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Anti-PcrV titers in non-cystic fibrosis patients with *Pseudomonas aeruginosa* respiratory tract infection

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ABSTRACT

Objective: The epidemiology and role of the anti-PcrV titer in non-cystic fibrosis patients with *Pseudomonas aeruginosa* airway tract infections is not fully understood. This study was performed to compare the anti-PcrV titers of patients with and without *P. aeruginosa* respiratory tract infections.
Methods: This prospective cohort study was conducted at Hokkaido University Hospital in Japan. Participants had blood and sputum specimens collected on admission. They were divided into two groups based on their sputum culture results. Those with a *P. aeruginosa* infection were assigned to the *P. aeruginosa* (PA) group and those without a *P. aeruginosa* infection were assigned to the non-PA group. Serum anti-PcrV titers were measured using a validated ELISA.
Results: Of the 44 participants, 15 were assigned to the PA group and 29 were assigned to the non-PA group. In the PA group, 10/15 participants (66.7%) had an anti-PcrV titer >1000 ng/ml compared to 3/29 participants (10.3%) in the non-PA group ($p < 0.001$). In the PA group, two of the five participants with an anti-PcrV titer <1000 ng/ml died of recurrent *P. aeruginosa* pneumonia; the other three participants did not develop pneumonia.
Conclusion: The anti-PcrV titers in participants with *P. aeruginosa* infection varied considerably. Patients with low anti-PcrV titers and refractory *P. aeruginosa* infections need to be monitored closely.

Introduction

Pseudomonas aeruginosa is an important bacterial pathogen that causes acute life-threatening lower respiratory tract infections (LRTI), which occasionally require mechanical ventilation (Kollef et al., 2014). In addition to disease due to acute infection, *P. aeruginosa* airway colonization or chronic *P. aeruginosa* infection is associated with more frequent exacerbations and a higher mortality rate in individuals with several diseases, including cystic fibrosis (Li et al., 2005), bronchiectasis (Finch et al., 2015; Martínez-García et al., 2007), chronic obstructive pulmonary disease (COPD) (Murphy et al., 2008; Murphy, 2009), and diffuse panbronchiolitis (DPB) (Homma et al., 1983). Given the recent emergence of multidrug-resistant *P. aeruginosa* (Micek et al., 2015), the therapeutic effectiveness of current therapies for *P. aeruginosa* infection of the lower respiratory tract is becoming increasingly limited.

Recently, immunity against the type III secretion system (TTSS), one of the major virulence factors of *P. aeruginosa*, has attracted attention as a key determinant of *P. aeruginosa* infection (Sawa et al., 2014). Among the component proteins of TTSS, the PcrV antigen protects against *P. aeruginosa* infection. Several researchers have found that, in animals, anti-PcrV antibodies have a protective effect, which is enhanced when combined with antibiotic treatment (Sawa et al., 1999; Shime et al., 2001; Faure et al., 2003; Imamura et al., 2007). With regard to clinical application, several approaches have been developed for the therapeutic use of anti-PcrV antibodies (François et al., 2012; Thaden et al., 2016; Jain et al., 2018).

Previous epidemiological studies have shown that in children with cystic fibrosis and chronic *P. aeruginosa* infection of their

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respiratory tract, serum anti-PcrV antibodies increase significantly over time (Milagres et al., 2009).

To date, the number of reports describing the epidemiology and role of anti-PcrV titers in *P. aeruginosa* infections in individuals without cystic fibrosis has been limited. In a study of anti-PcrV titers in 114 patients with acute *P. aeruginosa* bacteremia, none of the patients had protective levels of PcrV titers during the acute phase of their bacteremia, despite the detection of PcrV in 99% of the *P. aeruginosa* isolates (Thaden et al., 2016). In contrast to this finding, Yasumoto et al. (2016) reported that serum anti-PcrV titers were elevated in 10.6% of 198 study participants without a history of *P. aeruginosa* infection who were anesthetized for scheduled surgeries. Currently, the epidemiology and clinical significance of anti-PcrV IgG titers in *P. aeruginosa* respiratory tract infections in individuals without cystic fibrosis is not fully understood.

This study was conducted to measure the anti-PcrV titers in patients with acute or chronic *P. aeruginosa* respiratory tract infections and to compare the titers with those of patients without *P. aeruginosa* infection.

Materials and methods

This prospective observational study was conducted over a 14-month period (February 2017 to March 2018) in the First Department of Medicine at Hokkaido University Hospital in Sapporo, Japan. The Human Subjects Review Committee of the Hospital approved the study protocol (016-0119). Written informed consent for sputum and blood sample collection was obtained from all of the study participants. The primary objective was to evaluate the anti-PcrV titers in non-cystic fibrosis patients with *P. aeruginosa* respiratory tract infections and to determine whether these were higher or not when compared with those of non-cystic fibrosis patients without *P. aeruginosa* respiratory tract infections. Exploratory objectives were to evaluate the relationship between anti-PcrV titers and *P. aeruginosa* respiratory tract infection.

Study participants and protocol

After confirming the sampling procedure (February 2017–September 2017), all patients who met the eligibility criteria and who consented to participate were enrolled (Figure 1). Participants were divided into two groups according to the presence of *P. aeruginosa* in sputum cultures. Those with *P. aeruginosa* isolated from their sputum on enrolment were assigned to the PA group and those without *P. aeruginosa* isolated from their sputum were assigned to the non-PA group, for comparison purposes. Patients with a history of *P. aeruginosa* infection but a negative sputum culture on being screened for the study were excluded. The participants were followed up until March 2018.

The inclusion criterion for chronic infection due to *P. aeruginosa* was defined as a patient with an evident previous history of *P. aeruginosa* isolation, with one or more isolates of *P. aeruginosa* from sputum during the study period. Patients who had pneumonia on admission and *P. aeruginosa* isolated on sputum culture were included in the PA group as acute *P. aeruginosa* pneumonia cases.

In the non-PA group, most of the participants had infectious pneumonia caused by an organism other than *P. aeruginosa*. Patients with an initial diagnosis of acute pneumonia and a final diagnosis of acute respiratory distress syndrome (ARDS), a drug-induced lung injury, or an acute exacerbation of interstitial pneumonia were also included in the non-PA group. Clinically stable patients with chronic lower respiratory tract disease, who did not require any additional medication and had no *P. aeruginosa* isolated on sputum culture were also included in the non-PA group.

In this study, infectious pneumonia was defined as follows: patients with a body temperature of >38 °C or <36 °C and a newly developed lesion detected by chest radiography consistent with the diagnosis of pneumonia. Findings on computed tomography (CT) scans were considered as supporting evidence. Signs and symptoms included two or more of the following: cough, purulent sputum, abnormal findings on auscultation, signs of respiratory failure, signs of dyspnea, and worsening of the tracheal aspirate fluid in patients on mechanical ventilation. One of the following was required: leukocytosis or leukopenia (white blood cell count $>10 \times 10^9$/l or $<4.5 \times 10^9$/l, respectively), band neutrophils >15%, pulse rate >120 beats/min, or systolic hypotension. In this study, infectious pneumonia was classified as community-acquired pneumonia (CAP) or hospital-acquired pneumonia (HAP) according to the American Thoracic Society guidelines (Mandell et al., 2007; American Thoracic Society and Infectious Diseases Society of America, 2005; Kalil et al., 2016).

Radiological examination

Previous chest CT scans were reviewed retrospectively and evaluated. In order to evaluate structural lung abnormalities related to *P. aeruginosa* infection, the following radiological patterns were sought: bronchiolitis (small nodules <5 mm in diameter, arranged in a centrilobular fashion), bronchiectasis (1.5 times as wide as a nearby vessel and a lack of bronchial tapering on sequential slices), traction bronchiectasis, and emphysema (Poletti et al., 2006; Yamazaki et al., 2016; Naito et al., 2017; Kadowaki et al., 2015).

Antimicrobial susceptibility testing and the detection of TTSS genes

The clinical isolates on culture were confirmed to be *P. aeruginosa* by biochemical testing and mass spectrometry (matrix-assisted

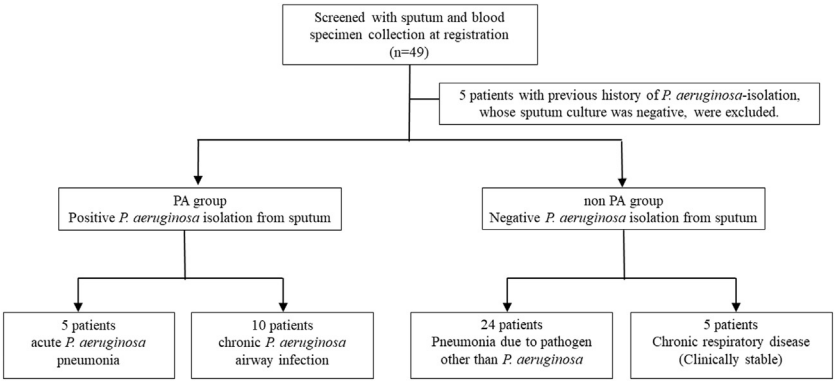

Figure 1. Flow chart illustrating the distribution of patients in this study.

laser desorption/ionization time-of-flight, MALDI-TOF). Minimum inhibitory concentrations (MIC) were then determined by broth microdilution method with cation-adjusted Mueller–Hinton broth (Becton Dickinson, Japan) according to the recommendations of the Clinical and Laboratory Standards Institute (CLSI, 2015). The following antimicrobials were used: doripenem (DRPM) (Shionogi & Co., Ltd, Japan), ceftazidime (CAZ) (Chem-Impex, USA), ciprofloxacin (CPFX) (LKT Laboratories, USA), and tobramycin (TOB) (Sigma-Aldrich, USA). Crude genomic DNA was extracted from each isolate by heat lysis. Cellular debris was removed by centrifugation, and then supernatant containing DNA was subjected to PCR. The PCR mixture was composed of Go Taq Master Mix (Promega, USA), 1.5% dimethyl sulfoxide (DMSO), 250 pM of each primer, and DNA template, in a total volume of 20 μl. Amplification reactions were performed in a thermocycler programmed as follows: an initial denaturation at 94 °C for 5 min was followed by 25 cycles of 94 °C for 30 s, 55 °C for 30 s, and 74 °C for 1 min. The primers used for amplification are shown in the **Supplementary Material** (Table S1). The PCR products were separated by electrophoresis on 1% or 2% agarose gel consisting in 1 × TAE (Tris-Acetate-Ethylenediaminetetraacetic acid). After electrophoresis, the gels were stained with ethidium bromide. Then, the gels were washed with the distilled water and the PCR products were detected as the fluorescence of ethidium bromide by fatty acid synthases (Nippon Genetics, Japan).

Anti-PcrV titer measurement

Anti-PcrV titers were quantified using an ELISA, as described previously (Yasumoto et al., 2016). Polystyrene microtiter plates (MaxiSorp; Nunc, Roskilde, Denmark) were coated with anti-biotin antibody and blocked for 2 h with blocking buffer (50 mM Tris–HCl, pH 7.5 containing 100 g/l sucrose and 20 g/l Block Ace). After three washes with wash buffer (90 g/l NaCl, 1 g/l Tween20, and 5 g/l ProClin 150), 75 μl of biotinylated-recombinant PcrV at 133 ng/ml was added to the wells and incubated for 1.5 h, and then washed with wash buffer. Then, 50 μl of 2000-fold plasma samples and 25 μl assay buffer (0.1 M NaCl, 1 mM $MgCl_2$, 0.1 mM $ZnCl_2$, 1 g/l Tween20, 10 g/l bovine serum albumin (BSA), 10 mg/l mouse gammaglobulin, 0.5 g/l NaN_3 in 0.05 M Tris–HCl, pH7.5) were added and incubated overnight at 4 °C. After washing with wash buffer, 75 μl alkaline phosphatase (ALP)-labeled anti-human IgG (H + L) (Promega, USA) at 33.3 ng/ml was added to the wells and incubated for 1.5 h. Next, 75 μl of substrate solution (CDP Star with Emerald II solution; Thermo Fisher Scientific, USA) was added and the solution was incubated for 15 min. Finally, the chemiluminescence of each well was measured using an ARVO SX reader (PerkinElmer, USA). In the ELISA assays, the human anti-PcrV mAb 6F5 (Shionogi & Co., Ltd, Japan) was utilized as a standard to quantify the anti-PcrV titers as the concentration of mAb 6F5 equivalents.

Statistical analysis

All continuous variables were expressed as the mean ± standard deviation or median (interquartile range). Differences in anti-PcrV titer between groups were evaluated using Fisher's exact test. *p*-Values of <0.05 were considered to be statistically significant. For subgroup analyses according to anti-PcrV titer, 1000 ng/ml was set as the cut-off value to classify titers as high or low.

Results

Participant characteristics

The clinical characteristics of the 15 participants in the PA group are summarized in Table 1, and those of the 29 participants in the non-PA group are summarized in Table 2.

Of the 15 participants in the PA group, five (33.3%) had acute *P. aeruginosa* pneumonia on admission and chronic *P. aeruginosa* respiratory tract infections; three (20.0%) had DPB, five (33.3%) had bronchiolitis and were using immunosuppressants, two (13.3%) had idiopathic pulmonary fibrosis, and two (13.3%) had COPD. Six of the participants in the PA group were on corticosteroids or immunosuppressants (two post organ transplantation, two for collagen vascular disease, one for bronchial asthma, and one for thymoma). Ten of the participants in the PA group had been colonized with *P. aeruginosa* for >2 years and five had been colonized for <2 years. Five of the participants had had recurrent *P. aeruginosa* pneumonia after colonization, which had required admission to hospital or the administration of intravenous antibiotics.

Of the 29 participants in the non-PA group, 19 (65.5%) had CAP and two (6.9%) had HAP. One had ARDS, one had exacerbation of interstitial pneumonia, and one had a drug-induced lung injury. Among the clinically stable participants in the non-PA group, two

Table 1
Clinical characteristics of patients in the PA group.

	Acute pneumonia (*n* = 5)	Chronic LRTI (*n* = 10)	Total (*n* = 15)
Age, years (mean ± SD)	68 ± 7.0	67.7 ± 10.5	67.8 ± 9.2
Sex: number of female patients (%)	2 (20.0)	7 (70.0)	9 (60.0)
Underlying pulmonary disease, *n* (%) of patients			
Diffuse panbronchiolitis	0 (0.0)	3 (30.0)	3 (20.0)
Bronchiolitis with immunosuppressant usage	2 (40.0)	3 (30.0)	5 (33.3)
Idiopathic pulmonary fibrosis	2 (40.0)	0 (0.0)	2 (13.3)
COPD	1 (20.0)	1 (10.0)	2 (13.3)
Others	0 (0.0)	3 (30.0)	3 (20.0)
Diabetes mellitus, *n* (%)	0 (0.0)	3 (30.0)	3 (20.0)
Corticosteroid use, *n* (%)	0 (0.0)	4 (40.0)	4 (26.7)
Immunosuppressant use, *n* (%)	2 (40.0)	3 (30.0)	5 (33.3)
Secondary hypogammaglobulinemia, *n* (%)	1 (20.0)	2 (20.0)	3 (20.0)
Duration of *P. aeruginosa* colonization, *n* (%)			
<2 years	3 (60.0)	2 (20.0)	5 (33.3)
>2 years	2 (40.0)	8 (80.0)	10 (66.7)
Recurrent *P. aeruginosa* pneumonia after colonization, *n* (%)	3 (60.0)	2 (20.0)	5 (33.3)
Chest CT scan findings, *n* (%)			
Bronchiolitis	2 (40.0)	8 (80.0)	10 (66.7)
Bronchiectasis	1 (20.0)	6 (60.0)	7 (46.7)
Traction bronchiectasis	3 (60.0)	1 (10.0)	4 (26.7)
Emphysema	1 (20.0)	3 (30.0)	4 (26.7)

LRTI, lower respiratory tract infection; SD, standard deviation; COPD, chronic obstructive pulmonary disease; CT, computed tomography.

Table 2
Clinical characteristics of patients in the non-PA group.

	Acute pneumonia ($n=24$)	Clinically stable patients ($n=5$)	Total ($n=29$)
Age, years (mean ± SD)	67.3 ± 9.9	67.6 ± 14.1	67.4 ± 10.4
Sex: number of female patients (%)	9 (37.5)	1 (20.0)	10 (34.5)
Underlying disease, *n* (%) of patients			
Community-acquired pneumonia	19 (79.2)	–	19 (65.5)
Hospital-acquired pneumonia	2 (8.3)	–	2 (6.9)
Others	3 (12.5)	5 (100.0)	8 (27.6)
Diabetes mellitus, *n* (%)	3 (12.5)	2 (40.0)	5 (17.2)
Corticosteroid use, *n* (%)	6 (25.0)	3 (60.0)	9 (31.0)
Immunosuppressant use, *n* (%)	2 (8.3)	1 (20.0)	3 (10.3)
Smoking history (current or ex-smoker), *n* (%)	13 (54.2)	0 (0.0)	13 (44.8)
Present history of LRTI due to *P. aeruginosa*, *n* (%)	0 (0.0)	0 (0.0)	0 (0.0)
Chest CT scan findings, *n* (%)			
Bronchiolitis	1 (4.2)	0 (0.0)	1 (3.4)
Bronchiectasis	4 (16.7)	1 (20.0)	5 (17.2)
Traction bronchiectasis	2 (8.3)	2 (40.0)	4 (13.8)
Emphysema	4 (16.7)	0 (0.0)	4 (13.8)

SD, standard deviation; LRTI, lower respiratory tract infection; CT, computed tomography.

Table 3
Results of serum anti-PcrV titers in this study.

Anti-PcrV antibody	PA group			Non-PA group			*p*-Value[a]
	Acute pneumonia ($n=5$)	Chronic LRTI ($n=10$)	Total, *n* (%) ($n=15$)	Acute pneumonia ($n=24$)	Clinically stable patients ($n=5$)	Total, *n* (%) ($n=29$)	
<1000	1	4	5 (33.3)	21	5	26 (89.7)	<0.005
>1000	4	6	10 (66.7)	3	0	3 (10.3)	<0.005

LRTI, lower respiratory tract infection.
[a] *p*-Value of total PA group vs. total non-PA group; *p*-values were obtained by Fisher's exact test.

had idiopathic interstitial pneumonia, two were infected with non-tuberculous mycobacteria, and one was on treatment for tuberculosis. Nine of the participants in the non-PA group were on corticosteroids or immunosuppressants (three for rheumatoid arthritis, two for lymphoma after bone marrow transplantation, two for idiopathic interstitial pneumonia, and two for sarcoidosis). None of the participants in the non-PA group had a history of *P. aeruginosa* isolation from sputum or any other specimens.

All participants in the study had CT scans. Bronchiolitis and bronchiectasis were significantly more common in participants in the PA group than in those in the non-PA group (bronchiolitis: 67% vs. 3%, $p<0.05$; bronchiectasis: 46.7% vs. 17.2%, $p<0.05$). Structural lung abnormalities were significantly more common among participants in the PA group than among those in the non-PA group (100% vs. 37.9%; $p<0.005$).

Distribution of anti-PcrV titers in the study group

Table 3 summarizes the anti-PcrV titer results according to group and type of condition (acute or chronic). The titers ranged from 19 ng/ml to 21 830 ng/ml. The median (interquartile range) of anti-PcrV titers (in ng/ml) according to subgroup were as follows: acute *P. aeruginosa* pneumonia, 3520 (2528–3688); chronic *P. aeruginosa* infection, 2027 (251–3451); infectious pneumonia/acute respiratory failure in participants in the non-PA group, 87 (72–131); clinically stable participants in the non-PA group, 65 (55–210). In the PA group, 10/15 participants (66.7%) had an anti-PcrV titer >1000 ng/ml, compared to 3/29 patients (10.3%) in the non-PA group ($p<0.001$).

Microbiological data

The characteristics of *P. aeruginosa* isolated from participants are summarized in Table 4. Of the 15 clinical isolates, all strains were *exoS*-positive and *exoU*-negative. Of the strains, three (20.0%) were resistant to DRPM, two (13.3%) were resistant to CAZ, and four (26.7%) were resistant to CPFX.

Clinical characteristics of patients in the PA group with low serum anti-PcrV titers

In the PA group, five participants (33.3%) had low anti-PcrV titers in spite of having *P. aeruginosa* infection. Their clinical characteristics are summarized in Table 5.

Both participants with low anti-PcrV titers and recurrent *P. aeruginosa* pneumonia had a poor response to treatment. Patient A had four admissions to hospital within 2 years for intravenous antibiotic treatment of *P. aeruginosa* pneumonia, and patient E had more than 10 admissions within 5 years. Patient A died due to intractable multidrug-resistant *P. aeruginosa* pneumonia that did not respond to antibiotics, including colistin. Patient E also had intractable *P. aeruginosa* pneumonia that did not respond to antibiotics, and died due to deterioration of dyspnea caused by severe *P. aeruginosa* pneumonia. Notably, patient E did not have any signs of immunodeficiency: his white blood cell count, CD4 count,

Table 4
Type III secretary protein genotype in *Pseudomonas aeruginosa* from the lower respiratory tract.

Type III secretory protein genotype	$n=15$
pcrV positive	14 (93.3)
exoS positive	15 (100.0)
exoU positive	0 (0.0)
exoT positive	15 (100.0)
exoY positive	13 (86.7)
MIC	
Doripenem >8	3 (20.0)
Ceftazidime >8	2 (13.3)
Tobramycin >4	0 (0.0)
Ciprofloxacin >4	4 (26.7)

Data are presented as *n* (%). MIC, minimum inhibitory concentration.

Table 5
Characteristics and outcomes of all five patients in the PA group with a lower serum anti-PcrV titer.

Patient	Age, sex	Underlying disease	Previous history	Immunodeficiency	Immunosuppressant usage	Anti-PcrV titer	Recurrent *P. aeruginosa* pneumonia after colonization	CT manifestation	Duration of *P. aeruginosa* colonization	Prognosis
A	57 M	Post bone marrow transplantation	Malignant lymphoma	Secondary hypogammaglobulinemia	None	111	Frequent recurrence	Traction bronchiectasis	2 years 4 months	Died due to *P. aeruginosa* pneumonia
B	72 F	Thymoma, post thymectomy	NTM infection	Secondary hypogammaglobulinemia	None	160	None	Bronchiolitis	1 year 4 months	Alive
C	67 M	Polymyositis, chronic bronchitis	–	None	TAC + steroid	122	None	Bronchiolitis	5 years	Alive
D	83 F	Diffuse panbronchiolitis	Colon cancer	None	None	475	None	Bronchiolitis + emphysema	16 years	Alive
E	66 M	Diffuse panbronchiolitis	–	None	Steroid	176	Frequent recurrence	Bronchiolitis + bronchiectasis	5 years	Died due to *P. aeruginosa* pneumonia

CT, computed tomography; M, male; F, female; NTM, non-tuberculous mycobacteria; TAC, tacrolimus.

and serum IgG were not decreased. He had DPB, but he did not have a medical history of any other specific underlying illness, including AIDS, collagen disease, and congenital immunodeficiency. Therefore, his low anti-PcrV titer could not be explained.

Discussion

In this study, serum anti-PcrV titers were evaluated in patients with and without *P. aeruginosa* LRTI. Compared to participants without *P. aeruginosa* infection, those with *P. aeruginosa* of their LRTI had significantly higher anti-PcrV titers. Notably, both participants who died due to recurrent *P. aeruginosa* pneumonia had low anti-PcrV titers.

People with structural lung abnormalities, such as cystic fibrosis, bronchiectasis, DPB, idiopathic pulmonary fibrosis, and emphysema, have a high incidence of *P. aeruginosa* airway tract colonization or infection (Poletti et al., 2006; Yamazaki et al., 2016; Naito et al., 2017; Kadowaki et al., 2015; Arancibia et al., 2002). In the present study, three participants in the PA group had DPB and 10 had radiological findings of bronchiolitis, which may reflect *P. aeruginosa* colonization of their bronchioles, and all participants in the PA group had structural lung abnormalities. In addition, the five participants in the PA group who had acute *P. aeruginosa* pneumonia at enrolment, also had a history of chronic *P. aeruginosa* respiratory tract infection. The majority of participants in the PA group had chronic *P. aeruginosa* respiratory tract infections with structural lung abnormalities, particularly bronchiolitis and bronchiectasis.

In this study, 10/15 participants in the PA group (66.7%) and 3/29 participants in the non-PA group (10.3%) had anti-PcrV titers >1000 ng/ml. Similar to previous reports (Moss et al., 2001; Banwart et al., 2002; Milagres et al., 2009), the elevation in anti-PcrV titers is likely to reflect sensitization against chronic *P. aeruginosa* infection of the respiratory tract.

With regard to the participants in the non-PA group who had elevated anti-PcrV titers, past exposure to *P. aeruginosa*, although not evident via microbiological tests, was considered possible from the features of their underlying diseases: two had pulmonary non-tuberculous mycobacteria infections and one had a congenital immunodeficiency. Among the 10 participants with anti-PcrV titers >1000 ng/ml in the PA group, five did not develop any acute *P. aeruginosa* pneumonia, while the other five did. Among those five participants, recurrent *P. aeruginosa* pneumonia was observed in three patients; however, neither of the patients in the PA group with higher anti-PcrV titers had a fatal course due to *P. aeruginosa* infection.

Of note, this study included five patients in the PA group with low anti-PcrV titers. This indicates that anti-PcrV levels are not necessarily correlated with chronic *P. aeruginosa* respiratory tract infection. As high anti-PcrV titers were observed in several participants on immunosuppressants, the reason for the low anti-PcrV titers among some participants in the PA group could not be attributed to immunosuppressive therapy alone. The absence of a history of *P. aeruginosa* pneumonia in three of the patients with low anti-PcrV titers demonstrates that low anti-PcrV titers do not necessarily contribute to susceptibility to *P. aeruginosa* respiratory tract infection. The low anti-PcrV titer of patient E, one of the two participants who died due to recurrent *P. aeruginosa* pneumonia, could not be explained. This raises the possibility that some people with recurrent *P. aeruginosa* pneumonia may have an unknown underlying immunodeficiency.

It appears that this study is the first to report the variability in anti-PcrV titers among patients with chronic *P. aeruginosa* respiratory tract infections without cystic fibrosis. The results suggest that low anti-PcrV titers are not necessarily correlated with susceptibility to *P. aeruginosa* infection, although low anti-PcrV titers may occasionally be associated with recurrent *P. aeruginosa* pneumonia and death due to *P. aeruginosa* pneumonia.

Regarding the relationship between antibody levels and chronic *P. aeruginosa* infection, Suarez-Cuartin et al. (2017) reported higher anti-*P. aeruginosa* antibody levels in patients with bronchiectasis and chronic *P. aeruginosa* infection. The antibody levels were determined using a validated ELISA kit. In their study, the sensitivity and specificity of anti-*P. aeruginosa* IgG was 95% and 74.4%, respectively, but contrary to the present study findings, the researchers did not report an association between low anti-*P. aeruginosa* antibody levels and chronic *P. aeruginosa* infection. Until now, there have been very few descriptive studies that have examined the relationship between *P. aeruginosa* respiratory tract infection and antibodies to *P. aeruginosa*.

It is believed that this study is the first to demonstrate a clinical relationship between low antibody levels and recurrent *P. aeruginosa* respiratory tract infection in people without cystic fibrosis. This finding may partially explain the mechanisms underlying recurrent *P. aeruginosa* respiratory tract infection. The results of this study show that low anti-PcrV titers are not always attributable to immunosuppressant therapy or hypogammaglobulinemia, and raise the possibility of the existence of an immunodeficiency against a restricted bacterial factor. Moreover, the variation in clinical outcome among participants with low anti-PcrV titers indicates that factors other than antibodies may contribute to the control of *P. aeruginosa* infection. Further studies are necessary to examine the epidemiology and pathology of anti-PcrV titers in *P. aeruginosa* respiratory tract infection.

This study has several limitations. First, it was conducted at a single center and included a relatively small number of participants. Second, the number of participants in the *P. aeruginosa* group was too small to assess the role of anti-PcrV titers in each phase of acute or chronic infection.

Although anti-PcrV titers largely reflect past *P. aeruginosa* infection and do not become elevated immediately after the onset of acute *P. aeruginosa* infection (Thaden et al., 2016), further studies with a focus on the acute phase or the chronic phase would also be worthwhile to elucidate the relationship between anti-PcrV titers and *P. aeruginosa* respiratory tract infection more accurately.

In conclusion, this study demonstrated that anti-PcrV titer levels vary considerably in people with chronic *P. aeruginosa* respiratory tract infections. Anti-PcrV titers are not always correlated with *P. aeruginosa* infection and its prognosis, and people with refractory *P. aeruginosa* sometimes have low anti-PcrV titers. Patients with refractory *P. aeruginosa* infections and low anti-PcrV titers may have a poorer prognosis and thus need to be monitored closely. Further research is necessary to elucidate the role of anti-PcrV titers in the prevention of *P. aeruginosa* infection of the lower respiratory tract.

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Ethical approval

The Human Subjects Review Committee of Hokkaido University Hospital approved the study protocol (016-0119). Written informed consent for sputum and blood sample collection was obtained from all study participants.

Author contributions

KN designed and interpreted the experiments and prepared the manuscript. KN and YY, with assistance of HK, HK, and MS, collected the clinical data. TF and KH contributed bacterial experiments. MY, TH, HM, and TO contributed the other experimental data. All authors contributed to discussions throughout the work.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.ijid.2019.08.008.

References

American Thoracic Society, Infectious Diseases Society of America. Guidelines for the management of adults with hospital-acquired, ventilator-associated, and healthcare-associated pneumonia. Am J Respir Crit Care Med 2005;171(4):388–416.

Arancibia F, Bauer TT, Ewig S, Mensa J, Gonzalez J, Niederman MS, et al. Community-acquired pneumonia due to gram-negative bacteria and *Pseudomonas aeruginosa*: incidence, risk, and prognosis. Arch Intern Med 2002;162(1):1849–58.

Banwart B, Splaingard ML, Farrell PM, Rock MJ, Havens PL, Moss J, et al. Children with cystic fibrosis produce an immune response against exoenzyme S, a type III cytotoxin of *Pseudomonas aeruginosa*. J Infect Dis 2002;185(2):269–70.

Faure K, Fujimoto J, Shimabukuro DW, Ajayi T, Shime N, Moriyama K, et al. Effects of monoclonal anti-PcrV antibody on *Pseudomonas aeruginosa*-induced acute lung injury in a rat model. J Immune Based Ther Vaccines 2003;1(1):2.

Finch S, McDonnell MJ, Abo-Leyah H, Aliberti S, Chalmers JD. A comprehensive analysis of the impact of *Pseudomonas aeruginosa* colonization on prognosis in adult bronchiectasis. Ann Am Thorac Soc 2015;12(11):1602–11.

François B, Luyt CE, Dugard A, Wolff M, Diehl JL, Jaber S, et al. Safety and pharmacokinetics of an anti-PcrV PEGylated monoclonal antibody fragment in mechanically ventilated patients colonized with *Pseudomonas aeruginosa*: a randomized,double-blind, placebo-controlled trial. Crit Care Med 2012;40(8):2320–6.

Homma H, Yamanaka A, Tanimoto S, Tamura M, Chijimatsu Y, Kira S, et al. Diffuse panbronchiolitis. A disease of the transitional zone of the lung. Chest 1983;83(1):63–9.

Imamura Y, Yanagihara K, Fukuda Y, Kaneko Y, Seki M, Izumikawa K, et al. Effect of anti-PcrV antibody in a murine chronic airway *Pseudomonas aeruginosa* infection model. Eur Respir J 2007;29(5):965–8.

Jain R, Beckett VV, Konstan MW, Accurso FJ, Burns JL, Mayer-Hamblett N, et al. KB001-A, a novel anti-inflammatory, found to be safe and well-tolerated in cystic fibrosis patients infected with *Pseudomonas aeruginosa*. J Cyst Fibros 2018;17(4):484–91.

Kadowaki T, Yano S, Wakabayashi K, Kobayashi K, Ishikawa S, Kimura M, et al. An analysis of etiology, causal pathogens, imaging patterns, and treatment of Japanese patients with bronchiectasis. Respir Investig 2015;53(1):37–44.

Kalil AC, Metersky ML, Klompas M, Muscedere J, Sweeney DA, Palmer LB, et al. Management of adults with hospital-acquired and ventilator-associated pneumonia: 2016 clinical practice guidelines by the infectious diseases society of America and the American thoracic society. Clin Infect Dis 2016;63(5):e61–111.

Kollef MH, Chastre J, Fagon JY, François B, Niederman MS, Rello J, et al. Global prospective epidemiologic and surveillance study of ventilator-associated pneumonia due to *Pseudomonas aeruginosa*. Crit Care Med 2014;42(10):2178–87.

Li Z, Kosorok MR, Farrell PM, Laxova A, West SE, Green CG, et al. Longitudinal development of mucoid *Pseudomonas aeruginosa* infection and lung disease progression in children with cystic fibrosis. JAMA 2005;293(5):581–8.

Mandell LA, Wunderink RG, Anzueto A, Bartlett JG, Campbell GD, Dean NC, et al. Infectious diseases society of America/American thoracic society consensus guidelines on the management of community-acquired pneumonia in adults. Clin Infect Dis 2007;44 Suppl 2:S27–72.

Martínez-García MA, Soler-Cataluña JJ, Perpiñá-Tordera M, Román-Sánchez P, Soriano J. Factors associated with lung function decline in adult patients with stable non-cystic fibrosis bronchiectasis. Chest 2007;132(5):1565–72.

Micek ST, Wunderink RG, Kollef MH, Chen C, Rello J, Chastre J, et al. An international multicenter retrospective study of *Pseudomonas aeruginosa* nosocomial pneumonia: impact of multidrug resistance. Crit Care 2015;19:219.

Milagres LG, Castro TL, Garcia D, Cruz AC, Higa L, Folescu T, et al. Antibody response to *Pseudomonas aeruginosa* in children with cystic fibrosis. Pediatr Pulmonol 2009;44(4):392–401.

Moss J, Ehrmantraut ME, Banwart BD, Frank DW, Barbieri JT. Sera from adult patients with cystic fibrosis contain antibodies to *Pseudomonas aeruginosa* type III apparatus. Infect Immun 2001;69(2):1185–8.

Murphy TF, Brauer AL, Eschberger K, Lobbins P, Grove L, Cai X, et al. *Pseudomonas aeruginosa* in chronic obstructive pulmonary disease. Am J Respir Crit Care Med 2008;177(8):853–60.

Murphy TF. *Pseudomonas aeruginosa* in adults with chronic obstructive pulmonary disease. Curr Opin Pulm Med 2009;15(2):138–42.

Naito K, Yamasaki K, Yatera K, Akata K, Noguchi S, Kawanami T, et al. Bacteriological incidence in pneumonia patients with pulmonary emphysema: a bacterial floral analysis using the 16S ribosomal RNA gene in bronchoalveolar lavage fluid. Int J Chron Obstruct Pulmon Dis 2017;12:2111–20.

Poletti V, Casoni G, Chilosi M, Zompatori M. Diffuse panbronchiolitis. Eur Respir J 2006;28(4):862–71.

Sawa T, Shimizu M, Moriyama K, Wiener-Kronish JP. Association between *Pseudomonas aeruginosa* type III secretion, antibiotic resistance, and clinical outcome: a review. Crit Care 2014;18(6):668.

Sawa T, Yahr TL, Ohara M, Kurahashi K, Gropper MA, Wiener-Kronish JP, et al. Active and passive immunization with the *Pseudomonas* V antigen protects against type III intoxication and lung injury. Nat Med 1999;5(4):392–8.

Shime N, Sawa T, Fujimoto J, Faure K, Allmond LR, Karaca T, et al. Therapeutic administration of anti-PcrV F(ab')(2) in sepsis associated with *Pseudomonas aeruginosa*. J Immunol 2001;167(10):5880–6.

Suarez-Cuartin G, Smith A, Abo-Leyah H, Rodrigo-Troyano A, Perea L, Vidal S, et al. Anti-*Pseudomonas aeruginosa* IgG antibodies and chronic airway infection in bronchiectasis. Respir Med 2017;128:1–6.

Thaden JT, Keller AE, Shire NJ, Camara MM, Otterson L, Huband M, et al. *Pseudomonas aeruginosa* bacteremic patients exhibit nonprotective antibody titers against therapeutic antibody targets PcrV and Psl exopolysaccharide. J Infect Dis 2016;213(4):640–8.

Yamazaki R, Nishiyama O, Sano H, Iwanaga T, Higashimoto Y, Kume H, et al. Clinical features and outcomes of IPF patients hospitalized for pulmonary infection: a Japanese cohort study. PLoS One 2016;11(12)e0168164.

Yasumoto H, Katoh H, Kinoshita M, Shimizu M, Hamaoka S, Akiyama K, et al. Epidemiological analysis of serum anti-*Pseudomonas aeruginosa* PcrV titers in adults. Microbiol Immunol 2016;60(2):114–20.