The Importance of Obligate Anaerobes and the *Streptococcus anginosus* Group in Pulmonary Abscess: A Clone Library Analysis Using Bronchoalveolar Lavage Fluid

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Key Words

Lung abscess · Obligate anaerobes · *Streptococcus anginosus* group · 16S ribosomal RNA · Bronchoalveolar lavage fluid · *Fusobacterium* spp.

Abstract

Background: Appropriate antibiotic use reduces the mortality of patients with lung abscess; however, 40–60% of the bacterial etiologies in these patients have remained unknown with the culture methods. Obligate anaerobes and the *Streptococcus anginosus* group are common pathogens in lung abscess, but a precise evaluation of these bacteria by ordinary culture methods seems to be difficult due to upper respiratory tract contamination. **Objectives:** The aim of this study was to assess the microbiota of lung abscess by bronchoalveolar lavage fluid (BALF) using the molecular method in comparison to culture methods. **Methods:** BALF samples obtained from the affected lesions and sputum samples of

59 patients with lung abscess were evaluated. The microbiota in BALF was analyzed according to the molecular method using the 16S ribosomal RNA gene. **Results:** Fifty-six of the 59 BALF samples were positive in polymerase chain reaction analysis. *Fusobacterium* spp. (23.7%) were most frequently detected, followed by the *S. anginosus* group (15.3%), as the predominant phylotypes. Obligate anaerobes were detected in 42.4% of the BALF specimens as the predominant phylotypes, whereas the detection rate of obligate anaerobes was 13.6% by culture methods using BALF. In addition, the detection rate among those patients in whom the phylotype of obligate anaerobes was detected in >5% of the lung microbiota according to the molecular method was 86.5% in the 'mixed-bacterial' infection group. **Conclusions:** The findings by the molecular method suggest that obligate anaerobes play important roles in the pathogenesis of lung abscess and provide additional bacterial information regarding conventional culture methods.

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Introduction

Lung abscess is a microbial infection defined as a circumscribed collection of pus in the lung parenchyma, leading to cavity formation and/or an air-fluid level within the cavity [1]. Appropriate antibiotic use for the treatment of lung abscess has contributed to a decrease in mortality [2], which ranges from 2.4 to 20% [1, 3–5]. A precise understanding of the bacterial etiology of lung abscess is important for proper antibiotic selection and duration of treatment.

Obligate anaerobes and streptococci, which may often occur due to aspiration of oropharyngeal specimens [6], are common, while other facultative bacteria are less common, as bacteria causative of lung abscess [4]; 26–80% of lung abscesses are caused by obligate anaerobes [2, 4, 7, 8].

Sputum cultures are generally not useful in the diagnosis of causative pathogens of lung abscess, due to unavoidable bacterial contamination in the upper respiratory tract, although this method is commonly used. On the other hand, bacteriological assessments of lung abscess using bronchoscopic examination, transthoracic needle aspiration, and surgically resected tissues have also been reported in some studies [2–4, 6, 8]. The precise detection of obligate anaerobes requires anaerobic culture conditions and an appropriate duration of culture, and routine aerobic bacterial culturing may not identify the true pathogens in the lung [3]. Indeed, previous reports have shown that in 37.3–64.3% of patients, the lung abscesses have an unknown etiology [1, 2, 4], and a recent report from Taiwan showed that *Klebsiella pneumoniae* was the most important pathogen in lung abscess [4]. Thus, the bacterial etiology of lung abscess is still not fully understood.

Molecular biological methods, particularly sequence-based approaches using the 16S ribosomal RNA (rRNA) gene, have recently been used as culture-independent methods for identifying causative bacteria in respiratory infections [9, 10]. We previously reported the microbiota in patients with community-acquired pneumonia (CAP) [11], healthcare-associated pneumonia (HCAP) [12] using bronchoalveolar lavage fluid (BALF) specimens, and bacterial pleurisy using pleural effusion [13] according to a bacterial 16S rRNA gene sequencing clone library analysis. The method we used has beneficial effects in that it can estimate bacterial species because of the relatively longer length of sequences (approx. 600 bp) compared to next-generation sequencing. Using this method, important roles for obligate anaerobic bacteria and oral strep-

tococci in lower respiratory tract infections have been demonstrated among patients with CAP [11], HCAP [12], and bacterial pleuritis [13] when compared to ordinary culture methods.

In this study, we investigate the lung microbiota in BALF specimens from lung abscess patients using both the clone library method and conventional culture methods.

Patients and Methods

Study Population

This study was a prospective multicenter trial targeting patients with lung abscess on whom a bronchoscopic examination was performed. This study was conducted at the University of Occupational and Environmental Health, Japan, as well as in affiliated community hospitals between October 2013 and April 2015. This study was approved by the Human and Animal Ethics Review Committee of the University of Occupational and Environmental Health, Japan (No. 09-118, UMIN000011840). All patients provided their written informed consent prior to enrollment in the study. Clinical information and laboratory and radiological findings were collected.

Definitions

Lung abscess was diagnosed when the following three criteria were fulfilled: (1) the presence of new areas of infiltrations with a cavity or low-density area within the infiltrates on chest radiographs and/or CT; (2) new clinical findings including at least two of the following: a fever, sputum production, coughing, chest pain, and leukocytosis (peripheral white blood cell count $\geq 10,000/\mu\text{l}$), and (3) exclusion of other causes.

Sample Collection

Bronchofiberscopy was performed, avoiding any contamination from the upper respiratory tract if possible, and BALF was obtained from abscess lesions of the affected lung identified on chest radiographs and/or chest CT using 40 ml of sterile saline as previously described [11, 12]. Sputum specimens were also evaluated in patients with sputum production.

Total Bacterial Cell Count and Cell Lysis Efficiency Analysis

Total bacterial cell counts and the efficiency of cell lysis were evaluated using epifluorescence microscopy as previously described [11–13].

Microbiological Evaluation Using Conventional Culture Methods

Bacterial culture of the BALF and sputum specimens was performed using a semiquantitative method [11, 12]. All positive culture results were described as microbial identification [12].

DNA Extraction and Polymerase Chain Reaction, Clone Library Construction, and Nucleotide Sequence Determination

DNA samples were extracted from the BALF, and 16S rRNA gene amplifications were performed with the universal primer sets (E341F and E907R) using a polymerase chain reaction (PCR) ther-

Fig. 1. Patient inclusion and exclusion flow diagram. CRP = C-reactive protein.

mocycler (GeneAmp PCR system 9700; Applied Biosystems, Foster City, Calif., USA) [11, 12]. Cloning of the PCR products was performed using the TOPO TA Cloning Kit (Invitrogen, Carlsbad, Calif., USA). The nucleic acid sequences of 96 randomly selected clones in each clone library were determined using a 3130xl Genetic Analyzer (Applied Biosystems).

Homology Search

Highly accurate sequences selected by Phred quality values were trimmed from the primer and vector regions, and only the sequences with good quality were used for the analysis. The detected sequences were compared with an in-house database containing the 16S rRNA gene sequences of 5,870 type strains using the basic local alignment search tool algorithm. The 16S rRNA gene sequences of type strains were obtained from the DNA Data Bank of Japan (<http://www.ddbj.nig.ac.jp/>) and the Ribosomal Database Project (<http://rdp.cme.msu.edu/>) [11–13].

Definition of Mono- or Mixed-Bacterial Infection Groups

Patients belonged to the ‘mono-bacterial’ infection group when the most predominant phylotype comprised over 80% of the detected bacterial phylotypes in each BALF sample using the clone library method, while patients who did not fall into this category were assigned to the ‘mixed-bacterial’ infection group [11, 12].

Statistical Analysis

Fisher’s exact test for tables (2×2) and the Mann-Whitney test were used as appropriate. All analyses were performed using the SPSS software package (version 19). $p < 0.05$ was considered to be statistically significant.

Results

Patient Characteristics

During the study period, 135 of 674 outpatients who underwent bronchofiberscopy were enrolled, and eventually 59 patients with lung abscess were evaluated (fig. 1). The baseline characteristics of these 59 patients are presented in table 1. The average age was 68.3 ± 10.6 years, the ratio of males was 76.3%, and 66.1% of the patients had 1 or more comorbid diseases. The average time from symptom onset to hospital visit was 13.6 ± 14.8 days, and cavity formation was found in 50.8% of the patients.

Total Bacterial Cell Count

Positive PCR results detecting the 16S rRNA gene in the BALF specimens were found in 94.9% of the patients using the clone library method; the total bacterial cell counts ranged from 1.2×10^4 to 8.9×10^8 cells/ml (median: 1.0×10^6), whereas all those in patients with negative PCR results were $<10^4$ cells/ml. The cell lysis efficiency was maintained at $\geq 80\%$ in all specimens.

Results of Culture Methods Using Sputum and BALF

The conventional culture methods identified bacteria in 66.1 and 42.4% of the patients using BALF and sputum

Table 1. Clinical and laboratory parameters of the 59 patients with lung abscess

Age, years	68.3±10.6
Gender (male/female)	45/14
BMI	20.5±3.9
Comorbidities	
Neoplastic diseases	5 (8.5)
Cerebrovascular diseases	4 (6.8)
Chronic pulmonary diseases	15 (25.4)
Cardiac diseases	9 (15.3)
Liver diseases	3 (5.1)
Renal diseases	2 (3.4)
Diabetes mellitus	15 (25.4)
Collagen vascular diseases	4 (6.8)
No comorbidity	20 (33.9)
Smoking history	40 (67.8)
Current	19 (32.2)
Duration of symptoms, days	13.6±14.8
Symptoms	
Fever	36 (61.0)
Cough	32 (54.2)
Sputum	28 (47.5)
Chest pain	11 (18.6)
Previous antibiotic treatment	19 (32.2)
Laboratory findings	
White blood cell counts, /μl	12,500±8,499
C-reactive protein, mg/dl	11.9±8.6
Albumin, g/dl	3.0±0.5
BUN ≥21 mg/dl	4 (6.8)
Glucose ≥250 mg/dl	3 (5.1)
Hct <30%	13 (22.0)
Radiographic findings	
Right upper lobe	12 (20.3)
Right middle lobe	13 (22.0)
Right lower lobe	15 (25.4)
Left upper lobe	4 (6.8)
Left lower lobe	11 (18.6)
Bilateral	4 (6.8)
Cavity formation	30 (50.8)
Pleural effusion	9 (15.3)
PSI score	88.5±30.6
In-hospital mortality	3 (5.1)

Data are presented as n (%) or means ± SD. BMI = Body mass index; PSI = pneumonia severity index.

specimens, respectively (table 2). BALF culture demonstrated that streptococci (except for *Streptococcus pneumoniae* and the *S. anginosus* group) (27.1%) were most frequently cultured, followed by *Staphylococcus aureus* (16.9%) and *Staphylococcus* spp. (except *S. aureus*) (10.2%). Obligate anaerobes were cultured in 13.6% of the patients using BALF specimens. Sputum culture

demonstrated that streptococci (except for *S. pneumoniae* and the *S. anginosus* group) (25.4%) were most frequently cultured, followed by *Corynebacterium* spp. (20.3%) and *S. aureus* (13.6%). In 16 (27.1%) and 14 (23.7%) of the 59 patients, 2 or more bacterial species were isolated from BALF and sputum specimens, respectively. The average numbers of bacteria in these patients were 3.3 and 4.0 using BALF and sputum specimens, respectively.

Predominant Bacterial Phylotypes Using the Clone Library Method

Fusobacterium spp. (23.7%) were most frequently detected as the predominant phylotype, followed by the *S. anginosus* group (15.3%), streptococci (except for *S. pneumoniae* and the *S. anginosus* group) (11.9%), *Parvimonas* spp. (6.8%), and *S. pneumoniae* (5.1%) (table 2). The details for all patients obtained by the molecular method are shown in online supplementary table S1 (for all online suppl. material, see www.karger.com/doi/10.1159/000447976). Using the clone library method, obligate anaerobic phylotypes were detected in 25 patients (42.4%) as the predominant phylotypes, and the 16S rRNA gene was amplified in 18 (90%) of 20 patients in whom no bacteria were identified in the BALF cultures [obligate anaerobes (n = 7; *Fusobacterium* spp., *Parvimonas* spp., and *Porphyromonas* spp.), the *S. anginosus* group (n = 4), and streptococci (except for *S. pneumoniae* and the *S. anginosus* group; n = 3)].

Evaluation of the Proportion of Microbiota according to the Clone Library Method

Among the 56 (94.9%) of the 59 patients in whom bacterial phylotypes were detected by the clone library method, 19 (33.9%) and 37 patients (66.1%) were categorized into the ‘mono-bacterial’ infection and the ‘mixed-bacterial’ infection groups, respectively. In the mono-bacterial infection group, the *S. anginosus* group was most frequently detected in 6 of the 19 patients (31.6%) as the predominant phylotype, whereas obligate anaerobes (>5% of the bacterial flora in each sample) were detected in 32 of the 37 patients (86.5%) in the mixed-bacterial infection group (fig. 2). Conversely, *S. anginosus* group phylotypes (>5% of the bacterial flora in each sample) were detected in only 6 of the 37 patients (16.2%). The ratio of mono-bacterial infection among patients in whom the *S. anginosus* group was detected as the predominant phylotype was significantly higher than that among patients with obligate anaerobes (table 3).

Table 2. The results of the conventional culture method and the predominant phylotypes according to the molecular method in 59 patients

	Conventional culture method		Clone library method in BALF: predominant phylotype
	sputum	BALF	
Gram-positive pathogens	47 (79.7)	48 (81.4)	22 (37.3)
<i>S. pneumoniae</i>	4 (6.8)	3 (5.1)	3 (5.1)
<i>S. aureus</i>	8 (13.6)	10 (16.9)	2 (3.4)
Methicillin-susceptible <i>S. aureus</i>	3 (5.1)	3 (5.1)	
Methicillin-resistant <i>S. aureus</i>	1 (1.7)	4 (6.8)	
Unknown ^a	4 (6.8)	3 (5.1)	
<i>Staphylococcus</i> spp. (except for <i>S. aureus</i>)	6 (10.2)	6 (10.2)	
<i>S. anginosus</i> group	2 (3.4)	4 (6.8)	9 (15.3)
<i>Streptococcus</i> spp. (except for <i>S. pneumoniae</i> and <i>S. anginosus</i> group)	15 (25.4)	16 (27.1)	7 (11.9)
<i>Granulicatella</i> spp.			1 (1.7)
<i>Corynebacterium</i> spp.	12 (20.3)	9 (15.3)	
<i>Bacillus</i> spp.		1 (1.7)	
Gram-negative pathogens	20 (33.9)	18 (30.5)	9 (15.3)
<i>Haemophilus influenzae</i>	2 (3.4)	2 (3.4)	2 (3.4)
<i>Haemophilus</i> spp. (except for <i>H. influenzae</i>)	4 (6.8)	5 (8.5)	
<i>Moraxella catarrhalis</i>	1 (1.7)		1 (1.7)
<i>Klebsiella</i> spp.	2 (3.4)	3 (5.1)	2 (3.4)
<i>Pseudomonas aeruginosa</i>	1 (1.7)	1 (1.7)	2 (3.4)
<i>Escherichia coli</i>	1 (1.7)		
<i>Enterobacter</i> spp.	2 (3.4)	1 (1.7)	1 (1.7)
<i>Acinetobacter</i> spp.	1 (1.7)		
<i>Neisseria</i> spp.	6 (10.2)	5 (8.5)	1 (1.7)
<i>Raoultella</i> spp.		1 (1.7)	
Anaerobic pathogens		8 (13.6)	25 (42.4)
<i>Fusobacterium</i> spp.			14 (23.7)
<i>Parvimonas</i> spp.		3 (5.1)	4 (6.8)
<i>Prevotella</i> spp.		3 (5.1)	2 (3.4)
<i>Porphyromonas</i> spp.			2 (3.4)
<i>Veillonella</i> spp.		1 (1.7)	
<i>Actinomyces</i> spp.		1 (1.7)	
<i>Dethiosulfovibrio</i> spp.			1 (1.7)
<i>Eubacterium</i> spp.			1 (1.7)
<i>Propionibacterium</i> spp.			1 (1.7)
Oral bacteria		1 (1.7)	
Mixed infection	14 (23.7)	16 (27.1)	37 (62.7)
Aerobes only	14 (23.7)	10 (16.9)	
Anaerobes only	0 (0.0)	0 (0.0)	
Mixture of aerobic and anaerobic pathogens	0 (0.0)	6 (10.2)	
No pathogen identified	34 (57.6)	20 (33.9)	3 (5.1)
Data are presented as n (%). Percentages refer to the total number of patients (n = 59).			
^a Drug susceptibility was not analyzed.

Fig. 2. Proportion of microbiota in the mono-bacterial and mixed-bacterial infection groups as determined by the clone library method. **a** Mono-bacterial group. **b** Mixed-bacterial group.

Comparison of the Backgrounds of Patients in Whom Facultative Bacteria, the *S. anginosus* Group, and Obligate Anaerobes Were Detected as the Predominant Phylotypes

The clinical and laboratory parameters of the patients in whom facultative bacteria, the *S. anginosus* group, and

obligate anaerobes were detected as the predominant phylotypes are shown in table 3. Patients with the *S. anginosus* group were significantly older than those with obligate anaerobes, and cerebrovascular diseases were significantly more frequently observed in patients with the *S. anginosus* group than in patients with facultative bac-

Table 3. Clinical characteristics of the patient groups with facultative bacteria, the *S. anginosus* group, and obligate anaerobes

	Facultative bacteria (n = 22)	<i>S. anginosus</i> group (n = 9)	Obligate anaerobes (n = 25)	p value
Age, years	68.7±10.5	76.0±6.8	64.8±9.9	≤0.05 ¹
Males	17 (77.3)	7 (77.8)	19 (76.0)	n.s.
BMI	19.7±3.6	21.2±5.9	20.8±3.4	n.s.
Comorbidities				n.s.
Neoplastic diseases	5 (22.7)	0 (0.0)	0 (0.0)	≤0.05 ²
Cerebrovascular diseases	0 (0.0)	3 (33.3)	1 (4.0)	≤0.05 ³
Chronic pulmonary diseases	7 (31.8)	3 (33.3)	4 (16.0)	n.s.
Cardiac diseases	5 (22.7)	2 (22.2)	2 (8.0)	n.s.
Liver diseases	0 (0.0)	0 (0.0)	3 (12.0)	n.s.
Renal diseases	1 (4.5)	0 (0.0)	1 (4.0)	n.s.
Diabetes mellitus	3 (13.6)	1 (11.1)	11 (44.0)	≤0.05 ²
Collagen vascular diseases	2 (9.1)	0 (0.0)	2 (8.0)	n.s.
No comorbidity	9 (40.9)	3 (33.3)	6 (24.0)	n.s.
Smoking history	14 (63.6)	8 (88.8)	18 (72.0)	n.s.
Current	4 (18.2)	2 (22.2)	13 (52.0)	≤0.05 ²
Duration of symptoms, days	9.0±10.4	15.2±9.1	17.2±19.2	≤0.05 ⁴
Symptoms				n.s.
Fever	13 (59.1)	4 (44.4)	17 (68.0)	n.s.
Cough	11 (50.0)	5 (55.6)	14 (56.0)	n.s.
Sputum	9 (40.9)	5 (55.6)	14 (56.0)	n.s.
Chest pain	3 (13.6)	2 (22.2)	5 (20.0)	n.s.
Preantibiotic treatment	6 (27.3)	4 (44.4)	6 (24.0)	n.s.
Laboratory findings				
White blood cell count, / μ l	10,596±7,237	17,602±15,672	12,922±5,176	n.s.
C-reactive protein, mg/dl	12.8±8.2	16.6±11.9	9.3±7.4	n.s.
Albumin, g/dl	2.9±0.58	2.8±0.5	3.0±0.5	n.s.
BUN $\geq$ 21 mg/dl	4 (18.2)	0 (0.0)	0 (0.0)	≤0.05 ²
Glucose $\geq$ 250 mg/dl	0 (0.0)	1 (25.0)	2 (13.3)	n.s.
Hct <30%	9 (40.9)	1 (11.1)	3 (12.0)	≤0.05 ²
Radiographic findings				
Right upper lobe	6 (27.3)	1 (11.1)	4 (16.0)	n.s.
Right middle lobe	2 (9.1)	3 (33.3)	7 (28.0)	n.s.
Right lower lobe	7 (31.8)	2 (22.2)	5 (20.0)	n.s.
Left upper lobe	1 (4.5)	0 (0.0)	3 (12.0)	n.s.
Left lower lobe	4 (18.2)	3 (33.3)	3 (12.0)	n.s.
Bilateral	3 (13.6)	0 (0.0)	1 (4.0)	n.s.
Cavity formation	13 (59.1)	3 (33.3)	11 (44.0)	n.s.
Pleural effusion	6 (27.3)	1 (11.1)	2 (8.0)	n.s.
Mono-bacterial group	8 (36.4)	6 (66.6)	5 (20.0)	≤0.05 ¹
PSI score	104±38	89±20	75±17	≤0.05 ⁵
In hospital mortality	3 (13.6)	0 (0.0)	0 (0.0)	n.s.

Data are presented as n (%) or means $\pm$ SD. BMI = Body mass index; PSI = pneumonia severity index; n.s. = not significant.

¹ Statistically significant difference between the *S. anginosus* and the obligate anaerobes groups.

² Statistically significant difference between the obligate anaerobes and facultative bacteria groups.

³ Statistically significant difference between the *S. anginosus* and facultative bacteria/obligate anaerobes groups.

⁴ Statistically significant difference between the facultative bacteria and *S. anginosus* groups.

⁵ Statistically significant difference between the obligate anaerobes and facultative bacteria/*S. anginosus* groups.

teria or obligate anaerobes. However, patients with diabetes mellitus (DM) as a comorbidity were significantly more often observed to have obligate anaerobes isolated than facultative bacteria. Abnormal findings of blood urea nitrogen (BUN; ≥ 21 mg/dl) and hematocrit (Hct; $< 30\%$) were significantly more frequently observed in patients with facultative bacteria than in patients with obligate anaerobes. Peripheral white blood cell counts, serum levels of C-reactive protein, and radiological findings were not significantly different between the three groups.

Discussion

In this study, the clone library method showed the utility of the molecular method in detecting obligate anaerobes and streptococci, suggesting that anaerobes play important roles in the pathogenesis of lung abscess. Additionally, we suggest that the *S. anginosus* group is especially important in elderly patients with comorbid cerebrovascular diseases.

Obligate anaerobes and the *S. anginosus* group are well known to be causative pathogens of lung abscess, whereas the etiology is unknown in 37.3–64.3% of lung abscess patients [1, 2, 4]. It was also reported that *K. pneumoniae* was an important pathogen in Taiwanese lung abscess patients [4]. Thus, the etiology of lung abscess has remained unclear. The molecular method we used has the potential merits that no conditional preparation is required and bacterial species are estimated. On the other hand, our method has the problem of how to reduce the influence of upper respiratory tract contamination in the evaluation of the bacterial flora of lower respiratory infections. Although the bronchoscopic evaluation we used in the present study unavoidably had the potential to introduce upper respiratory tract contamination, we had previously reported that PCRs for detecting the 16S rRNA gene in BALF specimens were negative for all patients ($n = 30$) with interstitial pneumonia (noninfectious causes) and that a total bacterial cell count of $> 10^4$ cells/ml is a useful criterion for indicating bacterial infection in BALF [11]. Thus, the present method using bronchofiberscopy is very useful for evaluating obligate anaerobes and oral bacteria [11–13]. In addition, the molecular method could evaluate the bacterial flora in 94.9% of the BALF specimens from patients with lung abscess in this study – as compared to 66.1% using the culture methods. Therefore, we believe that our results provide valuable information for understanding the etiology of patients with lung abscess.

Our data demonstrated that obligate anaerobes were most frequently detected as the predominant phylotypes, and these rates were obviously high in comparison to the rates of anaerobes in our previous reports on CAP (15.6%) and HCAP (9.8%) [11, 12]. Especially, *Fusobacterium* spp. accounted for half of the obligate anaerobes detected, and all of the 14 cases of *Fusobacterium* spp. were determined to be *F. nucleatum*, which has been reported to be a major anaerobic bacterium in lung abscess patients [5]. Thus, we reconfirmed the clinical importance of these bacterial species in lung abscess patients.

Members of the *S. anginosus* group are facultative anaerobic pathogens that colonize the upper airways and tend to form pus due to their unique characteristics [14–16]. In addition to obligate anaerobes, the *S. anginosus* group is common in the etiology of patients with lung abscess [2, 17–19], and it was the second most common bacterial phylotype detected in the present study. The detection rate of these bacteria was less than half of the cases in cultures in this study, and the prevalence of these oral bacteria might be underestimated in real-world clinical settings. Similarly to a former report, which described a link between the aspiration of oral flora and lung abscess development [20], our results also indicate the important and close relationship between oral bacterial flora and lung abscess. Therefore, physicians should be more aware of the *S. anginosus* group as primary pathogens of lung abscess.

Mixed infections have been recognized as being important in the pathogenesis of lung abscess, and have been reported in 21–50% of cases [2, 4, 6]. Moreover, obligate anaerobes and streptococci are common in lung abscess patients with mixed infections [5, 6, 21]. Obligate anaerobe phylotypes ($> 5\%$ of the microbiota in the BALF specimens) were detected in 86.5% of the mixed-bacterial group using the molecular method, and obligate anaerobes had previously been detected in 67.7% of CAP [11] and 46.0% of HCAP [12] patients, respectively. Our results by the molecular method may thus indicate that obligate anaerobes play a greater role in patients with lung abscess than in pneumonia patients [11, 12].

In the present study, similarly to previous observations [1, 4, 5], lung abscess was predominantly observed in adult males with comorbidities, patients with a history of unconsciousness, smoking, and long duration from the onset of symptoms to the hospital visit, especially among those patients with lung abscess caused by obligate anaerobes (table 1). No differences were observed in the duration from the onset of symptoms to the hospital visit between patients with lung abscess caused by aerobes and

those with lung abscess caused by obligate anaerobes, although significant differences were found between patients with lung abscess caused by the *S. anginosus* group and those with lung abscess caused by aerobic bacteria. As complications in lung abscess patients, DM is one of the well-known risk factors for lung abscess [2], and the incidence of morbidities in DM patients with periodontal diseases is approximately two or three times that in healthy individuals [22, 23]. In comparison with aerobes, obligate anaerobes were significantly more frequently detected in patients with DM as the predominant phylo-types in this study, indicating that obligate anaerobes were some of the main bacteria in patients with lung abscess complicated with DM, although dental conditions were not evaluated in this study. In addition, abnormal findings for BUN and Hct were significantly more frequently observed in patients with aerobes than in those with obligate anaerobes as the most predominant phylo-types in the present study. Patients with lung abscess complicated with anemia showed significantly higher mortality rates in addition to basic physical conditions, including underlying diseases [1], and elevated BUN and decreased Hct values were risk factors for severe pneumonia. Physicians should, therefore, also be aware of these abnormal laboratory findings in lung abscess patients.

There are several limitations associated with this study [11–13]. First, although we believe that our method using BALF is a useful tool for evaluating obligate anaerobes and/or oral streptococci, the influence of possible contamination of the upper respiratory tract should be considered. Second, because it took several days to obtain the final results with the clone library method we used, we could not apply the bacterial information obtained by the molecular method to the selection of antibiotics during

this study. Furthermore, we mainly used antimicrobial agents that were effective against obligate anaerobes in the present study; thus, we could not analyze the influence in the selection of antibiotic treatment according to the results of molecular analysis. Third, approximately 100 clones per library were analyzed, suggesting that bacterial 16S rRNA gene sequences present at small fractions (<1% of each sample) may be undetectable.

In conclusion, obligate anaerobes and the *S. anginosus* group were more often detected by use of the molecular method than by conventional culture methods in patients with lung abscess. These results suggest that physicians should consider the importance of these bacteria as pathogens of lung abscess and also take this information into consideration when selecting antibiotics that are effective against obligate anaerobes.

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