Table 6. Changes in Hemodynamic Parameters at Baseline and at Week 12 of Administration in Subjects

Hemodynamic parameter	Baseline (n=20)		12 week of administration (LOCF) (n=20)			
	Actual value		Actual value		Changes from baseline	
	Mean±SD	95%CI	Mean±SD	95%CI	Mean±SD	95%CI
Systolic pulmonary artery pressure (mmHg)	75.3±18.5	66.6, 84.0	72.0±20.9	62.2, 81.7	−3.4±13.4	−9.6, 2.9
Diastolic pulmonary artery pressure (mmHg)	30.1±12.4	24.2, 35.9	26.9±11.9	21.3, 32.5	−3.2±8.3	−7.0, 0.7
Systolic systemic arterial pressure (mmHg)	115.4±17.5	107.2, 123.5	116.1±16.1	108.5, 123.6	0.7±16.5	−7.0, 8.4
Diastolic systemic arterial pressure (mmHg)	68.3±14.8	61.3, 75.2	65.2±14.7	58.3, 72.1	−3.1±9.0	−7.3, 1.2
Mean systemic arterial pressure (mmHg)	88.5±19.0	79.6, 97.4	87.7±18.7	78.9, 96.4	−0.9±12.9	−6.9, 5.2
Pulmonary capillary wedge pressure (mmHg)	8.48±2.48	7.31, 9.64	9.15±3.15	7.68, 10.62	0.68±3.14	−0.79, 2.14
Right atrial pressure (mmHg)	6.6±3.4	5.0, 8.2	6.4±3.6	4.6, 8.1	−0.3±4.4	−2.3, 1.8
Cardiac index ($L \cdot min^{-1} \cdot m^{-2}$)	2.35±0.78	1.98, 2.71	2.67±0.99	2.20, 3.13	0.32±0.62	0.03, 0.61
Heart rate (beats/min)	73.59±15.05	66.54, 80.63	69.45±15.98	61.97, 76.93	−4.14±7.45	−7.62, −0.65
Pulmonary resistance index (dyne·s/cm^5/m^2)	1,581.31±791.94	1,210.67, 1,951.95	1,199.31±660.73	890.09, 1,508.54	−382.00±491.80	−612.17, −151.83
Systemic vascular resistance (dyne·s/cm^5)	1954.86±945.04	1,512.57, 2,397.16	1,689.09−606.04	1,405.45, 1,972.73	−265.77±785.52	−633.41, 101.86
Systemic vascular resistance index (dyne·s/cm^5/m^2)	3,127.11±1,564.66	2,394.82, 3,859.39	2,717.22±1,027.45	2,236.35, 3,198.08	−409.89±1271.30	−1,004.88, 185.09
Mixed blood oxygen saturation index (%)	65.37±9.74	60.81, 69.93	68.28±5.82	65.56, 71.00	2.91±9.05	−1.33, 7.15
Atrial blood oxygen saturation (%)	92.930±6.877	89.711, 96.149	93.370±3.799	91.592, 95.148	0.440±5.437	−2.104, 2.984
Atrial oxygen tension (mmHg)	74.36±15.63	67.05, 81.67	72.35±12.09	66.69, 78.00	−2.02±11.17	−7.24, 3.21
Mixed venous oxygen tension (mmHg)	36.55±4.33	34.46, 38.64	37.12±2.67*	35.83, 38.40*	0.57±4.35*	−1.53, 2.66*

*n=19.
n, no. of evaluated patients; LOCF, last observation carried forward; SD, standard deviation; CI, confidence interval.

criteria: primary endpoints are assessed; there is no violation of the inclusion and exclusion criteria that could possibly affect primary endpoints; any combination-prohibited drug with possible effects on primary endpoints is not used during the study period; and the medication adherence 80–100%. Summary statistics and 95% confidence intervals for the means were calculated with respect to the actual values of the 6-min walking distance, hemodynamic parameters, Borg dyspnea score, and plasma BNP concentration and to their changes from baseline.

The WinNonlin software version 4.1 (Pharsight Corp.; Mountain View, CA, USA) was used to determine pharmacokinetic parameters of sildenafil and its metabolite according to the noncompartment model method in subjects who did not receive any other therapeutic drug for PAH, who met all inclusion criteria for pharmacokinetic assessment and who did not fall under any exclusion criteria for the assessment. All subjects who received at least 1 dose of sildenafil were assessed for safety.

Results

Subject disposition and analysis sets are shown in **Table 1**. Twenty-one subjects were enrolled, 2 of whom discontinued the trial. Therefore, 19 subjects completed the trial. Of those who discontinued, 1 showed insufficient efficacy and 1 had an adverse event.

Among 21 subjects who were enrolled, 1 and 5 were excluded from FAS and PPS, respectively. The former patient was excluded due to no postdose measurement of all endpoints for efficacy. The latter patients were excluded from PPS because of the following reasons: 2 subjects violated the inclusion/exclusion criteria; 1 subject was excluded from FAS; 1 subject was not evaluated for primary endpoints; and 1 subject ingested a combination-prohibited drug.

The demographic characteristics of subjects and their features at baseline are shown in **Table 2**. The percentages of males and females were as follows: 19.0% (4 males) and 81.0% (17 females). Subjects had different baseline WHO functional classes: 7 with class II and 14 with class III.

The types of PAH and duration of disease are shown in **Table 3**. Among the subjects, 6, 5, 10 were diagnosed with idiopathic PAH, familiar PAH, and PAH associated with underlying disorders, respectively.

Therapeutic drugs for PAH and basic therapeutic drugs for PAH, which were administered in combination with sildenafil during the study period, are shown in **Table 4**. The major therapeutic drug for PAH, which was used in combination therapies during the study period, was beraprost, and the major basic therapeutic drugs for PAH were diuretics and oxygen therapy. Among the subjects, 9 received beraprost in combination with sildenafil; 12 received sildenafil alone.

Efficacy

The actual values of 6-min walking distance at baseline and at weeks 8 and 12 of administration, as well as changes in 6-min walking distance from baseline are shown in **Table 5**. At week 8 of administration, 6-min walking distance improved statistically significantly ($P<0.0001$) by 87.5 m from baseline. Therefore, the distance was shown to have improved as much at week 8 of administration as at week 12 of administration. At week 12 of administration, the 6-min walking distance had also improved statistically significantly ($P<0.0001$) by 84.2 m from baseline.

The actual values of hemodynamic parameters (mean PAP,

PVR, and CO) at baseline and week 12 of administration are shown in Table 6. The mean PAP and PVR at week 12 of administration decreased as compared with baseline, and CO increased as compared with baseline. The mean PAP and PVR decreased as compared with baseline, and CO increased as compared with baseline.

Among the subjects, only 1 subject showed deterioration in WHO functional class from class II at baseline to class III at week 12 of administration; 6 subjects improved (5 from class III to class II and 1 from class III to class I). Other 13 subjects sustained their class at baseline.

Hemodynamic parameters other than mPAP, PVR, and CO were also assessed. In the subjects, consequently, the change (mean±SD) in PVR index in last observation carried forward (LOCF) at week 12 of administration from baseline was –382.00±491.80 dyne·s/cm^5/m^2; therefore, PVR decreased. Furthermore, the actual value (mean±SD) of PVR index in the LOCF at week 12 of administration was 1,199.31±660.73 dyne·s/cm^5/m^2.

The changes (mean±SD) in Borgs dyspnea score in the LOCF at weeks 8 and 12 of administration from baseline were –0.84±1.89 and –0.95±1.94, respectively. Therefore, the scores decreased as compared with the baseline value (mean±SD: 3.10±1.45).

Plasma BNP concentrations showed an average decrease of 78.00 pg/ml at week 4 of administration as compared with the baseline value (mean±SD: 216.52±204.70 pg/ml) and also sustained decreases also at weeks 8 and 12 of administration.

Pharmacokinetics

The pharmacokinetics of sildenafil and its metabolite at steady state in the repeated oral administration of 20 mg t.i.d. was examined in 7 subjects. Consequently, the mean T_{max} of sildenafil was approximately 1.1 h after administration. The mean values (coefficients of variation) of C_{max}, AUC_{0-8}, $C_{ss,av}$, and C_{trough} of sildenafil at steady state were 164.88 ng/ml (45.4%), 545.14 ng·h/ml (54.1%), 68.14 ng/ml (54.1%), and 19.608 ng/ml (63.4%), respectively. Therefore, relatively large interindividual variations were observed. Furthermore, sildenafil underwent the first-pass effect and rapidly produced its metabolite, and the mean T_{max} value was approximately 1.6 h after administration. The mean values (coefficients of variation) for C_{max} and AUC_{0-8} of the metabolite were 87.27 ng/ml (35.1%) and 365.85 ng·h/ml (51.0%), respectively.

Safety

There were 36 episodes of undeniable causality in 16 cases (76.2%) among 21 subjects. There were no cases of serious and severe adverse events of undeniable causality. However, 2 subjects temporarily reduced the dose of the drug or discontinued its administration because of adverse events of undeniable causality. The major adverse events of undeniable causality were headache (10 cases, 22.7%) and flushing (8 cases, 18.2%); all the events were mild or moderate.

Therefore, there were no safety concerns about laboratory values, vital signs, and electrocardiographic findings.

Discussion

In the present multicenter, collaborative, open-label trial, PAH patients taking sildenafil showed sustained improvement in the 6-min walking distance at weeks 8 and 12 of administration without any serious adverse events causally related to sildenafil administration.

PAH is considered to be caused by pulmonary endothelial dysfunction, by the imbalance between vasoconstrictive factors such as endothelin and thromboxane, and vasorelaxant factors such as prostacyclin and nitric oxide, followed by pulmonary arterial vasoconstriction, and by the proliferation of the vascular wall including endothelium, smooth muscle and adventitia, resulting in increased resistance of pulmonary blood flow through pulmonary arterioles.[18–20] At present, there are 3 categories of effective drugs for PH which present one of the following pharmacological mechanisms. Endothelin receptor antagonists (eg, bosentan) suppress vasoconstriction induced by a vasoconstrictive factor endothelin, dilate pulmonary arteries, and regresses the proliferation of vascular wall cells.[21] Prostacyclin and nitric oxide–which are secreted from the pulmonary artery endothelium, relax smooth muscle cells, and inhibit the proliferation and promote apoptosis of vascular walls– are depleted in PAH patients. Cyclic adenosine monophosphate stimulated by prostacyclin in smooth muscle cells has a potent vasodilating and antiproliferative ability, and a synthetic prostacyclin, epoprostenol, is the first effective drug in the treatment of PH. Nitric oxide stimulates cGMP, which in turn produces cGMP with potent vasodilating and antiproliferative ability. cGMP is degraded by PDE-5 which has been reported to be increased in PAH.[22] Sildenafil inhibits PDE-5 and increases guanosine monophosphate, and alleviates PH.

Six-minute walking distance is a reliable index of functional capacity in patients with PAH and has been widely used as the primary endpoint in most clinical trials designed for patients with PAH.[23–25] In the present subjects, the 6-min walking distance increased by 87.5 m and 84.2 m at weeks 8 and 12 of administration, respectively; these values were greater than those reported with other therapeutic drugs for PAH [eg, epoprostenol (47 m),[23] bosentan (44 m),[24] and beraprost (63 m)[25]].

The efficacy and safety of sildenafil for PAH patients have been demonstrated in uncontrolled[18–20] and controlled[8,16] clinical trials. The controlled clinical trial with the largest number of patients was the Sildenafil Use in Pulmonary Arterial Hypertension (SUPER) study, in which 278 patients were enrolled; the mean 6-min walk distance and mean PVR significantly increased by 45 m and decreased by 122 dyne·s/cm^5, respectively, as compared with the placebo group.[9] Our study afforded comparable results in efficacy. Safety profiles also were almost equivalent between the SUPER study and ours. Namely, any laboratory changes of clinical concern were not observed in these studies. One patient each in the placebo group and the sildenafil 20 mg group died from right heart failure and from acute embolism and urosepsis, respectively, in the SUPER study in contrast to the present trial in which no deaths occurred. The SUPER study reported 1 serious adverse event of undeniable causality in one patient receiving 20 mg of sildenafil in contrast to none in the present trial. The incidences of headache in the SUPER study and ours were 46.0% and 22.7%, respectively.

Changes in hemodynamic parameters (mean PAP, PVR, and CO) at week 12 of administration improved as compared with baseline and indicated a decrease in the mean PAP and an increase in CO. However, no statistically significant changes were found in systemic arterial pressure and heart rate. Therefore, improvement in CO did not involve increases in systemic artery pressure and heart rate. The improvement in CO was also corroborated by an increase in mixed venous oxygen saturation. Decreases in right atrial pressure, systolic pulmonary arterial pressure, and diastolic pulmonary arterial pressure suggested the overall improvement in right heart

function by the administration of sildenafil. The secondary endpoints–changes in other hemodynamic parameters, changes in Borg dyspnea scores, and changes in plasma BNP concentrations–also improved. These results indicated the efficacy of sildenafil which was orally administered to PAH patients at a regimen of 20 mg t.i.d. for 12 weeks.

Study Limitations

The principal limitation of the present trial is the fact that it was not a double-blind, controlled study, because of ethical considerations. Therefore, the possibility of investigator or selection bias cannot be excluded completely with regard to the endpoints examined, especially functional capacity. Another limitation of the present trial was that it did not enroll any PAH patients with WHO class IV. This has possibly favored the clinical outcomes of enrolled subjects with respect to their background at baseline. Although the number of enrolled subjects was as low as at 21, the present study is the first systematically designed study that provides clinical evidence for the efficacy, safety, and pharmacokinetic profile of sildenafil in Japanese patients with PAH.

Conclusions

Sildenafil 20 mg t.i.d. was effective for patients with PAH through improvements in the 6-min walking distance, hemodynamic parameters, Borg dyspnea scores, and plasma BNP concentration after 12-week oral administration. Furthermore, sildenafil showed relatively large interindividual variations in pharmacokinetic parameters, was well tolerated by the patients, and did not elicit any concerns about safety based on the results from laboratory tests, vital signs, and electrocardiography.

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INTERNAL MEDICINE

□ CASE REPORT □

Respiratory Failure of Williams-Campbell Syndrome is Effectively Treated by Noninvasive Positive Pressure Ventilation

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Abstract

Williams-Campbell syndrome is a rare disease, characterized by a congenital deficiency of cartilage in the fourth to sixth order bronchi, leading to chronic respiratory failure with recurrent pulmonary infections. An effective and practical treatment has not yet been established. A 31-year-old man who was diagnosed as Williams-Campbell syndrome by inspiratory and expiratory computed tomography findings developed recurrent pulmonary infections and showed progressive deterioration of dyspnea. Domiciliary NPPV was administered, followed by a dramatic improvement of respiratory failure and a decrease in the episodes of pulmonary infections. NPPV may have an advantage in adults with Williams-Campbell syndrome who have severe respiratory failure and recurrent pulmonary infections.

Key words: noninvasive positive pressure ventilation (NPPV), Williams-Campbell syndrome, bronchiectasis

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Introduction

Williams-Campbell syndrome a rare disease first reported in 1960, is characterized by a congenital deficiency of cartilage in the fourth to sixth order bronchi (1). The chest high resolution computed tomography (CT) findings show severe bronchiectasis collapsing in the expiratory phase (2); this syndrome demonstrates obstructive pulmonary disorder and recurrent pulmonary infections (1, 3). Many cases are usually found in childhood, resulting in death during childhood due to recurrent pulmonary infections (3). The effective and practical treatment is still unknown except for home oxygen therapy and lung transplantation (4). Here, we describe an adult case of Williams-Campbell syndrome in whom noninvasive positive pressure ventilation (NPPV) is effective as a supportive treatment.

Case Report

A 31-year-old man who had been treated for bronchial asthma and recurrent pneumonia since childhood was diagnosed as having Williams-Campbell syndrome by inspiratory and expiratory CT at the age of 30. He had a history of admission to the hospital due to pneumonia once or twice a year. Even after the diagnosis of Williams-Campbell syndrome was established, however, he still developed recurrent pulmonary infections and showed progressive deterioration of dyspnea. He was admitted to our hospital for the treatment of his respiratory condition at the age of 31.

On examination, his height was 166.5 cm, his body weight was 85.4 kg and he had a reddish face, central cyanosis, bilateral pretibial edema, but no clubbed fingers. Chest examination demonstrated wheezes and rhonchi over the bilateral lung fields. He did not have any past history of sinusitis.

Arterial blood gas analysis showed severe respiratory fail-

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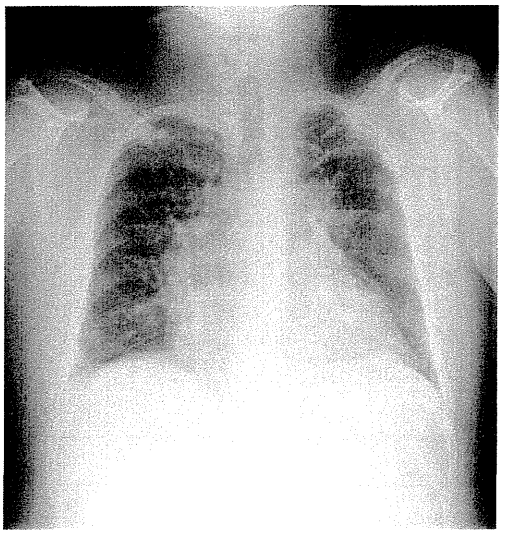

Figure 1. Chest X-ray on admission.

ure (pH 7.26, PaO_2 39.2 mmHg, $PaCO_2$ 79.7 mmHg). Laboratory data on admission demonstrated polycythemia (Hb 20.9 g/dL), but there was no elevation of white blood cells (8,200/mm^3) or CRP (0.31 mg/dL), although there was a marked elevation of brain natriuretic peptide (BNP) (175 pg/mL). Although respiratory functional tests one year previously demonstrated a mixed obstructive and restrictive ventilator defect [VC 2.31 L (50.5% of predicted), FEV_1 1.36 L (34.0% of predicted, FEV_1/FVC 56.7%, TLC 4.39 L (77.2% of predicted), RV 2.08 L (146.4% of predicted), D_{LCO} 24.97 mL/min/mmHg (84.4% of predicted), D_{LCO}/V_A 7.64 mL/min/mmHg/L (136.4% of predicted)], respiratory function tests on admission demonstrated more severe pulmonary function disorders [VC 1.46 L (36.2% of predicted), FEV_1 0.63 L (16.9% of predicted), FEV_1/FVC 44.4%]. Echocardiogram showed normal wall motion of the left ventricle (ejection fraction 54%), marked elevation of tricuspid regurgitation pressure gradient (52.1 mmHg) and D-shape of the left ventricle due to enlargement of the right ventricle. Chest x-ray showed reticular shadow, pulmonary congestion in the bilateral lung fields and enlargement of the heart (Fig. 1). A thin slice chest CT scan was obtained in both the inspiratory and expiratory phases. CT showed reticular shadow and bronchiectasis in bilateral lung fields in the inspiratory CT. CT also showed bilateral cystic bronchiectasis and collapse of the dilated bronchi in the expiratory phase (Fig. 2A and 2B). Figure 3A and 3B show an airway tree in the inspiratory and expiratory phases. These images demonstrate the collapse of the affected bronchi in the expiratory phase.

We considered that recurrent pulmonary infections resulted in irreversible pulmonary disorder and continuous hypoxemia caused secondary polycythemia and heart failure. Therefore, we initiated bilevel noninvasive positive pressure ventilation (NPPV) in addition to supplementary oxygen therapy. The ventilation was set to S/T mode; inspiratory positive airway pressure (PAP) of 16 cmH_2O, expiratory PAP of 6 cmH_2O, and the respiratory rate of 20 times/min with 4 L/min oxygen. Arterial blood gas analysis at discharge showed marked improvement of respiratory condition (pH 7.33, PaO_2 90.5 mmHg, $PaCO_2$ 62.4 mmHg at 4 L/min of O_2). The patient received domiciliary NPPV during sleep and supplementary oxygen therapy after discharge. Laboratory data 2 months after the initiation of NPPV showed marked improvement of polycythemia and heart failure (Hb 14.4 g/dL, BNP 5.94 pg/mL). Respiratory condition was maintained fairly well after the initiation of NPPV. Arterial blood gas analysis one year after the initiation of NPPV showed pH 7.36, PaO_2 88.8 mmHg, $PaCO_2$ 67.4 mmHg at 3 L/min of O_2. Pulmonary functional tests showed an improvement [VC 1.92 L (42.4% of predicted), FEV_1 0.94 L (23.8% of predicted), FEV_1/FVC 48.7%]. He gained 6 kg through the two years after the initiation of NPPV. He has not been admitted to hospital for 26 months since the initiation of NPPV. His episodes of pulmonary infections have dramatically decreased after the initiation of NPPV. Health related-quality of life (QOL) of the patient has dramatically improved since the initiation of NPPV.

Discussion

The present case demonstrates that domiciliary NPPV may have an advantage in adult patients with Williams-Campbell syndrome who have severe respiratory failure and recurrent pulmonary infections. This is important information for respiratory physicians since Williams-Campbell syndrome is considered as an intractable disorder.

Williams and Campbell first reported five pediatric cases of Williams-Campbell syndrome in 1960 (1). The most common clinical features of this disease include cough, wheezing and recurrent pulmonary infection episodes developing in early infancy (5) and the number of cases with long-term follow-up remains limited. Patients who survive into adulthood demonstrate recurrent pulmonary infections, and have limitations on physical activity (6).

There is no well-established treatment for Williams-Campbell syndrome except for lung transplantation and home oxygen therapy. Lung transplantation is the only definitive treatment. Palmer et al reported a 28-year-old man who underwent sequential lung transplantation for Williams-Campbell syndrome (4). In their case, although the patient's pulmonary function dramatically improved after transplantation, he developed recurrent bacterial pulmonary infections and died 13 months after transplantation. Lung transplantation carries two high risks. One is the risk of complications due to surgery, and the other is pulmonary infection related to proximal airway collapse and immunosuppressive agents. In contrast, NPPV has a limited risk of complications. NPPV has been reported to improve hypercapnic ventilatory failure, quality of life and survival in restrictive chest wall disease and neuromuscular disease (7, 8). It was also reported that NPPV shows improvement of chronic respiratory

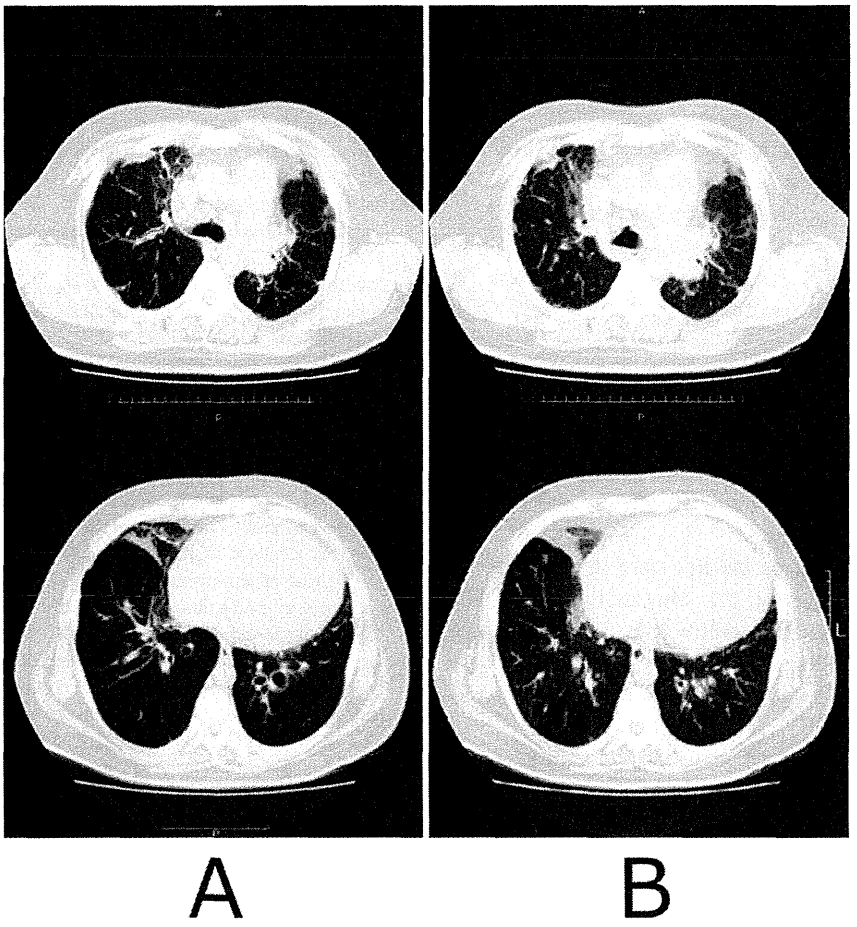

Figure 2. **Chest CT in both the inspiratory and expiratory phase. Chest CT showed reticular shadow in bilateral upper and middle lobe. Inspiratory CT (A) shows bronchiectasis and expiratory CT (B) shows collapse of the affected bronchi.**

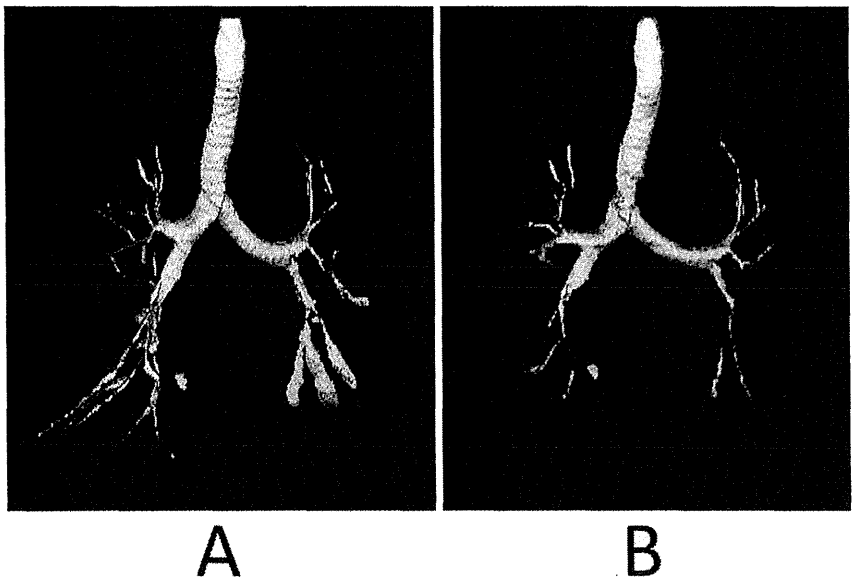

Figure 3. **Three-dimensional CT of inspiratory (A) and expiratory (B) airway tree showed collapse of the affected bronchi.**

failure in patients with bronchiectasis (9). NPPV combined with long-term home oxygen therapy decreases carbon dioxide retention and improves dyspnea and health-related QOL in hypercapnic chronic obstructive pulmonary disease (COPD) (10). Moreover, long-term NPPV may decrease acute exacerbation and recurrent hospitalization in stable hypercapnic COPD (11) and decrease the level of $PaCO_2$ (12).

Williams-Campbell syndrome is an obstructive disorder similar to COPD and NPPV has the possibility of improving dyspnea, carbon dioxide retention, and health-related QOL and the possibility of decreasing acute exacerbation in advanced adult cases of Williams-Campbell syndrome. The prevention of recurrent hospitalization due to pulmonary infections leads to an improvement in the prognosis and health-related QOL. Thus, we think NPPV may be a noninvasive and practical supportive treatment for adults with Williams-Campbell syndrome.

In conclusion, we describe the first case in which domiciliary NPPV was effective for an adult patient with Williams-Campbell syndrome. NPPV may have an advantage in adults with Williams-Campbell syndrome who have severe respiratory failure and recurrent pulmonary infections.

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

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CHEST Original Research

SLEEP DISORDERS

Differences in Breathing Patterning During Wakefulness in Patients With Mixed Apnea-Dominant vs Obstructive-Dominant Sleep Apnea

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Background: **Mixed apneas share both central and obstructive components and are often treated as if they are obstructive events. The hypothesis is that patients with obstructive sleep apnea syndrome (OSAS) who exhibit a majority of mixed apneas will differ in ventilatory control from those with predominantly obstructive apneas during wakefulness; moreover, this difference could affect nasal continuous positive airway pressure (CPAP) adherence.**

Methods: **In a retrospectively derived case-control study, 5 min of respiratory inductance plethysmography signals during wakefulness prior to sleep onset were extracted from a diagnostic polysomnogram in these groups: (1) mixed apnea-dominant OSAS (mix-OSAS) (n = 36), (2) obstructive apnea-dominant OSAS (pure-OSAS) (n = 20), (3) central apnea-dominant sleep apnea syndrome (pure-CSAS) (n = 6), and (4) control subjects (n = 10). Breathing patterning was compared between the groups using the coefficient of variation (CV) for breath-to-breath inspiration time (TI), expiration time (TE), TI + TE (Ttot), and tidal volume, and an information theory-based metric of signal pattern variability (sample entropy). Subsequent CPAP adherence over 12 months was determined in OSAS groups.**

Results: **Breath-to-breath CV parameters and sample entropy in the mix-OSAS group were significantly greater as compared with the pure-OSAS and control groups. In a subanalysis, CV and sample entropy were similar in the mix-OSAS and the pure-CSAS groups. CPAP adherence was significantly poorer in mix-OSAS compared with pure-OSAS.**

Conclusions: **During wakefulness, both breath patterning and sample entropy in mix-OSAS are similar to pure-CSAS and more variable than in pure-OSAS. In addition, CPAP adherence was decreased in patients with mix-OSAS, which may be related to basic differences in respiratory control.**

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Abbreviations: AHI = apnea-hypopnea index; CompSAS = complex sleep apnea syndrome; CPAP = continuous positive airway pressure; CV = coefficient of variation; EMG = electromyogram; ESS = Epworth sleepiness scale; mix-OSAS = mixed apnea-dominant obstructive sleep apnea syndrome; OSA = obstructive sleep apnea; OSAS = obstructive sleep apnea syndrome; pure-CSAS = central apnea-dominant sleep apnea syndrome; pure-OSAS = obstructive apnea-dominant obstructive sleep apnea syndrome; RIP = respiratory inductance plethysmography; TE = expiration time; TI = inspiration time; Ttot = inspiration time + expiration time

Obstructive sleep apnea syndrome (OSAS) is a major public health problem with a prevalence estimated at approximately 4% of adults in both Western and Asian countries.[1,2] Nasal continuous positive airway pressure (CPAP) therapy for OSAS has been the most effective and widely used treatment.[3-5] However, approximately 25% to 50% of patients with OSA will either refuse to try or will not tolerate CPAP therapy.[6] Furthermore, some patients do not respond to CPAP treatment, either without symptom improvements or without reductions in overall respiratory events. Finally, central apneas can emerge with initiation of CPAP therapy, a condition that has been called "complex sleep apnea."[7] Taken together, these facts indicate significant variability of the OSAS phenotype.

Mixed apneas are characterized by a relative lack of respiratory effort during the initial event period followed by efforts against an occluded upper airway. According to the American Academy of Sleep Medicine Task Force in 1999, mixed apneas are pathophysiologically considered to be a part of obstructive apneas.[8] Thus, patients whose apneas are mostly mixed receive the clinical diagnosis of OSAS, even though respiratory events in these patients may be primarily central as opposed to obstructive. This may contribute to the fact that some patients with OSAS do not show benefit from CPAP therapy.

In this study, we hypothesized that even before sleep onset there would be a fundamental difference in the breathing pattern between patients with mixed apnea-dominant OSAS (mix-OSAS) and those with obstructive apnea-dominant OSAS (pure-OSAS); moreover, this difference may affect CPAP acceptance and compliance. To examine these hypotheses, the breathing pattern during wakefulness was analyzed using conventional (linear) analysis of tidal volume and frequency, as well as nonlinear analysis of the respiratory signal, an approach that does not depend on breath identification. In addition, subsequent CPAP compliance was compared between OSAS groups.

The Bottom Line

How does this work advance the field?

Patients with mostly mixed apneas are diagnosed with and treated for obstructive sleep apnea syndrome. More irregular breathing during wakefulness and poor adherence to continuous positive airway pressure in patients with primarily mixed apneas identifies clinically important variability in the obstructive sleep apnea syndrome phenotype.

What are the clinical implications?

Patients with mixed apneas may have fundamental differences in respiratory control, which manifest as increased breathing pattern variability during wakefulness. An assessment of resting breathing pattern variability during wakefulness may be able to identify these patients who are less likely to adhere to subsequent continuous positive airway pressure therapy.

Materials and Methods

Subjects

Subjects were selected from 987 patients referred to a sleep laboratory with suspected sleep-disordered breathing who underwent diagnostic polysomnography between 2003 and 2008. The research database held values for the total number of apneas according to the each type (obstructive, central, and mixed) for each patient and measures of sleep latency, length, and state. Forty-four patients with mix-OSAS (4.4% of the sample) were identified and compared with patients with pure-OSAS (n = 20) and control subjects (n = 10) randomly extracted from the same database. A group with central apnea-dominant sleep apnea syndrome (pure-CSAS) (n = 7) was also identified. Data were collected on the Epworth sleepiness scale (ESS), medical history, and current medications.

Inclusion Criteria

Mix-OSAS was defined as an apnea-hypopnea index (AHI) > 20, in which the number of mixed apneas during the diagnostic study was > 30% of the total number of apneic events. Pure-OSAS was defined by an AHI > 20 in a patient in whom all of the apneas were obstructive apneas. The definition of pure-CSAS was a central apnea index > 5, where the total number of central apneas was greater than the number of obstructive apneas, or the presence of Cheyne-Stokes respiration. Patients with AHI < 5 were defined as control subjects.

Analysis of the Respiratory Signal

Approximately 5 min of stable respiratory signal data before sleep onset were extracted from the diagnostic polysomnography. Respiratory signals were generated by the sum of chest and abdominal signals using respiratory inductance plethysmography (RIP). The stable respiratory signal was identified using the respiratory signal itself as well as electromyogram (EMG) (chin and limb) to detect any body movements. When the amplitude of the EMG signal was high, that part of the signal was considered to be during movement and inappropriate for analysis. In the analytic phase of the study, investigators were blinded to the group assignment, and each 5-min record of respiratory signal during EEG staging of wakefulness was analyzed for breath-to-breath inspiration time (TI), expiration time (TE), TI + TE (Ttot), and tidal volume. To assess breathing irregularity, the coefficient of variation (CV) ([SD/mean] × 100) for each parameter was calculated.

Sample entropy is calculated from the same data sample (RIP-sum signals) but is not dependent on information of tidal volume or frequency per se. Rather, it is a statistical measure of the predictability or regularity of the data set and is defined as the logarithm of the difference between the probability that a vector X is within a chosen distance r in m-dimensional space and the probability that the vector X is within the same chosen distance r in

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m + 1-dimensional space.[9] The probability densities are normally estimated using the method suggested by Grassberger and Procaccia.[10] In the present study, sample entropy of the raw respiratory signal (sampled at 10 Hz) was calculated using standard parameters (m = 2 and $r = 0.2 \times SD$).[9] This information theory-based metric reflects linear and nonlinear determinants of temporal pattern variability with high values denoting less self-similarity and greater complexity. Comparisons of sample entropy of the original time series were made with the sample entropy of surrogate data sets (n = 19) to provide a statistical comparison between the surrogate data sets as well as to provide a means for comparing results of analyzing surrogate data and surrogate and original data through computational algorithms. Surrogate data were computed using the iterated amplitude-adjusted Fourier transform by moving the data into the frequency domain for adjustment and then back into the time domain while ensuring that both the frequency distribution (power spectrum/autocorrelation function) and the amplitude distribution are maintained.[11,12] Sample entropy was computed over multiple time delays from unity up to one cycle length.[13] Values were averaged across time lags excluding those with high linear correlations as defined by the first minimum of the mutual information function. Average sample entropy (excluding small lags) was reported for the surrogate and original data for each group.

Sleep Study

Data acquisition started from 9:00 PM and continued until 6:00 AM on the following morning. Polysomnography was performed using a polygraph system (EEG7414; Nihon Kohden; Tokyo, Japan). EEG (C3-A2, C4-A1), bilateral electrooculogram, submental EMG, ECG, and bilateral anterior tibial EMG were recorded. Airflow was monitored using an oronasal thermal sensor and/or nasal air pressure transducer. Thoracic and abdominal respiratory movements were monitored using RIP (Respitrace; Ambulatory Monitoring Inc; Ardsley, New York). Oxyhemoglobin saturation and pulse rate were monitored using pulse oximetry with a finger probe (OLV-3100; Nihon Kohden). All the signals were digitized and stored on a personal computer. Apneas were defined as an episode of complete airflow cessation measured from the thermal sensor lasting > 10 s. Hypopneas were defined by ≥ 30% reduction in amplitude of the RIP-sum signal lasting > 10 s with ≥ 3% oxygen desaturation. AHI was calculated as the average number of apnea-hypopnea events per hour over the total sleep period.

Continuous Positive Airway Pressure

Patients who had an AHI > 20 and any symptoms related to OSAS were initiated on nasal CPAP (REMstar Auto; Respironics; Pittsburgh, Pennsylvania, or GoodKnight 420E; Tyco Mallinckrodt; Plaisir, France) with auto-titrating mode. All patients treated with CPAP visited our sleep laboratory every month, and CPAP compliance was monitored every month using data extracted from the memory of the CPAP equipment for at least 12 months. At the monthly visit to the laboratory, CPAP settings, including pressure range or CPAP mode (auto or fix mode), were modified by an expert physician if it was necessary. Eventually, most of the patients used CPAP with auto-titrating mode during the follow-up period. Good CPAP compliance was defined by use in > 75% of days with > 4 h usage each night; otherwise, it was considered to be poor compliance. CPAP acceptance was defined by whether a patient refuses CPAP within 1 month after CPAP administration.

Statistical Analysis

The differences in age, sleep-disordered parameters, and CV values between three groups (mix-OSAS, pure-OSAS, and control subjects) were detected by one-way analysis of variance. When the analysis of variance was significant, probing of differences within the model was done by *t* tests of estimated marginal means (simple main effects) with adjustments for multiple comparisons made via the Bonferroni correction. The difference in categorical variables between the three groups and the difference in CPAP acceptance and compliance were detected by χ^2 test for independence. For the subanalysis, comparison between pure-CSAS and mix-OSAS was done by *t* test. Differences with $P < .05$ were considered significant. All results were expressed as means ± SD. Statistical analysis was done with SPSS, version 10.0 for Windows software (SPSS Inc; Chicago, Illinois).

RESULTS

Subject Characteristics

Among 44 patients with mix-OSAS, eight were excluded from the analysis because sufficient respiratory signal data could not be extracted because of noise; thus, 36 patients with mix-OSAS were enrolled in the study. Table 1 shows subject characteristics for each group. Significant differences in ESS were not observed. There were significant differences in age, AHI, and BMI between the three groups; however, a post hoc test did not indicate significant differences between mix-OSAS and pure-OSAS groups. In addition, four subjects suffered from arrhythmias, including chronic atrial fibrillation (n = 3) and atrioventricular block (n = 1), in the mix-OSAS group. Another two patients in the mix-OSAS group had a past history of cerebral infarction. In contrast, no patients had a history of arrhythmias or cerebral infarction in the pure-OSAS and control groups. The use of medications for hypertension, hyperlipidemia, and diabetes mellitus were similar between groups. No patients were using opioid or hypnotic medications.

Table 1—*Subject Characteristics*

Characteristic	Mix-OSAS (n = 36)	Pure-OSAS (n = 20)	Control Subjects (n = 10)	*P* Value
Age, y	56.8 ± 13.9	49.9 ± 11.9	42.5 ± 9.6[a]	< .01
AHI, per h	65.8 ± 17.8	59.1 ± 15.8	3.2 ± 1.3[a]	< .001
ESS	11.2 ± 4.8	11.7 ± 7.0	8.0 ± 3.8	NS
BMI, kg/m²	28.2 ± 3.8	27.7 ± 4.0	24.4 ± 3.7[b]	< .05
Arrhythmia	4/36 (11.1)	0/20 (0)	0/10 (0)	NS
Hypertension	15/36 (41.7)	7/20 (35)	2/10 (20)	NS
Hyperlipidemia	8/36 (22.2)	3/20 (15)	1/10 (10)	NS
Diabetes mellitus	6/36 (16.7)	2/20 (10)	1/10 (10)	NS
Past history of cerebral infarction	2/36 (5.6)	0/20 (0)	0/10 (0)	NS

Data are shown as mean ± SD or No. (%). AHI = apnea-hypopnea index; ESS = Epworth Sleepiness Scale; mix-OSAS = mixed apnea-dominant obstructive sleep apnea syndrome; NS = not significant; pure-OSAS = obstructive apnea-dominant obstructive sleep apnea syndrome.

[a]Significant difference from mix-OSAS, $P < .01$.

[b]Significant difference from mix-OSAS, $P < .05$.

Breathing Irregularity During Rest Before Sleep Onset

Figure 1 shows examples of RIP-sum signals during wakefulness for two subjects with mix-OSAS and two subjects with pure-OSAS. These tracings highlight the more irregular breathing pattern prior to sleep onset in mix-OSAS as compared with pure-OSAS. The average interval between the end of the extracted respiratory signal and sleep onset time in mix-OSAS, pure-OSAS, and control subjects was 10.0 ± 18.2, 7.5 ± 7.1, and 18.5 ± 32.8 min, respectively (data are not shown). This suggests that the irregularity of the extracted respiratory signal was not affected by drowsiness or an oscillation to sleep state. The CV values for TI, TE, and Ttot in patients with mix-OSAS were significantly higher than in pure-OSAS and control subjects (TI: 31.5 ± 18.8, 14.5 ± 10.2, 15.9 ± 7.5; TE: 41.9 ± 23.5, 18.5 ± 8.1, 19.5 ± 6.0; Ttot: 29.7 ± 16.6, 13.0 ± 6.5, 14.3 ± 6.0, respectively). Moreover, the CV of tidal volume in the mix-OSAS group was significantly higher than in pure-OSAS and control groups (37.0 ± 13.3, 18.8 ± 8.6, 23.1 ± 10.4, respectively) (Fig 2). Sample entropy for the respiratory signal in the mix-OSAS group was greater than that in both the pure-OSAS and control groups (1.42 ± 0.15, 1.20 ± 0.16, 1.19 ± 0.25, respectively). These findings suggest that there is greater complexity, less predictability, and thus greater variability in the mix-OSAS group as compared with both the control and pure-OSAS groups. However, this difference between groups remained when sample entropy of surrogate data were measured, suggesting that this difference may come from linear determinants of pattern variability in these two groups (Fig 3).

CPAP Acceptance and Compliance

In the mix-OSAS group (n = 36), all patients were treated with CPAP; however, one subject's data for CPAP usage were not recorded. Excluding this subject, only 17 out of 35 patients with mix-OSAS (48.6%) had acceptable compliance and acceptance. On the other hand, in the pure-OSAS group (n = 20), three patients chose other treatments, including oral appliance, lateral positional sleep, and weight loss, rather than CPAP; thus 17 patients were treated with CPAP. One subject's data for CPAP usage were not recorded. Thus, 16 patients with pure-OSAS were eligible for analysis of CPAP compliance. Among them, 13 patients (81%) had a good compliance and acceptance, whereas only three patients refused or could not tolerate CPAP within 1 month (Table 2). The main reasons for poor CPAP acceptance and compliance were an uncomfortable feeling with CPAP and having a sensation of it being hard to breathe and fall asleep. Some reported removing CPAP without awareness during sleep. Also, none of the three patients who refused or could not tolerate CPAP treatment felt any improvement in symptoms such as excessive daytime sleepiness, morning headache, and sleep quality.

Comparison of Mix-OSAS and Pure-CSAS Groups

Among subjects with pure-CSAS, six patients were suitable for the analysis, as one patient did not have a sufficient length of stable respiratory signal for the analysis. The central apnea index for the pure-CSAS group was 15.3 ± 12.1. Their age and BMI were 64.7 ± 10.2 years and 25.3 ± 2.4, respectively, which were similar to the mix-OSAS group. Moreover, the

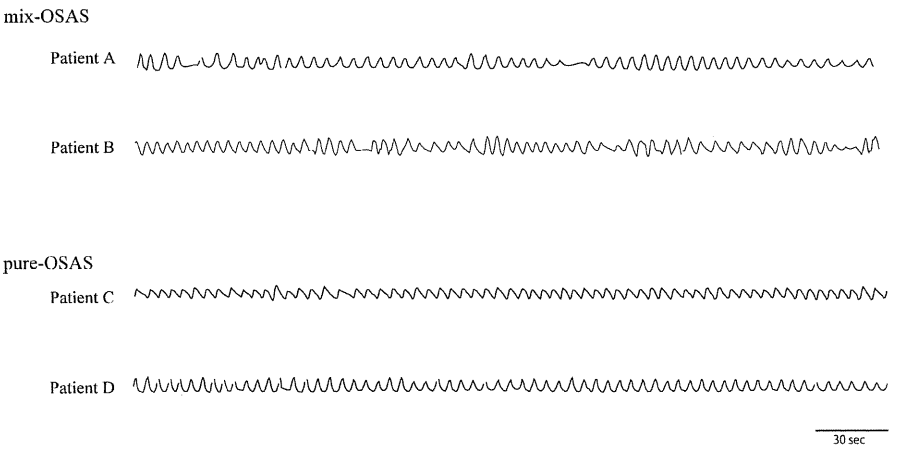

FIGURE 1. Respiratory inductance plethysmography (RIP)-sum tracings prior to sleep onset in two patients with mix-OSAS and two patients with pure-OSAS. Respiratory patterns are more irregular in mix-OSAS as compared with pure-OSAS. mix-OSAS = mixed apnea-dominant obstructive sleep apnea syndrome; pure-OSAS = obstructive apnea-dominant obstructive sleep apnea syndrome.

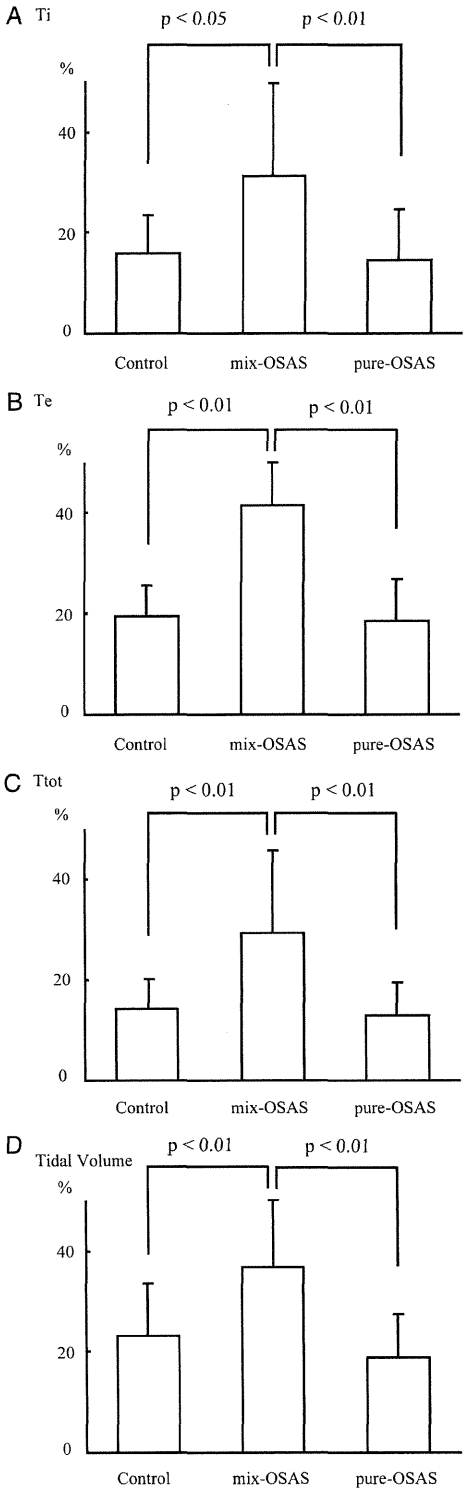

FIGURE 2. Coefficients of variation (CV) for breath-to-breath respiratory variables during resting breathing before sleep onset. Values are mean ± SD. A, Ti. B, Te. C, Ttot. D, Tidal volume. Resting breathing during wakefulness was more irregular in patients with mix-OSAS than in those with pure-OSAS and control subjects as expressed by higher CV values in all respiratory variables. Te = expiration time; Ti = inspiration time; Ttot = Ti + Te. See Figure 1 legend for expansion of other abbreviations.

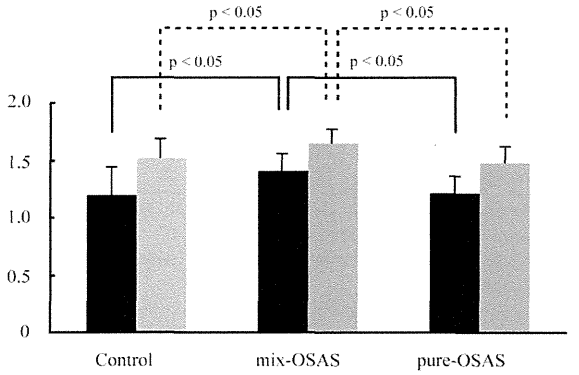

FIGURE 3. Sample entropy values for original data set (black bar) and surrogate data set (gray bar). Values are mean ± SD. See Figure 1 legend for expansion of abbreviations.

CV of TI, TE, Ttot, and tidal volume in pure-CSAS were 24.6 ± 6.5, 33.8 ± 9.2, 23.9 ± 7.6, and 34.6 ± 8.5, respectively, which were similar to the values for the mix-OSAS group. In addition, the sample entropy of the pure-CSAS group was 1.34 ± 0.17 bits, which was comparable to that observed in the mix-OSAS group ($P = .24$). However, the sample entropy of the surrogate data were 1.46 ± 0.20 bits in the pure-CSAS group, which was lower than that observed in the mix-OSAS group ($P < .05$). Taken together, these data suggest that the overall complexity and variability of the breathing pattern is similar between the pure-CSAS and the mix-OSAS groups, but that there are some differences in linear determinants of pattern variability.

DISCUSSION

The present study suggests breathing irregularity during wakefulness, as quantified by both linear and nonlinear metrics, is greater in patients with mix-OSAS as compared with patients with pure-OSAS and control subjects. Additionally, a secondary comparison indicated that breathing irregularity in patients with mix-OSAS is similar to those with pure-CSAS. This finding suggests an intrinsic pathophysiology in the respiratory control system for breathing rhythm and depth in patients with mix-OSAS. Furthermore, this instability of breathing at rest might have some predictive importance in regard to CPAP acceptance and compliance, as there was significantly poorer CPAP adherence in the mix-OSAS group as compared with the pure-OSAS group.

Breathing irregularity during wakefulness is associated with genetic diseases such as Rett syndrome,[14,15] with certain environments such as high altitude,[16,17]

Table 2—*CPAP Compliance and Acceptance*

CPAP Usage	Mix-OSAS (n = 35)[a]	Pure-OSAS (n = 16)[b]	*P* Value
Good compliance	17	13	< .01
Poor compliance	16	1	< .01
Poor acceptance	2	2	NS

CPAP = continuous positive airway pressure. See Table 1 legend for expansion of other abbreviations.

[a]One patient was excluded from the analysis because of loss of CPAP compliance data.

[b]Four patients were excluded from the analysis because three patients were given other treatments and one patient's CPAP data were lost.

with treatment with opioid medications,[18,19] and with medical conditions including heart failure[20-22] and cerebral infarction.[23,24] These phenomena reflect particular features of the respiratory control system involving respiratory rhythm generation and/or central and peripheral chemoreception. In the present study, we observed greater respiratory variability (as measured by CV of respiratory intervals) in the mix-OSAS group as compared with the pure-OSAS and control groups. This increase in breathing pattern variability was observed during a period of wakefulness, when it is rare for scoreable apnea and/or hypopnea events to occur. This finding suggests that the central respiratory control system in patients with mix-OSAS is different from those with pure-OSAS. To further investigate this difference, we quantified the morphology of the breathing pattern using sample entropy. This analysis identified a greater complexity and less predictability in the mixed group as compared with the control and obstructive groups. If this increased variability in the mixed group were due to nonlinear relationships in the data, we would expect that differences in sample entropy to be lost when looking at the surrogates. However, since these differences between the mixed and control and obstructive groups persisted on analysis of the surrogates, we concluded that the variability differences between the groups were primarily due to linear (stochastic) relationships in the data. The presence of these differences in the awake breathing patterns in these patients further supports the idea that there are fundamental differences in the respiratory control system in patients with mix-OSAS.

The pathogenesis for obstructive sleep apnea has been the focus of much study across the world. Anatomic features are key, but the neuromuscular control system also contributes to the pathogenesis of upper airway obstruction.[25-27] In this regard, OSA is already a fairly complex disease. Moreover, it has been proposed that the interaction of respiratory output to the upper airway and diaphragm may determine the expression of apnea types, such as central and obstructive.[28] Thus, individuals may manifest apneas with both obstructive and central components. The relative proportion of these components would depend on individual factors, which may be genetic or secondary to a medical condition. Taken together with our findings, we speculate that mixed apneas are closer to central apneas than to obstructive apneas. Although one can score each apnea as a mixed or obstructive apnea, the diagnosis must be OSAS because in the current American Academy of Sleep Medicine definition set a mixed apnea is considered as an obstructive apnea,[8] and "mixed sleep apnea syndrome" has not been defined. The present findings also suggest that variability in the OSAS phenotype may be one reason for the variability in CPAP treatment effectiveness for this group.

Poor CPAP compliance in the mix-OSAS group compared with the pure-OSAS group suggests that just opening the upper airway with a pressure splint is not always effective in patients with mix-OSAS. Among 36 patients with mix-OSAS, none had nasal disease; however, four patients had arrhythmias including chronic atrial fibrillation and two patients had a past history of cerebral infarction. As arrhythmias as well as cerebral infarction could affect respiration, the analyses were also performed in a subgroup excluding the six patients with these conditions. However, the significant differences in CV values between the mix-OSAS and pure-OSAS groups remained, suggesting that the presence of the arrhythmias and past history of cerebral infarction might be a surrogate marker and not necessarily the main reason for the respiratory irregularity observed in the mix-OSAS group.

Complex sleep apnea syndrome (CompSAS) is a novel category of sleep-disordered breathing that describes patients with obstructive apneas who develop frequent central apneas or Cheyne-Stokes respiration after successful application of CPAP.[29] It has been demonstrated that spectral analysis of ECG-based cardiopulmonary coupling distinguishes pure obstructive apnea from central or complex sleep apnea.[30] Moreover, patients with CompSAS show poor CPAP adherence.[31] Our study focused on mix-OSAS breathing detected during diagnostic PSG (before CPAP application). Although mixed-OSAS is distinct from CompSAS, similarities to CompSAS are relevant to our findings. Our study indicates that breath-to-breath analysis of breathing during wakefulness, which may be easier than spectral analysis of ECG-based cardiopulmonary coupling during sleep, might be able to not only distinguish mixed apnea dominant from pure obstructive sleep apnea but also predict CPAP adherence.

There are several potential limitations of the present work. First, arterial blood gas analysis was not

performed. Thus, a possible effect of hypocapnia on the irregular breathing during wakefulness in mix-OSAS or in those with central apneas cannot be excluded. However, if such a difference were present it would be another reason to suggest that the root cause for breathing irregularity is different. Second, repeat polysomnography with CPAP was not performed at follow-up. Of note, three patients with mix-OSAS had relatively high AHI (roughly around 10.0) during routine CPAP use as documented in the adherence report generated by CPAP equipment and obtained from CPAP memory. Thus, it is possible that patients with mix-OSA may be more likely to develop CPAP-emergent central apneas. Third, this was a retrospective clinical sample. Given the 4% to 5% prevalence of mix-OSAS, it would be difficult to do a prospective study to examine this issue. However, we point out that the recordings were extracted before a sleep study, and the matching to other groups was randomly done and analyzed in the same manner. In this regard there may be a bias that the recordings were acquired before sleep. Although all records were scored for state (in this case wakefulness using standard criteria) by investigators blinded to the group assignment, there may be differences in cortical control of breathing in patients with mix-OSAS and central apneas as compared with those with purely obstructive events. Whether the structure of breathing is also different at other times of the day during quiet wakefulness would need to be studied separately.

In summary, we conclude that irregular breathing during wakefulness and poor adherence to CPAP in the mix-OSAS group suggest distinct features of mix-OSAS as compared with pure-OSAS and control subjects. Mixed apneas may be part of central apneas rather than obstructive apneas, and specific or additional treatment using CPAP may be needed to treat patients with mixed apnea-dominant sleep apnea. Furthermore, an assessment of resting breathing pattern variability during wakefulness might be not only a window to explore the central respiratory control system but also a new tool to distinguish clinically important OSAS phenotypes.

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CHEST Original Research
CRITICAL CARE

Mucins Carrying Selectin Ligands as Predictive Biomarkers of Disseminated Intravascular Coagulation Complication in ARDS

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Background: **ARDS patients present with intrapulmonary and systemic coagulation abnormalities. We previously demonstrated that circulating KL-6/MUC1 could predict complications of disseminated intravascular coagulation (DIC) in ARDS. Recent studies indicate that circulating mucin can induce intravascular coagulation via interactions with selectin. We, therefore, investigated whether circulating mucins carrying selectin ligands are associated with DIC in ARDS.**
Methods: **We evaluated newly diagnosed patients with ARDS (n = 46) or bacterial pneumonia (n = 17), and healthy control subjects (n = 60). Using serum collected at diagnosis, circulating levels of KL-6/MUC1, KL-6/MUC1 carrying sialyl Lewis^a (SLAK), KL-6/MUC1 carrying sialyl Lewis^x (SLXK), and P-selectin glycoprotein ligand-1 (PSGL-1) were measured.**
Results: **Serum mucins with selectin ligands were significantly elevated in patients with ARDS compared with healthy control subjects. Significantly elevated levels of SLAK and SLXK were found in patients with ARDS subsequently complicated with DIC, as compared with those without DIC. In contrast, serum PSGL-1 levels were significantly decreased in ARDS patients with DIC. Furthermore, SLAK was discovered to be an independent predictor of DIC complication in ARDS. Using cutoff levels obtained by receiver operating characteristic curves, we found that these mucins can be used to distinguish between patients with ARDS with and without subsequently occurring DIC. Among the analyzed mucins, SLAK has the highest sensitivity and specificity for predicting future DIC development.**
Conclusions: **These results suggest that mucins with selectin ligands are novel markers for ARDS with future complications of DIC, and KL-6/MUC1 carrying selectin ligands may be involved in the pathogenesis of DIC in patients with ARDS.** ***CHEST 2011; 139(2):296–304***

Abbreviations: ALI = acute lung injury; APACHE = Acute Physiology and Chronic Health Evaluation; DIC = disseminated intravascular coagulation; ELISA = enzyme-linked immunosorbent assay; PSGL-1 = P-selectin glycoprotein ligand-1; ROC = receiver operating characteristic; SLAK = KL-6/MUC1 carrying sialyl Lewis^a; SLXK = KL-6/MUC1 carrying sialyl Lewis^x

Acute lung injury (ALI)/ARDS is a severe form of acute respiratory failure triggered by various intra- or extrapulmonary causes.[1] Previous studies suggest that enhanced coagulation and an impaired fibrinolytic system are key mediators in ALI/ARDS.[2,3] Controversial results from antiinflammatory therapies also highlight the importance of the coagulation and fibrinolytic systems in ALI/ARDS.[4-7] Patients with ARDS demonstrate systemic coagulation disorders, such as platelet consumption and disseminated intravascular coagulation (DIC). The presence of overt DIC in patients with ARDS is associated with a poorer prognosis, and the DIC scores of ALI/ARDS nonsurvivors are significantly higher than those of survivors.[8,9] Previous work from our laboratory showed that the development of DIC in patients with ARDS increases the mortality rate by more than twofold (from 32% to 83%).[10] In addition, DIC is known to be an independent factor for death in intensive care patients with severe sepsis and trauma,[11-13] which are the major causes of ALI/ARDS.[1] When complicated with DIC, the mortality rate doubles in patients with

severe sepsis.[13] Thus, DIC in patients with ALI/ARDS contributes to their extremely poor prognosis. These coagulation and fibrinolytic abnormalities typically focus on tissue factor and activated protein C, and clinical studies targeting these molecules are now under way.[4] To date, however, no evidence demonstrates direct improvements in the survival of patients with ALI/ARDS. In fact, a recent randomized trial of activated protein C failed to improve the prognosis of ALI.[14] Earlier diagnosis and treatment of DIC would contribute to a better outcome in patients with this disorder.[15] Therefore, further investigations to develop methods for the early identification and treatment of coagulation and fibrinolytic disorders are needed to improve the clinical outcome of patients with ALI/ARDS.

The selectins, a family of adhesion molecules expressed on various cell surfaces, mediate immunologic and inflammatory cell-cell reactions through selectin ligands. Antiselectin treatment has shown favorable outcomes in several experimental ALI/ARDS models.[16,17] Furthermore, levels of circulating soluble selectin molecules are associated with a prognosis of human ALI/ARDS, suggesting their importance in disease pathogenesis.[18,19] According to a recent review,[20] mucin-selectin interaction induces platelet aggregation. This interaction is involved in the mechanism of Trousseau syndrome, which is a carcinoma-induced coagulopathy. Many sugar chains, including selectin ligands, are conjugated to mucins, which can activate immune responses, cell-cell responses, and cell-cell adhesions via the sugar chains. Heavily glycosylated carcinoma mucins injected into mice can act as selectin ligands and this interaction results in the formation of disseminated platelet-rich microthrombi.[21] Moreover, the mucin-selectin-mediated intravascular coagulation does not depend on the existence of tissue factor or endotoxin.[20,21] However, the role of mucin-selectin interactions in the hypercoagulability of nonmalignant disease has not been defined completely at present.

Previously, we discovered KL-6, a sialylated sugar chain exclusively expressed on MUC1 mucin.[22,23] Until recently, KL-6/MUC1 had been used as a diagnostic and prognostic circulating marker for interstitial pneumonia, ALI/ARDS, and lung cancer.[24-29] Serum KL-6 level has been approved by Japan's Health Insurance Program as a diagnostic marker for interstitial lung diseases, and > 1 million samples are now examined per year. More recently, we demonstrated that KL-6/MUC1 was an independent predictor of DIC in patients with ARDS.[10] Increases in circulating levels of KL-6/MUC1 preceded DIC development, suggesting that KL-6/MUC1 may be involved in the pathophysiology of the coagulation abnormality in ARDS patients. On the other hand, we reported submolecular KL-6/MUC1 on which selectin ligands are expressed. We constructed an enzyme-linked immunosorbent assay (ELISA) system to measure KL-6/MUC1 carrying selectin ligand sialyl Lewis^a (SLAK) in sera.[30] The circulating level of SLAK was an independent prognostic factor in patients with lung adenocarcinoma, and higher levels of serum SLAK were observed in lung adenocarcinoma patients with distant metastasis.

We hypothesized that circulating KL-6/MUC1 with selectin ligands may be associated with intravascular coagulation in ARDS patients. To clarify this, serum levels of KL-6/MUC1 with selectin ligands were measured and compared with the level of P-selectin glycoprotein ligand-1 (PSGL-1), the mucin-like glycoprotein identified as a circulating functional ligand for P-selectin.

Materials and Methods

Subjects and Biochemical Parameters

This study was approved by the Institutional Review Board at Hiroshima University Hospital. Between April 2005 and September 2006, 46 consecutive patients newly diagnosed with ARDS at Hiroshima University Hospital were enrolled in this prospective cohort study. The diagnosis of ARDS was based on American-European Consensus Conference Committee criteria.[31] Briefly, ARDS patients were defined as those with acute-onset, severe hypoxemia (PaO_2/FIO_2 ratio < 200 mm Hg), diffuse bilateral pulmonary infiltrates on frontal radiograph, and absence of clinical evidence of left atrial hypertension. The inclusion criteria were (1) age ≥ 20 years and (2) absence of obvious malignant disease and chronic respiratory disease. For the enrollment period, one patient was excluded by the criteria because of gastric cancer.

Venous blood samples were collected and stored at −80°C within 24 h of ARDS diagnosis, with informed consent from patients or their families. All patients were assessed for a lung injury score, an Acute Physiology and Chronic Health Evaluation (APACHE) II score, a Sequential Organ Failure Assessment score, and the presence of systemic inflammatory response syndrome based on each recommended criterion.[10] All patients were treated with lung-protective ventilation. Whenever possible, venous blood samples were collected weekly for up to 2 weeks after the diagnosis of ARDS. Follow-up was calculated as the time between the date of diagnosis and the date of last contact or death,

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and all patients were provided a 60-day follow-up period. In accordance with the definition of DIC published by the International Society of Thrombosis and Haemostasis,[32] the DIC score for each patient was determined every other day for 60 days after diagnosis of ARDS. In brief, patients were scored on global coagulation tests (platelet count, fibrin degradation products, prothrombin time, and fibrinogen level), and the patients whose DIC score was >5 in their clinical course were diagnosed as complicated with overt DIC. To use this criteria, fibrin degradation product values of <10, 10 to 25, and >25 mg/L were assessed as no increase, moderate increase, and strong increase of fibrin degradation products, respectively. As previously described, the patients who presented with overt DIC during the clinical course of ARDS were described as "ARDS patients with DIC" and the patients who did not suffer from DIC during the whole clinical course were described as "ARDS patients without DIC."[10]

Each serum sample was analyzed for KL-6/MUC1, lactate dehydrogenase, and C-reactive protein. KL-6/MUC1 levels were measured with an ELISA from a commercially available kit (Eitest KL-6; Eisai; Tokyo, Japan). Each plasma sample was analyzed for fibrin degradation products and D-dimer with a commercially available latex agglutination kit (LPIA FDP-P and LPIA ace D-D dimer; Mitsubishi Chemical Medience Co; Tokyo, Japan). Serum high mobility group box 1, which has been found to be elevated in patients with DIC,[33] was also determined by a commercially available ELISA kit (HMGB1 ELISA Kit; Shino-Test Co; Tokyo, Japan). In addition, venous blood samples were collected from 60 age- and sex-matched healthy subjects and 17 patients with bacterial pneumonia with neither ALI/ARDS nor DIC. These subjects were assigned to either healthy or diseased control groups.

Measurement of Mucins Carrying Selectin Ligands

Serum KL-6/MUC1 molecules carrying selectin ligands were detected by sandwich ELISA. Because both sialyl Lewis^a and sialyl Lewis^x act as P-, L-, and E-selectin ligands on mucins, anti-KL-6/MUC1 antibody in combination with either anti-sialyl Lewis^a or anti-sialyl Lewis^x antibodies were used. SLAK was measured as previously described.[30]

Similarly, we constructed a sandwich ELISA system to detect KL-6/MUC1 carrying sialyl Lewis^x (SLXK) by using monoclonal mouse IgM antisialyl Lewis^x antibody (Chemicon International; Temecula, California). The pleural effusion in patients with lung cancer was used as a standard reference sample. One unit per milliliter was defined as the SLXK level, which was calculated from the pleural effusion (diluted 1:2,560; details of the measurement of SLXK are described in e-Appendix 1). PSGL-1, the major ligand for P-selectin, was also measured by a commercially available ELISA kit (Bender Medsystems GmbH; Vienna, Austria). The intra- and interassay coefficient of variation was evaluated in three independent experiments. Each assay was carried out with four replicates of four serum samples containing different concentrations of SLAK and SLXK.

Statistical Analysis

Data are shown as the median values and interquartile ranges. Differences between two groups were analyzed using the Mann-Whitney *U* test or Fisher exact test. The Kruskal-Wallis test was used to compare parameters among more than three groups. Univariate and multivariate analyses of predictive factors were performed using the Cox proportional hazard regression model to assess the combined influence of variables on the complications of DIC. Independent predictors for DIC complications at diagnosis of ARDS were identified from among the parameters whose *P* values were <.2 in a univariate analysis. In this step, DIC score-related parameters (DIC score, platelet count, and fibrin degradation products) were eliminated from the analysis, because these parameters were part of DIC itself. Hazard ratios and 95% CIs were computed according to the higher value of each group, except for sex and cause of ARDS. The median values were used to discriminate between the high and low groups. For the variables sex and cause of ARDS, male gender and direct cause were used, respectively. The circulating mucins (KL-6/MUC1, SLAK, SLXK, and PSGL-1) were further analyzed for their predictive capabilities for DIC complications by receiver operating characteristic (ROC) curves and Kaplan-Meier curves. Using the upper left corner coordinate point of the ROC curve, the optimum cutoff level for DIC prediction was determined. Differences among the Kaplan-Meier curves were assessed according to the log-rank test. The data were analyzed with a statistical software package (SPSS for Windows, version 12.0; SPSS Inc; Chicago, Illinois), and $P < .05$ was considered to indicate a significant difference.

Results

Mucins Carrying Selectin Ligands in Patients With ARDS and Control Groups

The serum levels of KL-6/MUC1 and mucins carrying selectin ligands (SLAK, SLXK, and PSGL-1) in

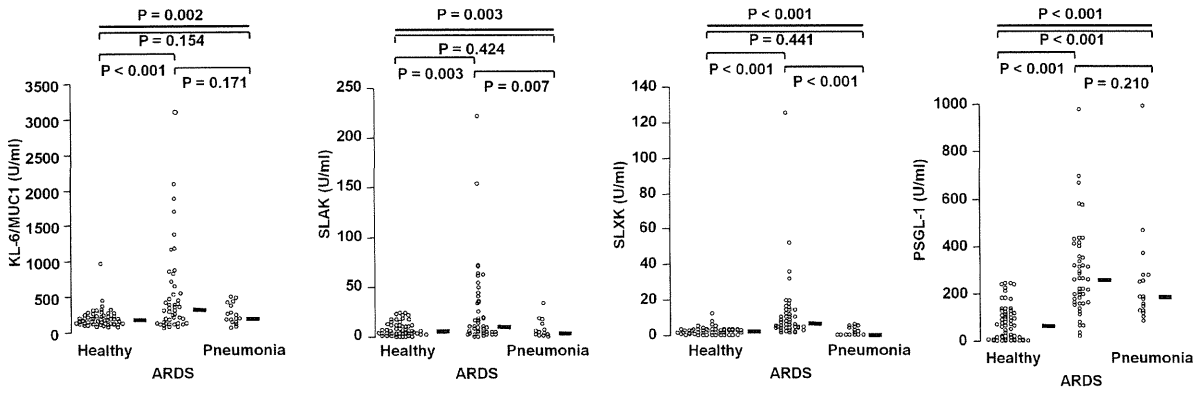

FIGURE 1. The serum levels of mucins (KL-6/MUC1, SLAK, SLXK, and PSGL-1) in healthy subjects, patients with ARDS, and patients with pneumonia uncomplicated by acute lung injury/ARDS. The thick bars denote the median. PSGL-1 = P-selectin glycoprotein ligand-1; SLAK = KL-6/MUC1 carrying sialyl Lewis^a; SLXK = KL-6/MUC1 carrying sialyl Lewis^x.

Table 1—*Characteristics of ARDS Patients at Diagnosis*

Characteristic	ARDS (N = 46) Without DIC (n = 32)	ARDS (N = 46) With DIC (n = 14)	*P* Value
Age, y	65.0 (59.8-73.3)	74.0 (65.3-76.0)	.123
Sex, male (female)	22 (10)	10 (4)	1.000
HMGB1, U/mL	4.6 (2.3-6.4)	5.2 (3.4-7.1)	.438
LDH, IU/mL	366 (247-452)	311 (251-569)	.747
CRP, mg/dL	14.5 (9.4-21.2)	15.5 (8.4-23.4)	.765
Platelet counts, $\times 10^9$/L	194 (117-225)	132 (83-162)	.041
D-dimer, μg/mL	6.5 (3.6-16.2)	9.2 (4.0-21.5)	.511
PaO_2/FIO_2 ratio	138 (122-167)	139 (102-176)	.711
Arterial pH	7.42 (7.29-7.47)	7.40 (7.32-7.44)	.642
Peak airway pressure, cm H_2O	21.5 (20.0-24.0)	24.0 (18.5-28.0)	.221
Mean airway pressure, cm H_2O	15.0 (13.0-16.0)	15.0 (13.5-18.5)	.583
PEEP, cm H_2O	8.0 (5.3-10.0)	8.0 (5.0-10.0)	.561
Lung injury score	2.33 (2.00-3.00)	2.84 (2.25-3.33)	.200
APACHE II score	18.5 (15.3-22.8)	23.0 (16.8-27.0)	.071
SOFA score	7.0 (4.0-8.0)	7.5 (6.0-9.3)	.311
SIRS criteria	3.0 (2.0-3.0)	2.5 (2.0-3.0)	.251
DIC score	2.0 (0.0-2.0)	2.0 (1.0-3.3)	.040
Cause, direct (indirect)	20 (12)	12 (2)	.169

Data are presented as observed numbers for sex and cause, and median (interquartile range) for other categories. Mann-Whitney *U* test or Fisher exact test were used to compare each parameter. APACHE = Acute Physiology and Chronic Health Evaluation; CRP = C-reactive protein; DIC = disseminated intravascular coagulation; HMGB1 = high mobility group box chromosomal protein 1; LDH = lactate dehydrogenase; PEEP = positive end-expiratory pressure; SIRS = systemic inflammatory response syndrome; SOFA = Sequential Organ Failure Assessment.

patients with ARDS and control subjects are shown in Figure 1. In patients with ARDS, serum levels of KL-6/MUC1, SLAK, SLXK, and PSGL-1 at diagnosis were significantly elevated compared with healthy control subjects. There were no significant differences in serum levels of KL-6/MUC1, SLAK, and SLXK between healthy control subjects and patients with bacterial pneumonia, whereas serum PSGL-1 was significantly higher in patients with bacterial pneumonia than in healthy control subjects. The overall intraassay coefficients of variation were calculated to be 2.9% and 7.5%, and the overall interassay coefficients of variation were calculated to be 6.7% and 9.0% for SLAK and SLXK, respectively.

Characteristics of ARDS Patients at Diagnosis

The characteristics of 46 patients newly diagnosed with ARDS included in the present study are shown in Table 1. Of 46 patients, 32 had a direct cause (26 had pneumonia and six had aspiration pneumonia) and 14 had an indirect cause (all 14 patients had extrapulmonary sepsis). When comparisons were made between ARDS patients with and without DIC, the levels of platelet counts and DIC scores were significantly different. There was no significant difference in serum high mobility group box 1 between ARDS patients with and without DIC (Table 1). In nonsurvivors of ARDS, DIC complications were frequent (11/18, 61%) compared with survivors (3/28, 11%, $P < .001$ by Fisher exact test). The mortality in ARDS patients with DIC (11/14, 79%) was significantly higher than in ARDS patients without DIC (7/32, 22%, $P < .001$ by Fisher exact test).

Circulating Levels of KL-6/MUC1, SLAK, and SLXK Are Increased in Patients With ARDS and DIC

At diagnosis of ARDS, the levels of KL-6/MUC1, SLAK, and SLXK were significantly higher in patients

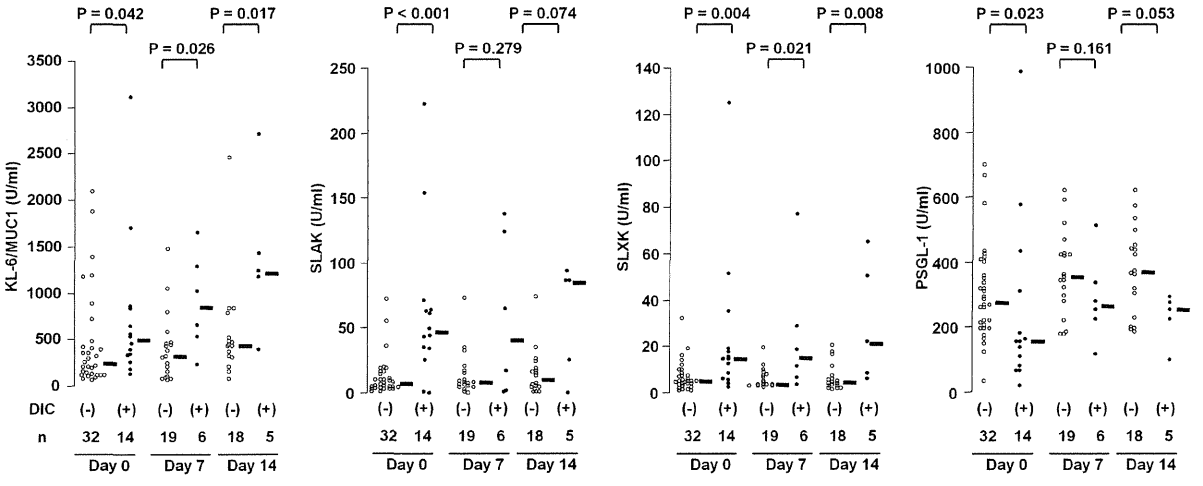

FIGURE 2. Serial measurements of the levels of circulating mucins (KL-6/MUC1, SLAK, SLXK, and PSGL-1) in ARDS patients with (●) or without (○) disseminated intravascular coagulation (DIC). The thick bars denote the median. See Figure 1 legend for expansion of abbreviations.

Table 2—*DIC Predictive Factors in Patients With ARDS*

Variables	Hazard Ratio	95% CI Lower	95% CI Upper	*P* Value
Univariate analysis				
Age ≥ 68 y	2.681	0.839	8.567	.096
Sex: male	1.080	0.338	3.450	.896
KL-6/MUC1 ≥ 324 U/mL	3.508	0.976	12.600	.054
SLAK ≥ 10.1 U/mL	7.845	1.750	35.164	.007
SLXK ≥ 6.4 U/mL	3.461	1.082	11.071	.036
PSGL-1 ≥ 258 U/mL	0.351	0.110	1.120	.770
HMGB1 ≥ 4.9 U/mL	1.326	0.460	3.827	.601
LDH ≥ 359 IU/mL	0.753	0.261	2.172	.600
CRP ≥ 14.8 mg/dL	1.520	0.527	4.386	.439
PaO_2/FIO_2 ratio ≥ 138	0.943	0.330	2.691	.912
Arterial pH ≥ 7.41	0.744	0.258	2.145	.584
Peak airway pressure ≥ 22 cm H_2O	1.746	0.571	5.341	.329
Mean airway pressure ≥ 15 cmH_2O	0.773	0.260	2.301	.644
PEEP ≥ 8 cmH_2O	0.805	0.270	2.398	.697
Lung Injury Score ≥ 2.67	2.947	0.922	9.419	.068
APACHE II score ≥ 20	2.927	0.916	9.353	.070
SOFA score ≥ 8	1.069	0.375	3.050	.901
SIRS criteria ≥ 3	0.712	0.247	2.055	.530
Cause of ARDS: direct	3.052	0.682	13.657	.145
Multivariate analysis				
Age ≥ 68 y	2.192	0.633	7.589	.216
KL-6/MUC1 ≥ 324 U/mL	1.163	0.183	7.403	.873
SLAK ≥ 10.1 U/mL	8.722	1.546	49.204	.014
SLXK ≥ 6.4 U/mL	1.155	0.226	5.904	.862
Lung Injury Score ≥ 2.67	1.288	0.286	5.804	.742
APACHE II score ≥ 20	3.986	0.894	17.779	.698
Cause of ARDS: direct	1.736	0.304	9.897	.535

Hazard ratios and 95% CIs were computed according to the higher value of each group, except for sex and cause of ARDS. The median values were used to discriminate between the high and low values of each group of variables, except for sex and cause of ARDS. For the variables sex and cause of ARDS, male gender and direct cause were used, respectively. PSGL-1 = P-selectin glycoprotein ligand-1; SLAK = KL-6/MUC1 carrying sialyl Lewis^a; SLXK = KL-6/MUC1 carrying sialyl Lewis^x. See Table 1 legend for expansion of other abbreviations.

with DIC than in those without DIC (Fig 2). In addition, the levels of KL-6/MUC1 and SLXK in patients with DIC were significantly higher for up to 2 weeks after the diagnosis of ARDS. The levels of SLAK at days 7 and 14 after diagnosis tended to be higher in patients with DIC; however, when compared with patients without DIC, this difference was not statistically significant. Furthermore, the circulating levels of PSGL-1 in patients with DIC were significantly lower at diagnosis and tended to be decreased for up to 2 weeks after the diagnosis (Fig 2). When the causes of ARDS were taken into consideration, the levels at diagnosis of KL-6/MUC1 and SLXK, but not of SLAK or PSGL-1, in patients with direct cause of ARDS were significantly higher than those in patients with indirect cause (data not shown).

Circulating Mucins With Selectin Ligands as DIC Predictors in ARDS

Mucins with selectin ligands were further examined for their predictive values of DIC complications in patients with ARDS. Multivariate analysis demonstrated that only serum SLAK at diagnosis was independently associated with DIC complications among the other tested factors with $P < .2$ in univariate analysis (Table 2).

ROC curves were drawn to determine the optimum cutoff levels of the measured circulating mucins for DIC complications. The area under the curve was the largest for SLAK among these mucins (Fig 3). Optimum cutoff levels were obtained from each ROC curve: 250 U/mL for KL-6/MUC1, 20 U/mL for SLAK, 11 U/mL for SLXK, and 190 U/mL for PSGL-1. Using these cutoff levels, the sensitivity, specificity, and positive likelihood for the development of DIC were determined: 85.7%, 53.1%, and 1.8 for KL-6/MUC1; 85.7%, 90.6%, and 9.1 for SLAK; 64.3%, 84.4%, and 4.1 for SLXK; and 71.4%, 84.4%, and 4.6 for PSGL-1. Therefore, the positive likelihood ratio among the molecules is the highest for SLAK.

In accordance with the cutoff levels determined by the ROC curves for DIC complications, the incidence of DIC for each mucin's (KL-6/MUC1, SLAK, SLXK, and PSGL-1) levels was further evaluated by Kaplan-Meier analysis (Fig 4). Development of DIC in patients with high levels of serum KL-6/MUC1, SLAK, and SLXK was significantly more frequent than in groups with low levels (Fig 4). In contrast, DIC complications were observed frequently in the PSGL-1 low group compared with the PSGL-1 high group.

Discussion

This study demonstrated that serum mucins carrying selectin ligands are elevated in patients at the time of ARDS diagnosis as compared with healthy control subjects, and such mucins are associated with subsequent complications of DIC. In patients with ARDS, circulating SLAK was found by multivariate analysis to be an independent predictor of future DIC complications, suggesting that this molecule is a novel, and seems to be a promising, biomarker. Additionally, KL-6/MUC1 carrying selectin ligands (SLAK and SLXK) are superior to the absolute amount of KL-6/MUC1 as a predictive marker for DIC complication. We also confirmed that ARDS patients with DIC have an extremely high mortality rate, which was consistent with our previous observations.[10]

This study also demonstrated differences in the dynamics of circulating mucins carrying selectin ligands; KL-6/MUC1 carrying selectin ligands and PSGL-1. Serum levels of SLAK and SLXK were significantly