.0)	23 (62.2)	14 (45.2)	7 (30.4)	15 (19.5)	4 (10.0)	2 (8.0)	66 (27.9)
	Former, n (%)	0 (0)	5 (13.5)	5 (16.1)	1 (4.4)	8 (10.4)	3 (7.5)	2 (8.0)	24 (10.1)
	Never, n (%)	3 (75.0)	9 (24.3)	12 (38.7)	15 (65.2)	54 (70.1)	33 (82.5)	21 (84.0)	147 (62.0)
	Total, n (%)	4 (100)	37 (100)	31 (100)	23 (100)	77 (100)	40 (100)	25 (100)	237 (100)

† Smoking is prohibited for those under 20 in Japan.

The characteristics of the 388 patients with sarcoidosis according to smoking status on the initial visit are shown in Table 5. In an attempt to explore the possible effects of smoking on the clinical manifestations of sarcoidosis, the clinical manifestations were compared between current smokers and never-smokers, excluding former smokers. Patients who were current smokers (n = 156) were significantly younger than never-smokers (n = 178) (29.5 years vs 55.0 years, $P < 0.001$) and showed a greater proportion of males (57.7% vs 17.4%, $P < 0.001$). Serum angiotensin-converting enzyme activity did not differ between current and never-smokers. Current smokers were more often referred to the hospital because of abnormal findings on chest radiography detected during regular medical check-up without any subjective symptoms ($P = 0.019$). In current smokers, as compared with never-smokers, the prevalence of patients with stage 0 (without pulmonary involvement) was lower (15.4% vs 25.8%, $P = 0.027$), while the combined prevalence of stage II and stage III was higher (37.8% vs 25.3%, $P = 0.019$). The prevalence of cutaneous involvement was lower in current smokers than in never-smokers (Table 5; 16.0% vs 28.1%, $P = 0.012$). However, these differences were not significant when adjusted for sex, age and the presence of symptoms at the initial visit (Table 6; lung parenchymal involvement, $P = 0.054$; cutaneous involvement, $P = 0.118$). Pulmonary function tests were available for 191 patients with sarcoidosis. Vital capacity did not differ between the smoking group and the non-smoking group, although forced expiratory volume in 1 s/forced vital capacity was marginally, although not significantly, lower in smoking group ($P = 0.087$).

DISCUSSION

The present study first examined the prevalence of smokers in Japanese patients with sarcoidosis at

Table 3 Prevalence of smokers in Kamishihoro town, Hokkaido investigated in 2006 by using the Japanese edition of the European Community Respiratory Health Survey

		Age							
	Smoking status	0–19†	20–29	30–39	40–49	50–59	60–69	70–79	Total
Male	Current, n (%)	0 (0)	93 (52.5)	129 (59.7)	120 (52.4)	154 (45.4)	94 (33.0)	60 (22.0)	650 (42.8)
	Former, n (%)	0 (0)	16 (9.0)	20 (9.3)	54 (23.6)	100 (41.8)	96 (33.7)	94 (34.4)	380 (25.0)
	Never, n (%)	1 (100)	68 (38.4)	67 (31.0)	55 (24.0)	85 (35.6)	95 (33.3)	119 (43.6)	490 (32.2)
	Total, n (%)	1 (100)	177 (100)	216 (100)	229 (100)	339 (100)	285 (100)	272 (100)	1520 (100)
Female	Current, n (%)	0 (0)	34 (22.5)	67 (26.7)	64 (28.2)	51 (13.8)	41 (11.9)	13 (5.7)	270 (17.1)
	Former, n (%)	0 (0)	14 (9.3)	15 (6.0)	23 (10.1)	17 (4.6)	19 (5.5)	18 (7.9)	106 (6.7)
	Never, n (%)	4 (100)	103 (68.2)	169 (67.3)	140 (61.7)	302 (81.6)	285 (82.6)	197 (86.4)	1200 (76.1)
	Total, n (%)	4 (100)	151 (100)	251 (100)	227 (100)	370 (100)	345 (100)	228 (100)	1576 (100)

† Smoking is prohibited for those under 20 in Japan.

Table 4 Prevalence of current smokers (%) by age in the general population (the National Nutrition Survey conducted by Japanese Ministry of Health, Labour and Welfare)

	Year	20–29	30–39	40–49	50–59	60–69	· 70	Total
Male	2000	60.8	56.6	55.1	54.1	37.0	29.4	47.4
	2001	58.9	58.1	58.4	49.6	35.9	29.0	45.9
	2002	53.3	57.1	54.3	48.1	34.7	28.3	43.3
	2003	55.8	56.8	55.4	54.4	35.7	26.6	46.8
	2004	51.3	57.3	51.4	47.7	33.3	24.0	43.3
	2005	48.9	54.4	44.1	42.5	34.0	20.0	39.3
	2006	45.1	53.3	46.5	46.2	34.8	19.9	39.9
	2007	47.5	55.6	49.1	42.3	32.8	18.6	39.4
	2008	41.2	48.6	51.9	41.2	32.6	19.1	36.8
Female	2000	20.9	18.8	13.6	10.4	6.6	4.0	11.5
	2001	16.1	16.0	11.7	9.7	6.5	3.4	9.9
	2002	17.4	17.2	14.4	9.4	7.5	2.9	10.2
	2003	19.2	18.1	15.5	10.7	6.4	4.2	11.3
	2004	18.0	18.0	13.7	13.7	7.6	4.5	12.0
	2005	18.9	19.4	15.1	12.4	7.3	2.6	11.3
	2006	17.9	16.4	13.8	9.2	6.4	2.8	10.0
	2007	16.7	17.2	17.9	9.3	7.3	3.7	11.0
	2008	14.3	18.0	13.4	9.5	4.9	3.2	9.1

initial diagnosis, which was 59.6% in men and 27.9% in women. Unexpectedly, compared with results from two large surveys (a nationwide survey of the prevalence of asthma conducted in 2006 in Kamishihoro town, Hokkaido, and the National Nutrition Survey conducted from 2000 to 2008), the prevalence of smokers in our study was not particularly low, in contrast with results from several studies from Western countries that have found a lower prevalence of smokers among patients with sarcoidosis.[5–7] According to the results from the A Case Controlled Etiologic Study of Sarcoidosis study, a large case-control study of sarcoidosis in the United States, only 10.0% of subjects were smokers.[7] A recent case-control study in North India also showed that only 12.2% of subjects were smokers.[16] The prevalence of current smokers in the present study (40.2%) thus appears substantially higher compared with these reports. In comparison with the results of a survey of the Hokkaido area, this prevalence was lower in any age decade except for men in their 30s (39.1% vs 59.7%). In particular, the prevalence of smokers in their 20s with sarcoidosis was extremely high (81.5% in males; 62.2% in females).

The epidemiological characteristics of sarcoidosis differ by geographical distribution probably because of differences in environmental exposures and predisposing genetic factors. Compared with the clinical manifestations of sarcoidosis reported from Western countries, sarcoidosis in Japan is known to be lower in prevalence, less severe in pulmonary manifestations, and more likely to display ocular and cardiac involvements.[17,18] Several studies have shown that the effects of genetic variants of certain genes on susceptibility to diseases are affected by smoking.[19–21] In addition, in animal studies, the effect of smoking on immunological responses was found to differ according to the strain of mouse.[22] Accordingly, differences in genetic background may be one of the potential explanations for the different effect of smoking on susceptibility to

Table 5 Characteristics of 388 patients with sarcoidosis divided according to smoking status

	Current (n = 156)	Former (n = 54)	Never (n = 178)	P†
Male, n (%)‡	90 (57.7)	30 (55.6)	31 (17.4)	<0.001
Age (median, range)§	29.5, 19–73	53, 20–78	55, 13–79	<0.001
Stage 0, n (%)‡	24 (15.4)	11 (20.4)	46 (25.8)	0.027
Stage I, n (%)‡	73 (46.8)	30 (55.6)	87 (48.9)	0.787
Stage II, n (%)‡	54 (34.6)	11 (20.4)	30 (16.9)	<0.001
Stage III, n (%)‡	5 (3.2)	2 (3.7)	15 (8.4)	0.076
Pulmonary involvement (Stage I–III), n (%)‡	132 (84.6)	43 (79.6)	132 (74.2)	0.027
Lung parenchymal involvement (Stage II–III), n (%)‡	59 (37.8)	13 (24.1)	45 (25.3)	0.019
Extra pulmonary involvement, n (%)‡	119 (76.3)	47 (87.0)	149 (83.7)	0.118
Ocular involvement, n (%)‡	100 (64.1)	35 (64.8)	114 (64.0)	0.918
Cutaneous involvement, n (%)‡	25 (16.0)	12 (22.2)	50 (28.1)	0.012
Cardiac involvement, n (%)‡	6 (3.8)	5 (9.3)	12 (6.7)	0.354
Serum ACE activity (median, range)§	24.4, 6.9–48.3	23.5, 0.9–57.8	24.4, 4.1–66.9	0.930
Reason to visit hospital (symptom/health check)‡	96/60	37/17	132/46	0.019
Pulmonary function test	(n = 86)	(n = 20)	(n = 85)	
VC (L) (median, range)§	3.67, 1.65–6.58	3.16, 2.70–5.38	2.87, 1.91–5.76	<0.001
% VC (%) (median range)§	108.5, 64.8–152.7	104.4, 77.9–132.5	109.9, 76.5–146.0	0.395
FEV_1 (L) (median, range)§	3.25, 1.44–4.80	2.40, 1.86–4.73	2.26, 1.42–4.92	<0.001
FEV_1/FVC (%) (median, range)§	79.7, 64.9–113.9	80.5, 65.7–98.0	82.3, 60.1–115.2	0.087

† P: current versus never.
‡ χ^2 test.
§ Mann–Whitney U test.
Pulmonary function test was available from 191 patients with sarcoidosis.
ACE, angiotensin converting enzyme; FEV_1, forced expiratory volume in 1 s; FVC, forced vital capacity; VC, vital capacity.

Table 6 The effect of smoking and the presence of lung parenchymal and cutaneous involvement, comparing current to never-smokers, adjusted for sex, age and the presence of symptoms

	Lung parenchymal involvement		Cutaneous involvement	
	OR (95% CI)	P†	OR (95% CI)	P†
Current smoking	1.33 (0.99–1.77)	0.054	0.78 (0.57–1.07)	0.118
Sex	1.32 (0.75–2.32)	0.328	0.62 (0.32–1.21)	0.158
Age (year)	1.01 (0.99–1.03)	0.156	1.00 (0.98–1.02)	0.897
Symptom‡	2.82 (1.69–4.71)	<0.001	0.47 (0.24–0.90)	0.023

† Logistic regression analysis.
‡ Presence of subjective symptoms at the initial visit.
CI, confidence interval; OR, odds ratio.

sarcoidosis in Japanese and Western populations. Of note, recent reports from Gupta et al. have shown that the prevalence of smokers was similar in healthy volunteers and patients with sarcoidosis in a case-control study of an Indian population.[16]

In the current study, lung parenchymal involvement was greater in current smokers than in never-smokers. There have been few reports that evaluated the association with lung parenchymal involvement. Douglas et al. reported a lower prevalence of smokers in patients with sarcoidosis, while compared with the patients with stage I, the prevalence was higher in patients with lung parenchymal involvement.[5] Staton et al. reported that ^{67}Ga uptake into the lungs was higher in smokers than in non-smokers.[23] This indicates that current smokers were having more lung inflammation, and this may be consistent with the present results. However, the mechanism is unknown, and the present results were not significant after adjusting for sex, age and the presence of subjective symptoms at the initial visit. Further studies need to be done to clarify the effect of smoking on the development of each organ involvement. Furthermore, pulmonary function also did not differ among the groups. This may be due to the younger median age of current smokers (29.5 years), suggesting that period of smoking exposure might be short.

Several limitations to this study must be taken into consideration when interpreting the results. First and foremost, this was not a case-control study, and patients with sarcoidosis were not drawn from the same population as that of the general Japanese surveys. Patients with sarcoidosis were diagnosed only at three referral centres in Sapporo City, so the present cohort may not be representative of the characteristics of general patients with sarcoidosis for the entire

Hokkaido area. Several potential confounding factors that may affect the prevalence of smokers and the development of sarcoidosis, such as socioeconomic status, occupation and information on passive smoking, were not considered. The present results were compared with those from two large surveys on the prevalence of smokers in the general Japanese population. The results from the European Community Respiratory Health Survey conducted in Kamishihoro town, Hokkaido are suitable for comparison with the results from the present cohort with regard to demographic/etiological factors. However, the European Community Respiratory Health Survey was conducted in 2006, so recent trends towards decreased smoking in Japan were not considered. In contrast, the National Nutrition Survey has been conducted annually and reports the average smoking rate in all of Japan. These individual surveys may be inadequate for accurate comparison but nevertheless provide helpful and meaningful comparisons for smoking rates in the present cohort. Second, no smoking index was obtained in this study, so the cumulative effects of smoking exposure on the development of sarcoidosis remain unclear. Third, in some patients, pathological diagnosis was obtained from an extrapulmonary organ; thus, not all patients with sarcoidosis underwent lung biopsies, and one cannot exclude the possible presence of other smoking-related pulmonary diseases in patients who had abnormal shadows on chest radiography. In addition, there was no gold standard for the evaluation of chest roentgenograms. Computed tomography scan may provide more information and would be needed to clarify these associations, although chest radiography is still an easy test to perform and not all patients will undergo computed tomography scan. As such, the tendency towards the higher prevalence of stage II lung parenchymal lesions in current smokers must be interpreted with caution.

In summary, 388 patients newly diagnosed with sarcoidosis were retrospectively identified at three hospitals in Sapporo City, Japan between 2000 and 2008, and smoking status on initial presentation was evaluated. The prevalence of smokers among patients with sarcoidosis was higher than that of the Japanese general population. These results are not consistent with the hypothesis derived from the Western literature that smoking prevalence is lower in sarcoidosis patient.

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Serum KL-6 concentrations are associated with molecular sizes and efflux behavior of KL-6/MUC1 in healthy subjects

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Background: Serum KL-6, a sialylated sugar chain on human MUC1, is used as a marker of interstitial lung diseases. We recently reported that efflux behavior of KL-6/MUC1 from the alveoli into the bloodstream assessed by molecular analysis differed according to genetically determined molecular sizes and influenced serum KL-6 concentrations in sarcoidosis. This study was designed to investigate associations between molecular size and efflux behavior of KL-6/MUC1, and factors contributing to serum KL-6 concentrations in healthy subjects.
Methods: Western blot analysis using anti-KL-6 antibody was performed on serum obtained from 250 healthy subjects.
Results: The efflux behavior of KL-6/MUC1 differed according to the genetically determined molecular sizes in healthy subjects. In subjects having low molecular size, there were significant associations between smoking status, aging, renal function and serum KL-6 concentrations. However, these associations were not significant in the subjects having higher molecular size and the efflux behavior of high molecular size was the only significant determinant of serum KL-6 concentrations.
Conclusions: This study showed an association between KL-6/MUC1 efflux based on molecular size and serum KL-6 concentrations in healthy subjects. We propose that the molecular size and efflux behavior of KL-6/MUC1 should be considered when interpreting serum KL-6 concentrations.

1. Introduction

Krebs von den Lungen-6 (KL-6) is a posttranslational modification for human mucin-1 (MUC1) and a mucinous sialylated sugar chain on MUC1 is recognized by anti-KL-6 mAb [1,2]. Serum KL-6 concentrations are specifically elevated in a majority of patients with interstitial lung diseases (ILDs) [3–6]. This phenomenon is considered to reflect the increased production of KL-6/MUC1 by regenerating type II epithelial cells in the lung, and/or enhanced permeability following destruction of the alveolar–blood interface [7,8]. Therefore, measurement of serum KL-6 is widely accepted, particularly in Japan, as a diagnostic test for ILDs and is a marker of disease activity. However, in some cases, we have experienced significant limitations in the interpretation of serum KL-6 concentrations, including its low diagnostic sensitivity.

Recent reports have shown that serum KL-6 concentrations are affected by several factors other than lung diseases [9–13]. Aging and long-term smoking are associated with increased concentrations in serum KL-6 [9]. Renal function can also influence interindividual variability, as KL-6/MUC1 is renally cleared [10]. There have been several reports describing the association between serum KL-6 concentrations and the genotype of a single nucleotide polymorphism (SNP) in exon 2 (rs4072037) of the MUC1 gene [11,12]. We recently reported a significant relationship between MUC1 genotypes and KL-6/MUC1 molecular size in bronchoalveolar lavage fluid (BALF); the A allele was linked with the low molecular size KL-6/MUC1, while the G allele was linked with high molecular size in subjects with sarcoidosis [13]. In addition, the efflux behavior of KL-6/MUC1 from the alveoli into the bloodstream assessed by molecular analysis differed according to KL-6/MUC1 molecular sizes and influenced serum KL-6 concentrations [13].

We believe that our findings provide new insights into understanding the efflux mechanisms of KL-6/MUC1, and the limitations in interpreting serum KL-6 concentrations. However, these results were based on an inherent study in subjects with sarcoidosis [13], which may present obstacles for better understanding the efflux mechanisms of KL-6/MUC1 characterized by molecular analysis. The

Abbreviations: BAL, bronchoalveolar lavage; BALF, bronchoalveolar lavage fluid; ILDs, interstitial lung diseases; KL-6, Krebs von den Lungen-6; MUC1, mucin-1; VNTR, variable number of tandem repeat.

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