process of a cell's reaction to intermittent hypoxia/reoxygenation. [18] Meanwhile, it has been reported that several inflammatory stimuli, such as interleukin 1β, stimulate systemic Ngal expression and secretion. NF-κB also has been shown to transactivate Ngal expression, suggesting that Ngal might be involved in inflammatory responses. [19,20].

Therefore, a positive correlation between OSA severity and systemic Ngal secretion through chronic inflammation seems possible. However, this relationship has never been investigated. Thus, we hypothesized that blood Ngal levels are elevated in patients with OSA and that its levels are modified by the treatment of OSA with continuous positive airway pressure (CPAP). In the present study, we measured plasma Ngal levels in patients with OSA and evaluated its utility in clinical practice.

Methods

Subjects

Study patients were consecutively recruited from the Sleep Unit of Kyoto University Hospital between January 2009 and May 2012. All had been referred to our sleep unit under suspicion of OSA with symptoms such as habitual snoring or daytime sleepiness. None had been previously diagnosed with or treated for OSA. Patients with overt renal failure (serum creatinine >1.3 mg/dl) or with any history of cardiovascular diseases, heart failure or arrhythmia were excluded because severe renal and/or heart failure can directly affect plasma Ngal levels. Also excluded were patients with pulmonary diseases, chronic infection, history of cancer or collagen disease. Since a consensus about the relationship between Ngal levels and metabolic syndrome has not yet been formed, we aimed to evaluate the correlations between risk factors for metabolic syndrome and plasma Ngal levels in actual clinical practice. We did not exclude patients with components of metabolic syndrome such as hypertension, diabetes and dyslipidemia even if they were under treatment for these comorbidities.[21–23] This study was approved by Kyoto University Graduate School and Faculty of Medicine Ethics Committee, and written informed consent was obtained from all patients.

Polysomnography and CPAP Implementation

The diagnosis of OSA was confirmed by polysomnography (SomnoStar pro, Cardinal Health, Dublin, OH, USA or Alice 4, Philips Respironics, Inc., Murrysville, PA, USA), which was started at 22:00 and ended at 6:00 the following morning. Surface electrodes were attached using standard techniques to obtain an electrooculogram, electromyogram of the chin and 12-lead electroencephalograph. Sleep stages were defined according to the criteria of Rechtchaffen and Kales. [24] Ventilation was monitored by inductive plethysmography (Respitrace QDC, Viasys Healthcare, Palm Springs, CA, USA). Airflow was monitored by a nasal pressure transducer and supplemented by an oronasal thermal sensor. Arterial oxygen saturation (SpO_2) was monitored continuously with a pulse oximeter.

Apnea was defined as the continuous cessation of airflow for more than 10 seconds and hypopnea was defined as a reduction in airflow of 30% or more lasting for 10 seconds or more accompanied by a decrease in SpO_2 of at least 4%. [25] Apnea-hypopnea index (AHI) values were calculated as the number of episodes of apnea and hypopnea per hour over the total sleep time. 4% oxygen desaturation index (ODI) values were defined as the

Table 1. Baseline characteristics and data on metabolic syndrome and its components in study patients.

	non OSA (n = 15)	mild OSA (n = 37)	moderate OSA (n = 24)	severe OSA (n = 26)	p
Age (y)	48.2±17.2	55.1±13.3	57.3±14.5	58.6±11.5	0.12
Sex (male), n(%)	8 (53.3)	24 (64.9)	18 (75)	18 (69.2)	0.56
Smoking status never/ex/current, n	2/3/10	5/13/19	3/5/16	3/8/15	0.88
Body mass index (kg/m^2)	23.5±3.7	25.1±4.6	24.8±4.1	29.2±7.8[a,b,c]	<0.01
Neck circumference (cm)	36.2±3.1	37.9±3.7	37.8±3.6	39.8±4.0[a]	0.03
Waist circumference (cm)	84.3±11.5	90.1±13.1	89.7±11.0	98.6±14.4[a,b]	0.01
Hip circumference (cm)	90.3±8.8	94.6±10.6	92.1±9.3	101.3±15.3[a,c]	0.01
Waist-to-hip ratio	0.93±0.07	0.95±0.05	0.97±0.04	0.97±0.03	0.04
SBP (mmHg)	119.8±16.1	124.2±16.3	127.3±12.9	127.4±16.0	0.40
DBP (mmHg)	72.7±11.9	76.7±11.5	78.6±11.2	76.2±12.0	0.50
Percentages of patients with metabolic syndrome or components of metabolic syndrome					
Hypertension, n (%)	7 (46.7)	20 (54.1)	15 (62.5)	19 (73.1)	0.16
Hyperglycemia, n (%)	5 (33.3)	7 (18.9)	4 (16.7)	5 (19.2)	0.56
Dyslipidemia, n(%)	6 (40.0)	16 (43.2)	11 (45.8)	13 (50.0)	0.98
Visceral fat accumulation, n(%)	7 (46.7)	22 (59.5)	18 (75.0)	23 (88.5)	0.01
Metabolic syndrome, n(%)	5 (33.3)	13 (35.1)	8 (33.3)	11 (42.3)	0.90
Percentages of patients under treatment for components of metabolic syndrome					
Hypertension, n (%)	2 (13.3)	14 (37.8)	9 (37.5)	12 (46.2)	0.16
Diabetes, n (%)	2 (13.3)	3 (8.1)	2 (8.3)	5 (19.2)	0.56
Dyslipidemia, n(%)	4 (26.7)	8 (21.6)	5 (20.8)	6 (23.1)	0.98

Data are expressed in mean ± SD or n (%).
OSA: obstructive sleep apnea; SBP: systolic blood pressure; DBP: diastolic blood pressure;
[a]$p<0.05$ vs non OSA; [b]$p<0.05$ vs mild OSA; [c]$p<0.05$ vs moderate OSA.
doi:10.1371/journal.pone.0054184.t001

Table 2. OSA parameters and laboratory profiles.

	non OSA (n = 15)	mild OSA (n = 37)	moderate OSA (n = 24)	severe OSA (n = 26)	p
Parameters of OSA					
Apnea hypopnea index/h	2.2±1.6	9.4±2.6	22.2±5.0[a,b]	50.5±19.8[a,b,c]	<0.01
4%ODI/h	1.8±1.6	8.2±3.3	20.7±6.0[a,b]	49.6±20.2[a,b,c]	<0.01
Minimum SpO_2 (%)	90.3±4.5	84.9±4.3	78.4±8.6[a,b]	71.3±12.2[a,b,c]	<0.01
Arousal index/h	24.3±12.1	22.8±9.6	30.7±13.2	43.4±18.3[a,b,c]	<0.01
Length of time SpO_2<90% (m)	3.2±5.4	11.4±17.4	37.3±52.2	121.1±107.8[a,b,c]	<0.01
Laboratory profiles					
FPG (mg/dl)	107.7±39.9	95.4±20.1	96.4±24.3	102.5±20.2	0.35
HbA1c (%)	5.80±1.22	5.49±0.65	5.45±0.91	5.74±0.86	0.45
Total cholesterol (mg/dl)	185.5±32.3	195.2±35.4	203.0±44.0	201.5±44.0	0.53
LDL cholesterol (mg/dl)	103.2±27.9	116.2±27.8	118.5±36.8	109.5±31.8	0.40
HDL cholesterol (mg/dl)	55.2±12.9	52.8±13.5	53.0±16.1	51.9±13.9	0.91
Triglycerides (mg/dl)	125.1±86.5	119.8±58.0	139.1±79.1	168.0±185.2	0.39
BNP (pg/ml)	14.4±8.7	20.6±24.4	22.4±31.8	21.5±18.7	0.73
Creatinine (mg/dl)	0.72±0.19	0.74±0.15	0.80±0.17	0.78±0.21	0.45
Ngal (ng/ml)	46.9±6.0	48.9±10.9	51.3±15.2	55.4±16.7	0.16

Data are expressed in mean ± SD or n (%).
OSA: obstructive sleep apnea; ODI: oxygen desaturation index; SpO_2: saturation of oxygen; FPG: fasting plasma glucose; LDL: low density lipoprotein; HDL: high density lipoprotein; BNP: brain natriuretic peptide; Ngal: neutrophil gelatinase associated lipocalin.
[a]$p<0.05$ vs non OSA; [b]$p<0.05$ vs mild OSA; [c]$p<0.05$ vs moderate OSA.
doi:10.1371/journal.pone.0054184.t002

number of desaturations ≥4% per hour of sleep. The length of time SpO_2<90% during sleep was calculated in each patient. Patients with central sleep apnea were excluded. OSA severity was defined by the AHI as follows: non OSA (AHI<5), mild OSA (5≤AHI<15), moderate OSA (15≤AHI<30) and severe OSA (30≤AHI).

Patients with an AHI ≥15 were candidates for nasal CPAP. Those who agreed with CPAP implementation underwent a second polysomnography with CPAP titration. We implemented CPAP with the auto adjusting positive airway pressure (PAP) function for all patients. Based on the second sleep study, minimum and maximum PAP were determined to abolish all respiratory events, arousal and desaturation events.

Follow-Up

At the three-month follow-up, we urged the patients to undergo a third sleep study to confirm whether an adjustment of the CPAP setting was necessary. To investigate the effect of CPAP treatment on plasma Ngal levels, at the third sleep study blood samples were collected in the same way as at the first sleep study. We also checked use time of the CPAP machine by reading the time counter on the CPAP machines. Similar to prior studies, we defined 'good compliance' as the use of CPAP for >4 h per night on >70% of nights and categorized the patients into two groups, those with 'good compliance' or 'poor compliance'. [26] We analyzed the data separately for each group and compared clinical variables before and after CPAP treatment.

Blood Sampling and Measurement of Plasma Ngal Level

Blood samples were drawn at 7:00 in the morning after the subjects had fasted beginning at 20:00 the previous night. Blood samples were centrifuged immediately at 3,000 rpm at 4°C for 10 min. The separated samples were stored −80°C until assay. Plasma Ngal concentrations were determined by an ELISA kit provided by Bioporto Diagnostics, Gentofte, Denmark. Intra- and inter-assay coefficients of variation for Ngal were 1.2–4.0% and 2.2–11.2%, respectively.

Definition of Metabolic Syndrome

In classifying patients based on the components of metabolic syndrome, we utilized Japanese criteria. [27] Waist circumference (WC) was measured at the level of the navel with the patient standing, and visceral fat accumulation was determined to be positive at WC ≥85 cm for men and ≥90 cm for women. A diagnosis of metabolic syndrome required the subject to have visceral fat accumulation and 2 or 3 of the following: (a) dyslipidemia (triglycerides ≥150 mg/dL and/or high-density lipoprotein cholesterol level <40 mg/dL, or specific treatment for these lipid abnormalities); (b) hypertension (systolic blood pressure ≥130 mmHg and/or diastolic blood pressure ≥85 mmHg, or treatment of previously diagnosed hypertension); and (c) hyperglycemia (fasting plasma glucose ≥110 mg/dL or specific treatment for diabetes mellitus). Anthropometric parameters and blood pressure were measured immediately after polysomnography recording ended.

Statistical Analysis

In the analysis of data, we classified the patients depending on the severity of OSA and compared their clinical backgrounds. We also compared plasma Ngal levels between patients with and without each component of metabolic syndrome to investigate the relationships between plasma Ngal levels and metabolic syndrome. Data were expressed as means ± standard deviation. The significance of intergroup differences based on the severity of OSA was determined by an analysis of variance. When a significant difference was found, we used the Tukey's honestly

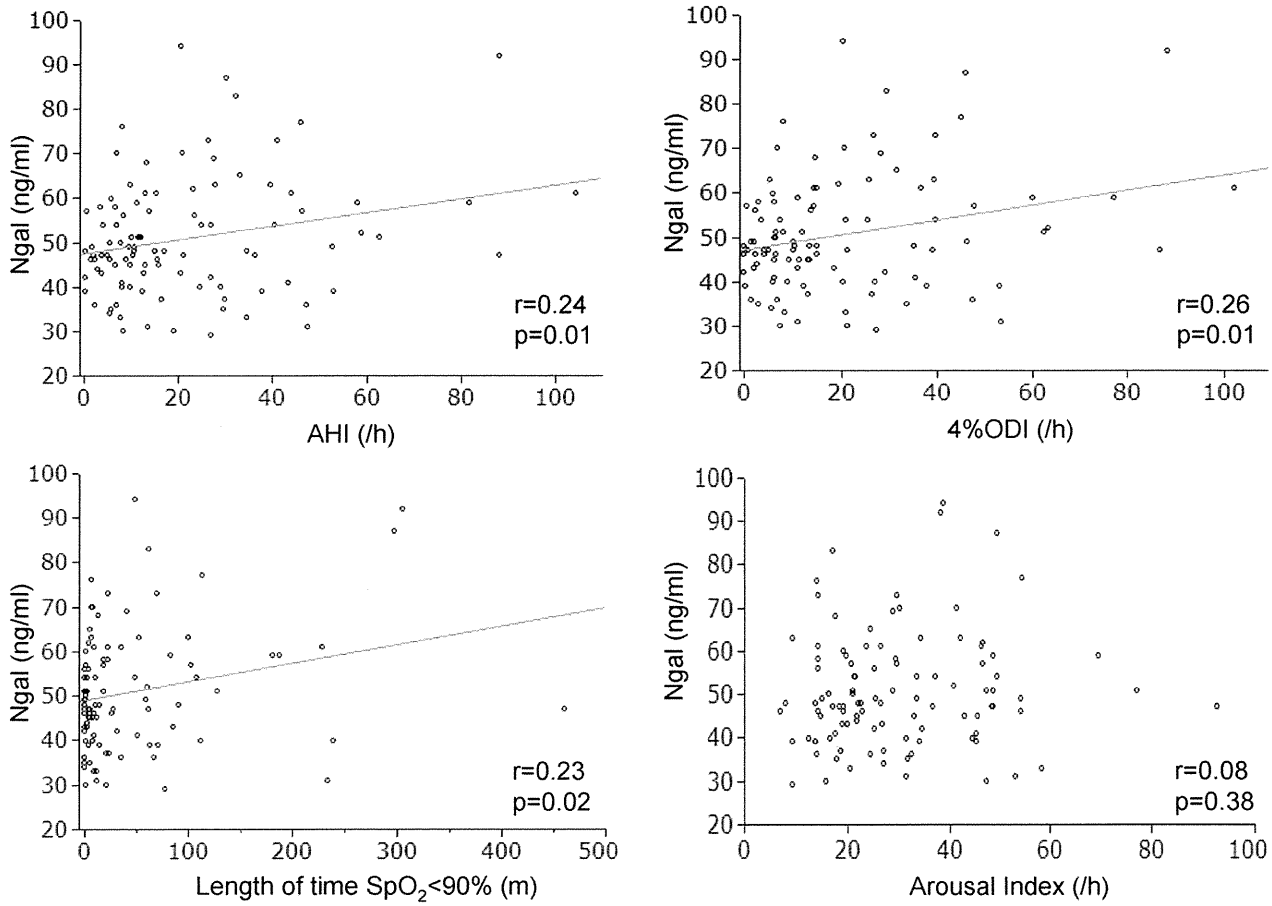

Figure 1. Simple correlations between plasma neutrophil gelatinase associated lipocalin (Ngal) levels and parameters of obstructive sleep apnea. AHI: apnea hypopnea index; ODI: oxygen desaturation index; SpO_2: saturation of oxygen.
doi:10.1371/journal.pone.0054184.g001

significant difference procedure to identify where the difference was significant. A chi-square test and the Mann-Whitney U test were used to compare categorical and continuous variables, respectively. We used Pearson's coefficient tests to evaluate the relationship between the plasma Ngal level and other continuous variables. Based on the results of this analysis, multiple regression analyses were performed to clarify the contribution rate of OSA and other comorbidities to systemic Ngal secretion. Wilcoxon signed rank test was used to compare clinical variables before and after CPAP treatment. Two-tailed p-values <0.05 were considered statistically significant. All statistical analyses were performed using JMP 7.0.2 statistical software (SAS Institute Inc., Cary, NC, USA).

Results

Baseline Characteristics of Study Patients

A total of 102 patients were studied, and their baseline characteristics are shown in table 1.Those with severe OSA were characterized by a significantly higher body mass index (BMI) than in the other three groups. The percentages of patients who fulfilled the criterion for visceral fat accumulation increased as the severity of OSA increased. Other anthropometric parameters with significant differences among the groups are also shown in table 1. With the exception of the parameters for OSA, there were no significant differences among the four groups in other clinical background factors. (Tables 1 and 2).

Plasma Ngal Levels in Patients at Diagnosis and follow up

Table 2 shows baseline plasma Ngal levels in the four groups, with no statistically significant differences found among them. However, simple linear regression analysis showed significant correlations of the plasma Ngal level with the following parameters of OSA: AHI ($r=0.24$, $p=0.01$), 4%ODI ($r=0.26$, $p=0.01$) and time of $SpO_2<90\%$ ($r=0.23$, $p=0.02$). (Figure 1) The plasma Ngal level was also correlated with values for serum low density lipoprotein (LDL) cholesterol ($r=-0.31$, $p<0.01$), triglycerides ($r=0.24$, $p=0.01$) and creatinine ($r=0.34$, $p<0.01$). On the other hand, none of anthropometric parameters and parameters associated with diabetes such as fasting plasma glucose and HbA1c levels showed significant correlations with Ngal levels.(Table 3) Furthermore, in the present cohort, significant differences were not found in plasma Ngal levels between patients with and without each of the components of metabolic syndrome. (Table 4).

For the multiple regression analysis, we chose 4%ODI as the representative variable for OSA severity as it had the best correlation with the Ngal level among OSA parameters in the simple correlation analysis. The analysis demonstrated that 4%ODI was associated with the Ngal level independently of creatinine and LDL-cholesterol levels. The contribution rate of 4% ODI to the Ngal level was 6.2% (Table 5).

Table 3. Simple correlations between plasma neutrophil gelatinase associated (Ngal) levels and clinical variables.

	r	p
Age (y)	0.04	0.62
Body mass index (kg/m^2)	0.13	0.17
Neck circumference (cm)	0.00	0.84
Waist circumference (cm)	0.00	0.91
Hip circumference (cm)	0.00	0.99
Waist-to-hip ratio	0.03	0.76
SBP (mmHg)	0.00	0.96
DBP (mmHg)	−0.13	0.17
FPG(mg/dl)	−0.08	0.39
HbA1c (%)	−0.06	0.52
Total cholesterol (mg/dl)	0.12	0.21
LDL-cholesterol (mg/dl)	−0.31	<0.01
HDL-cholesterol (mg/dl)	−0.18	0.06
Triglycerides (mg/dl)	0.24	0.01
BNP (pg/ml)	0.00	0.74
Creatinine (mg/dl)	0.34	<0.01

r: correlation coefficient; SBP: systolic blood pressure; DBP: diastolic blood pressure; FPG: fasting plasma glucose; LDL: low density lipoprotein; HDL: high density lipoprotein; BNP: brain natriuretic peptide.
doi:10.1371/journal.pone.0054184.t003

CPAP was implemented for 46 of the 50 patients with moderate or severe OSA. Of the 46 patients, 27 agreed to a follow-up sleep study. Just before the reevaluation, cardiac medicine was prescribed for one patient and an upper airway infection was found in another patient. These two patients were excluded from the analysis, and the remaining 25 patients were reevaluated. Thirteen were categorized into the good compliance group and the other 12 patients into the poor compliance group. Those in the good compliance group were significantly older than patients in the poor compliance group. The determined maximum and minimum PAP did not differ between the two groups.

After CPAP implementation, OSA was significantly improved in both groups. In the good compliance group, despite improvements in OSA, no significant change was noted in plasma Ngal levels from values before CPAP use. Furthermore, in the poor compliance group, Ngal levels were significantly elevated after CPAP implementation. There were not significant differences in the other confounding factors before and after CPAP treatment (Table 6).

Discussion

In this cross sectional evaluation, although significant differences in plasma Ngal levels were not found among groups classified according to the severity of OSA, parameters of OSA, such as 4%ODI and AHI per se, correlated with plasma Ngal levels in regression analysis. This suggests that OSA contributes, although weakly, to elevated plasma Ngal levels through nocturnal hypoxia. Because it has been reported that hypoxia induces an elevation in plasma Ngal levels in an experimental animal model, it is possible that OSA induces Ngal elevation through nocturnal intermittent hypoxia. [28] To the best of our knowledge, this is the first report to evaluate the relationship between the Ngal protein level and OSA severity in clinical practice.

Table 4. Plasma Ngal levels in patients with and without each component of metabolic syndrome.

	Plasma Ngal levels (ng/ml)		
	Comorbidity(+)	Comorbidity(−)	p
Hypertension	52.7±1.7	48.0±2.1	0.17
	(n=61)	(n=41)	
Hyperglycemia	48.4±14.5	51.4±13.0	0.27
	(n=21)	(n=81)	
Dyslipidemia	51.9±14.7	49.9±12.2	0.49
	(n=46)	(n=56)	
Visceral fat accumulation	50.9±14.1	50.5±11.6	0.92
	(n=70)	(n=32)	
Metabolic syndrome	51.8±16.7	50.2±11.1	0.93
	(n=37)	(n=65)	

Data are expressed in mean ± SD.
Ngal: neutrophil gelatinase associated lipocalin.
doi:10.1371/journal.pone.0054184.t004

The relationship between another protein in the lipocalin family and OSA has been investigated. Makino et al and Nena et al, respectively, investigated the relationship between the plasma level of retinol binding protein 4 (RBP-4), which also belongs to the lipocalin protein family, and OSA. [29,30] However, neither study found a correlation between RBP-4 levels and apnea-related indices. Although both Ngal and RBP-4 belong to the lipocalin family and share a common tertiary structure, these two proteins appear to have different patterns of regulation in response to inflammatory mediators. [21,23].

Our results also demonstrated a significant inverse correlation between Ngal and LDL cholesterol levels. Wallenius et al also reported such an inverse correlation in their epidemiological study. [21] However, in other studies, correlations between these two variables were not found. [23,31,32] Although this inverse correlation is possible, the results seem to vary depending on the clinical characteristics of the examined cohorts. Furthermore, the mechanisms of this correlation remain utterly unknown.

The relationship between Ngal and metabolic syndrome is quite controversial. Whereas Wang et al and Yan et al reported a close

Table 5. Multiple regression analyses using plasma neutrophil gelatinase associated lipocalin (Ngal) level as a dependent variable.

	p	β	r	R^2(%)
Body mass index (kg/m^2)	0.39	–		
4%ODI/h	<0.01	0.24	0.26	6.2
LDL-cholesterol (mg/dl)	<0.01	−0.29	−0.31	9.0
HDL-cholesterol (mg/dl)	0.71	–		
Triglycerides (mg/dl)	0.31	–		
Creatinine (mg/dl)	<0.01	0.28	0.34	9.5
Cumulative R^2				24.7

β: standard regression coefficient; r: correlation efficient; R^2: contribution rate; ODI: oxygen desaturation index; LDL: low density lipoprotein; HDL: high density lipoprotein.
doi:10.1371/journal.pone.0054184.t005

Table 6. Changes in clinical variables from baseline to after CPAP implementation.

	CPAP good compliance (n = 13)			CPAP poor compliance (n = 12)			
	before CPAP	after CPAP	p*	before CPAP	after CPAP	p*	p#
Ngal (ng/ml)	60.5±18.1	64.2±13.9	0.27	52.8±16.8	63.1±14.2	<0.01	-
4%ODI (/h)	33.1±16.7	1.1±1.9	<0.01	41.5±22.5	1.5±2.3	<0.01	-
Creatinine (mg/dl)	0.85±0.20	0.88±0.19	0.13	0.77±0.21	0.79±0.20	0.25	-
LDL cholesterol (mg/dL)	107.5±32.7	103.8±30.8	0.70	115.2±28.2	121.0±28.0	0.58	-
Body mass index (kg/m^2)	23.9±2.0	23.9±2.2	0.85	28.2±6.6	28.6±6.6	0.14	
Age (y)	67.5±8.3		-	54.5±12.2		-	0.01
Days with CPAP use >4 h (%)	85.8±9.6		-	43.3±20.7		-	<0.01
Maximum PAP (cmH_2O)	9.9±2.8		-	10.8±2.5		-	0.62
Minimum PAP (cmH_2O)	4.5±0.9		-	4.7±0.8		-	0.35

Data are expressed in mean±SD.
CPAP: continuous positive airway pressure; Ngal: neutrophil gelatinase associated lipocalin; ODI: oxygen desaturation index; LDL: low density lipoprotein; PAP: positive airway pressure; p*: p value for comparison with values before and after CPAP treatment; p# : p value for comparison between CPAP good compliance and CPAP poor compliance groups.
doi:10.1371/journal.pone.0054184.t006

association between Ngal and obesity or insulin resistance, Wallenius et al found no correlation between these risk factors. [21,22,31] Our results did not show any significant correlation between Ngal levels and obesity or diabetic indices. Also, in the present cohort there were no significant differences in Ngal levels between patients with and without metabolic syndrome. These results also seem to depend on the clinical characteristics of the cohorts. Specifically, we did not exclude patients under treatment for metabolic syndrome to investigate the utility of the plasma Ngal level in actual clinical practice. Because it was reported that the pharmaceutical treatment of diabetes and dyslipidemia can change plasma Ngal levels, treatment of metabolic syndrome in our cohort possibly affected the results. [31,33] Furthermore, in most of these studies, renal function was not taken into account as an explanatory variable of the Ngal level. Risk factors for metabolic syndrome induce latent renal function impairment and even a subtle change in renal function is known to affect blood and urinary Ngal levels. [34] Giaginis et al reported that plasma Ngal levels were higher in patients with than without hypertension and they speculated that the association between elevated Ngal levels and hypertension is secondary to the confounding effect of renal impairment. [35] In our study, even though we did not include patients with overt renal failure, the plasma Ngal levels correlated significantly with serum creatinine levels. Therefore, in evaluating the direct link between metabolic syndrome and Ngal levels, statistical correction for renal function seem to be necessary. In fact, Liu et al reported that the significant correlation between Ngal and insulin resistance detected in their study cohort disappeared after adjustment for serum creatinine values. [32].

Contrary to our expectation, plasma Ngal levels did not change even with the appropriate use of CPAP that effected an improvement in OSA. We could not confirm a direct causality between Ngal levels and OSA. Although we based the three-month treatment observation period on experiences in previous studies, we might need an extended period to find more remarkable changes in Ngal levels in the present cohort. [23,31].

Ngal levels were elevated after CPAP implementation in patients with poor compliance with CPAP. Because we did not include an actual control group that did not use CPAP, we could not judge whether the change in Ngal levels was caused by incomplete CPAP use or other reasons. Although we took every conceivable confounding factor into account, other determinants that we were not aware of might have influenced the results. In the cross sectional studies, the contribution rate of 4%ODI to Ngal levels was not large (6.2%). Therefore, it seems quite possible that other determinants negated the influence of the improvement in OSA. The presence of certain components of metabolic syndrome and their treatment in the present cohort might be among these determinants.

We recognize several limitations in the present study. First, the sample size was small, so it is not reasonable to extrapolate our data to the general population. In addition, the results of this study might have been influenced by the small sample size. Second, as we previously noted, we did not exclude patients with comorbidities such as hypertension and diabetes even if they were under treatment. It is possible that these comorbidities and their treatment affected the results. Third, as mentioned above, we did not have a planned control group without CPAP use. Therefore, we could not judge precisely whether changes in Ngal levels after CPAP implementation were caused by CPAP use or other reasons. Lastly, we did not measure C reactive protein (CRP) levels. Because inflammation has an influence on Ngal, results of measurement of high sensitive CRP would have been a good reflection of the inflammation status of patients and would have been helpful to achieve a more comprehensive understanding of the relationship between OSA and Ngal.

In summary, the present study provides the first clinical evidence demonstrating that plasma Ngal levels were positively but weakly associated with the severity of OSA. Plasma Ngal levels did not change after improvement in OSA, so we could not testify to the causality between Ngal levels and OSA severity. Because Ngal levels were just weakly correlated with the severity of OSA, changes in those levels appear to be influenced largely by other confounding factors. Thus, it would be difficult to use the Ngal level as a specific biomarker representing nocturnal hypoxia in OSA. The links between Ngal levels and metabolic syndrome remain controversial, and unrecognized determinants of plasma Ngal levels are likely to be present. Further studies are warranted to more comprehensively understand the regulation of Ngal in relation to OSA and metabolic syndrome.

Author Contributions

Conceived and designed the experiments: K. Murase K. Mori KC. Performed the experiments: K. Murase CY KA YC MA YH. Analyzed the data: K. Murase K. Mori. Contributed reagents/materials/analysis tools: YT KT T. Handa T. Hitomi TO MM. Wrote the paper: K. Murase K. Mori KC.

References

1. Kjeldsen L, Johnsen AH, Sengelov H, Borregaard N (1993) Isolation and primary structure of NGAL, a novel protein associated with human neutrophil gelatinase. The Journal of Biological Chemistry 268: 10425–10432.
2. Bao G, Clifton M, Hoette TM, Mori K, Deng SX, et al. (2010) Iron traffics in circulation bound to a siderocalin (ngal)-catechol complex. Nature Chemical Biology 6: 602–609. 10.1038/nchembio.402.
3. Mishra J, Ma Q, Prada A, Mitsnefes M, Zahedi K, et al. (2003) Identification of neutrophil gelatinase-associated lipocalin as a novel early urinary biomarker for ischemic renal injury. Journal of the American Society of Nephrology : JASN 14: 2534–2543.
4. Mishra J, Dent C, Tarabishi R, Mitsnefes MM, Ma Q, et al. (2005) Neutrophil gelatinase-associated lipocalin (NGAL) as a biomarker for acute renal injury after cardiac surgery. Lancet 365: 1231–1238. 10.1016/S0140–6736(05)74811-X.
5. Mori K, Lee HT, Rapoport D, Drexler IR, Foster K, et al. (2005) Endocytic delivery of lipocalin-siderophore-iron complex rescues the kidney from ischemia-reperfusion injury. The Journal of Clinical Investigation 115: 610–621. 10.1172/JCI23056.
6. Bolignano D, Lacquaniti A, Coppolino G, Donato V, Campo S, et al. (2009) Neutrophil gelatinase-associated lipocalin (NGAL) and progression of chronic kidney disease. Clinical Journal of the American Society of Nephrology : CJASN 4: 337–344. 10.2215/CJN.03530708.
7. Viau A, El Karoui K, Laouari D, Burtin M, Nguyen C, et al. (2010) Lipocalin 2 is essential for chronic kidney disease progression in mice and humans. The Journal of Clinical Investigation 120: 4065–4076. 10.1172/JCI42004.
8. Hemdahl AL, Gabrielsen A, Zhu C, Eriksson P, Hedin U, et al. (2006) Expression of neutrophil gelatinase-associated lipocalin in atherosclerosis and myocardial infarction. Arteriosclerosis, Thrombosis, and Vascular Biology 26: 136–142. 10.1161/01.ATV.0000193567.88685.f4.
9. Yndestad A, Landro L, Ueland T, Dahl CP, Flo TH, et al. (2009) Increased systemic and myocardial expression of neutrophil gelatinase-associated lipocalin in clinical and experimental heart failure. European Heart Journal 30: 1229–1236. 10.1093/eurheartj/ehp088.
10. Daniels LB, Barrett-Connor E, Clopton P, Laughlin GA, Ix JH, et al. (2012) Plasma neutrophil gelatinase-associated lipocalin is independently associated with cardiovascular disease and mortality in community-dwelling older adults: The rancho bernardo study. Journal of the American College of Cardiology 59: 1101–1109. 10.1016/j.jacc.2011.11.046.
11. Young T, Palta M, Dempsey J, Skatrud J, Weber S, et al. (1993) The occurrence of sleep-disordered breathing among middle-aged adults. The New England Journal of Medicine 328: 1230–1235. 10.1056/NEJM199304293281704.
12. Punjabi NM (2008) The epidemiology of adult obstructive sleep apnea. Proceedings of the American Thoracic Society 5: 136–143. 10.1513/pats.200709-155MG.
13. Nakayama-Ashida Y, Takegami M, Chin K, Sumi K, Nakamura T, et al. (2008) Sleep-disordered breathing in the usual lifestyle setting as detected with home monitoring in a population of working men in japan. Sleep 31: 419–425.
14. Kohler M, Stradling JR (2010) Mechanisms of vascular damage in obstructive sleep apnea. Nature Reviews.Cardiology 7: 677–685. 10.1038/nrcardio.2010.145.
15. Drager LF, Polotsky VY, Lorenzi-Filho G (2011) Obstructive sleep apnea: An emerging risk factor for atherosclerosis. Chest 140: 534–542. 10.1378/chest.10–2223.
16. Nicholl DD, Ahmed SB, Loewen AH, Hemmelgarn BR, Sola DY, et al. (2012) Declining kidney function increases the prevalence of sleep apnea and nocturnal hypoxia. Chest 141: 1422–1430. 10.1378/chest.11–1809.
17. Ahmed SB, Ronksley PE, Hemmelgarn BR, Tsai WH, Manns BJ, et al. (2011) Nocturnal hypoxia and loss of kidney function. PloS One 6: e19029. 10.1371/journal.pone.0019029.
18. Ryan S, Taylor CT, McNicholas WT (2005) Selective activation of inflammatory pathways by intermittent hypoxia in obstructive sleep apnea syndrome. Circulation 112: 2660–2667. 10.1161/CIRCULATIONAHA.105.556746.
19. Liu Q, Nilsen-Hamilton M (1995) Identification of a new acute phase protein. The Journal of Biological Chemistry 270: 22565–22570.
20. Jayaraman A, Roberts KA, Yoon J, Yarmush DM, Duan X, et al. (2005) Identification of neutrophil gelatinase-associated lipocalin (NGAL) as a discriminatory marker of the hepatocyte-secreted protein response to IL-1beta: A proteomic analysis. 91: 502–515.
21. Wallenius V, Elias E, Bergstrom GM, Zetterberg H, Behre CJ (2011) The lipocalins retinol-binding protein-4, lipocalin-2 and lipocalin-type prostaglandin D2-synthase correlate with markers of inflammatory activity, alcohol intake and blood lipids, but not with insulin sensitivity in metabolically healthy 58-year-old swedish men. Experimental and Clinical Endocrinology & Diabetes : Official Journal, German Society of Endocrinology [and] German Diabetes Association 119: 75–80. 10.1055/s-0030–1265212.
22. Yan QW, Yang Q, Mody N, Graham TE, Hsu CH, et al. (2007) The adipokine lipocalin 2 is regulated by obesity and promotes insulin resistance. Diabetes 56: 2533–2540. 10.2337/db07–0007.
23. Choi KM, Kim TN, Yoo HJ, Lee KW, Cho GJ, et al. (2009) Effect of exercise training on A-FABP, lipocalin-2 and RBP4 levels in obese women. Clinical Endocrinology 70: 569–574. 10.1111/j.1365–2265.2008.03374.x.
24. Rechtschaffen A, Kales A (1968) A manual of standardized terminology, techniques and scoring system for sleep stages of human subjects. Washington, DC: National Institutes of Health.
25. Iber C, Ancoli-Israel S, Chesson A, Quan S (2007) The AASM manual for the scoring of sleep and associated events: Rules, terminology and technical specifications. Westchester, IL, USA: American Academy of Sleep Medicine.
26. Collen J, Lettieri C, Kelly W, Roop S (2009) Clinical and polysomnographic predictors of short-term continuous positive airway pressure compliance. Chest 135: 704–709. 10.1378/chest.08–2182.
27. Niigaki M, Adachi K, Hirakawa K, Furuta K, Kinoshita Y (2012) Association between metabolic syndrome and prevalence of gastroesophageal reflux disease in a health screening facility in japan. Journal of Gastroenterology. 10.1007/s00535-012-0671-3.
28. Jiang W, Constante M, Santos MM (2008) Anemia upregulates lipocalin 2 in the liver and serum. Blood Cells, Molecules & Diseases 41: 169–174. 10.1016/j.bcmd.2008.04.006.
29. Makino S, Fujiwara M, Suzukawa K, Handa H, Fujie T, et al. (2009) Visceral obesity is associated with the metabolic syndrome and elevated plasma retinol binding protein-4 level in obstructive sleep apnea syndrome. Hormone and Metabolic Research = Hormon- Und Stoffwechselforschung = Hormones Et Metabolisme 41: 221–226. 10.1055/s-0028–1100411.
30. Nena E, Steiropoulos P, Tzouvelekis A, Tsara V, Hatzizisi O, et al. (2010) Reduction of serum retinol-binding protein-4 levels in nondiabetic obstructive sleep apnea patients under continuous positive airway pressure treatment. Respiration; International Review of Thoracic Diseases 80: 517–523. 10.1159/000295903.
31. Wang Y, Lam KS, Kraegen EW, Sweeney G, Zhang J, et al. (2007) Lipocalin-2 is an inflammatory marker closely associated with obesity, insulin resistance, and hyperglycemia in humans. Clinical Chemistry 53: 34–41. 10.1373/clinchem.2006.075614.
32. Liu X, Hamnvik OP, Petrou M, Gong H, Chamberland JP, et al. (2011) Circulating lipocalin 2 is associated with body fat distribution at baseline but is not an independent predictor of insulin resistance: The prospective cyprus metabolism study. European Journal of Endocrinology/European Federation of Endocrine Societies 165: 805–812. 10.1530/EJE-11–0660.
33. Fassett RG, Robertson IK, Ball MJ, Geraghty DP, Cardinal JW, et al. (2012) Effects of atorvastatin on NGAL and cystatin C in chronic kidney disease: A post hoc analysis of the LORD trial. Nephrology, Dialysis, Transplantation : Official Publication of the European Dialysis and Transplant Association - European Renal Association 27: 182–189. 10.1093/ndt/gfr193.
34. Ronco C (2007) N-GAL: Diagnosing AKI as soon as possible. Critical Care (London, England) 11: 173. 10.1186/cc6162.
35. Giaginis C, Zira A, Katsargyris A, Klonaris C, Theocharis S (2010) Clinical implication of plasma neutrophil gelatinase-associated lipocalin (NGAL) concentrations in patients with advanced carotid atherosclerosis. Clinical Chemistry and Laboratory Medicine : CCLM/FESCC 48: 1035–1041. 10.1515/CCLM.2010.211.

http://dx.doi.org/10.5665/sleep.2378

Flexible Positive Airway Pressure Improves Treatment Adherence Compared with Auto-adjusting PAP

Yuichi Chihara, MD, PhD[1]; Tomomasa Tsuboi, M.D, PhD[2]; Takefumi Hitomi, MD, PhD[2]; Masanori Azuma, MD[1]; Kimihiko Murase, MD[1]; Yoshiro Toyama, MD[1]; Yuka Harada, MD[1]; Kensaku Aihara, MD[1]; Kiminobu Tanizawa, MD[1]; Tomohiro Handa, MD, PhD[3]; Chikara Yoshimura, MD, PhD[2]; Toru Oga, MD, PhD[2]; Kazuhiko Yamamoto, MD, PhD[4]; Michiaki Mishima, MD, PhD[1]; Kazuo Chin, MD, PhD[2]

[1]Department of Respiratory Medicine, Kyoto University Graduate School of Medicine, Kyoto, Japan; [2]Departments of Respiratory Care and Sleep Control Medicine, Kyoto University Graduate School of Medicine, Kyoto, Japan; [3]Department of Rehabilitation, Kyoto University Hospital, Kyoto, Japan; [4]Departments of Allergy and Rheumatology, Tokyo University Graduate School of Medicine, Tokyo, Japan

Study Objectives: There are no clinical data comparing adherence and quality of life between auto-adjusting positive airway pressure (APAP) and two different flex positive airway pressure (PAP) devices (A-Flex, C-Flex) in patients with obstructive sleep apnea (OSA).

Design and Setting: Ninety-three patients in whom OSA was newly diagnosed were randomly assigned to receive 3 mo of APAP (n = 31), APAP with C-Flex (n = 31), or APAP with A-Flex (n = 31). Objective adherence was determined after 3 mo of CPAP treatment, and the Epworth Sleepiness Scale (ESS), Pittsburgh Sleep Quality Index (PSQI), and Calgary Sleep Apnea Quality of Life Index (SAQLI) were examined at baseline and after 3 mo. After 3 mo, patients in the APAP with A-Flex group and those in the APAP with C-Flex group were crossed over and those in the APAP group were switched to A-Flex for an additional 3 mo.

Measurements and Results: The groups were similar demographically. Treatment adherence during the first 3 mo was significantly greater in the APAP with C-Flex group (APAP with C-Flex: 5.19 ± 1.84 h/night versus APAP: 3.96 ± 1.66 h/night versus APAP with A-Flex: 4.27 ± 2.12 h/night, P = 0.04). There was a significant improvement in two of four of the SAQLI domain scores and in the ESS and PSQI in the APAP with C-Flex group. Adherence significantly improved among the poor compliers (< 4 h/night of use) in the APAP group after change to APAP with A-Flex (P = 0.01).

Conclusions: Of these three modes of PAP delivery, adherence was greatest with APAP with C-Flex.

Clinical Trial Registration: URL: http://www.clinicaltrials.gov. Unique identifier: NCT00873977.

Keywords: A-Flex, C-Flex, continuous positive airway pressure, obstructive sleep apnea, treatment adherence

Citation: Chihara Y; Tsuboi T; Hitomi T; Azuma M; Murase K; Toyama Y; Harada Y; Aihara K; Tanizawa K; Handa T; Yoshimura C; Oga T; Yamamoto K; Mishima M; Chin K. Flexible positive airway pressure improves treatment adherence compared with auto-adjusting PAP. *SLEEP* 2013;36(2):229-236.

INTRODUCTION

Continuous positive airway pressure (CPAP), the mainstay of treatment for moderate to severe obstructive sleep apnea (OSA), has been shown to normalize sleep architecture, reduce daytime sleepiness, enhance daily function, and decrease cardiovascular events.[1-3] Although CPAP is a highly effective treatment, adherence is suboptimal. To improve patient comfort and treatment adherence, various CPAP modalities have been developed. Auto-adjusting positive airway pressure (APAP) devices continuously adjust the pressure in real time according to that required to maintain upper airway patency. It has been reported that treatment compliance with APAP was equivalent to that with fixed PAP therapy.[4-6] Adherence with flexible CPAP (C-Flex; Philips Respironics, Murraysville, PA, USA), which flexes airway pressure on exhalation and inhalation on a breath-by-breath basis to reduce the work of breathing, has been reported to be significantly better compared with fixed PAP therapy in one study.[7] However, other studies reported that adherence with C-Flex and fixed positive airway pressure (PAP) therapy was similar.[8-11] To our knowledge, a comparison of treatment adherence of APAP with APAP with C-Flex has not been reported. Because the use of APAP for the treatment of OSA has increased, a prospective randomized study comparing APAP to APAP with C-Flex with respect to CPAP adherence is needed. In addition, a new CPAP device (A-Flex; Philips Respironics) is designed to further improve breathing comfort. Like C-Flex, A-Flex flexes pressure at the beginning of exhalation but also has a fixed 2 cm H_2O pressure difference between inspiration and expiration.

The aim of this study was to compare objective adherence to CPAP between APAP, APAP with C-Flex, and APAP with A-Flex over an initial 3-mo period. We also investigated daytime sleepiness, sleep quality, and quality of life (QOL). Additionally, after 3 mo, patients in the APAP with A-Flex group and the APAP with C-Flex groups were crossed over to use the alternate mode (C-Flex or A-Flex) to compare adherence. In addition, the APAP group was crossed over to the A-Flex for these final 3 mo.

METHODS

Study Participants

Patients in whom OSA was newly diagnosed (apnea-hypopnea index [AHI] > 20) were enrolled in the current study according to the Japanese Health Insurance System. Patients were excluded if they were younger than 20 y, had a major medical or psychiatric condition that would interfere with the demands of the study and adherence to PAP, or had ever used CPAP therapy. This study was approved by the Ethics Committee of Kyoto University. All patients gave written informed consent to participate.

Submitted for publication March, 2012
Submitted in final revised form June, 2012
Accepted for publication July, 2012
Address correspondence to: Kazuo Chin, MD, PhD, Departments of Respiratory Care and Sleep Control Medicine, Kyoto University Graduate School of Medicine, 54 Shogoin Kawahara-cho, Sakyo-Ku, Kyoto 606-8507, Japan; Tel: 81-75-751-3852; Fax: 81-75-751-3854; E-mail: chink@kuhp.kyoto-u.ac.jp

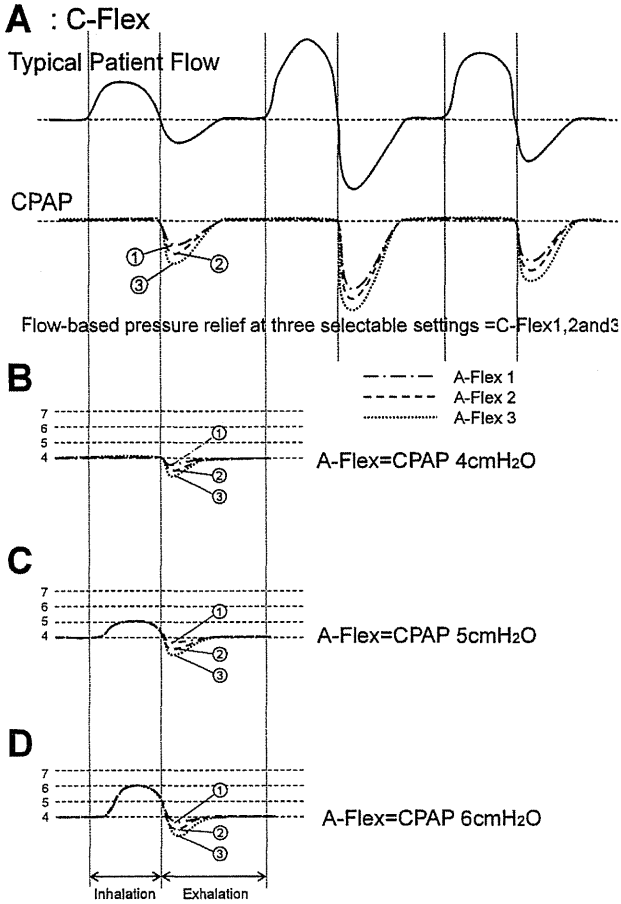

Figure 1—(A) C-Flex pressure relief reduced pressure at the beginning of exhalation and returned to the therapeutic pressure just before inhalation. The level of pressure relief varies based on the patient's expiratory flow and which of the three C-Flex settings (1, 2, and 3) has been selected. **(B-D)** A-Flex has the same function as C-Flex during expiration. At 4 cm H_2O CPAP, the A-Flex PAP machine is same as the C-Flex machine PAP **(B)**. At 5 cm H_2O CPAP, the A-Flex PAP machine can provide 1 cm H_2O pressure support at the inspiration **(C)**. From 6 or more cm H_2O CPAP, A-Flex machine can make 2 cm H_2O pressure supports at the inspiration **(D)**.

C-Flex and A-Flex PAP Machines

C-Flex pressure relief was developed to make CPAP therapy more comfortable by reducing pressure at the beginning of exhalation and returning to the therapeutic pressure just before inhalation (Figure 1A). The level of pressure relief varies based on the patient's expiratory flow and which of the three C-Flex settings (1, 2, and 3) has been selected.

Like C-Flex, A-Flex provides flow-based pressure relief at the beginning of exhalation. At 4 cm H_2O CPAP, the A-Flex PAP machine is the same as the C-Flex machine PAP (Figure 1B). However, at 5 cm H_2O CPAP, the A-Flex PAP machine can provide 1 cm H_2O pressure support at inspiration (Figure 1C). From 6 cm or more H_2O CPAP, the A-Flex machine can provide 2 cm H_2O pressure support at inspiration (Figure 1D). A-Flex has the same function as C-Flex during expiration (Figure 1B-D).

Study Design

The study was a randomized single-blind (patients blinded) crossover trial of APAP versus APAP with C-Flex (set to dip level 3) versus APAP with A-Flex (set to dip level 3). The dip level is the level of pressure reduction during expiration, and dip level 3 is the greatest pressure reduction during expiration. Data were collected at baseline and after 3 and 6 mo of PAP treatment. At baseline, we recorded the patients' demographic characteristics and polysomnographic data. In addition, subjective sleepiness, sleep quality, and health-related QOL were measured using the Epworth Sleepiness Scale (ESS),[12] Pittsburgh Sleep Quality Index (PSQI),[13] and Calgary Sleep Apnea Quality of Life Index (SAQLI).[14,15] The SAQLI consists of five domains: daily functioning (domain A), social interactions (domain B), emotional functioning (domain C), symptoms (domain D), and treatment-related symptoms (domain E). The total SAQLI score for domains A through D was obtained before and after CPAP treatment, and the score for domain E was factored in after the patient received CPAP. CPAP titration was performed with autotitration during full-night polysomnography (PSG) attended by sleep technicians and the pressure range was between 4 and 20 cm H_2O. For CPAP titration, each patient underwent full-night PSG on the allocated CPAP mode (i.e., APAP with C-Flex group underwent full-night PSG on APAP with C-Flex). The attending technicians checked for abnormal movements of the titrated PAP machine. However, they could not find any trouble during this trial.

The primary outcome was objective adherence after 3 mo of CPAP treatment. Objective adherence was downloaded from the memory card (Encore Pro Smartcard; Philips-Respironics) located in the PAP device. Secondary outcomes were ESS, PSQI, and SAQLI at 3 mo after the beginning of CPAP treatment.

Additionally, after 3 mo of PAP treatment, the APAP with A-Flex group and the APAP with C-Flex group were crossed over to the alternate mode (C-Flex or A-Flex) and the APAP group was switched to A-Flex for an additional 3 mo. The evaluations performed at 3 mo were repeated 6 mo after the start of CPAP treatment.

Polysomnography

The diagnosis of OSA was confirmed by PSG (SomnoStar Pro, Cardinal Health, Dublin, OH, USA), which was started at 22:00 and ended at 6:00 the following morning. Surface electrodes were attached using standard techniques to obtain an electrooculogram, electromyogram of the chin, and 12-lead electroencephalograph. Sleep stages were defined according to the criteria of Rechtschaffen and Kales.[16] Ventilation/respiratory effort was monitored by inductive plethysmography (Respitrace QDC, Viasys Healthcare, Palm Springs, CA, USA). Airflow was monitored by a nasal pressure transducer (PTAFlite, Pro-Tech Services Inc., Mukilteo, WA, USA) and supplemented by an oronasal thermal sensor (Sleepmate Technologies, Midlothian, VA, USA). Arterial oxygen saturation (SaO_2) was monitored continuously with a pulse oximeter (Adult Flex System, Nonin Medical, Plymouth, MN, USA).

Apnea was defined as the complete cessation of airflow and hypopnea as a clear decrease in airflow of 50% or more lasting for 10 sec or more accompanied by a decrease in SpO_2 of at least 3% and/or associated with arousal.[17] All AHI values were expressed as the number of episodes of apnea and hypopnea per h over the total sleep time. The lowest SpO_2 during sleep was calculated in each patient.

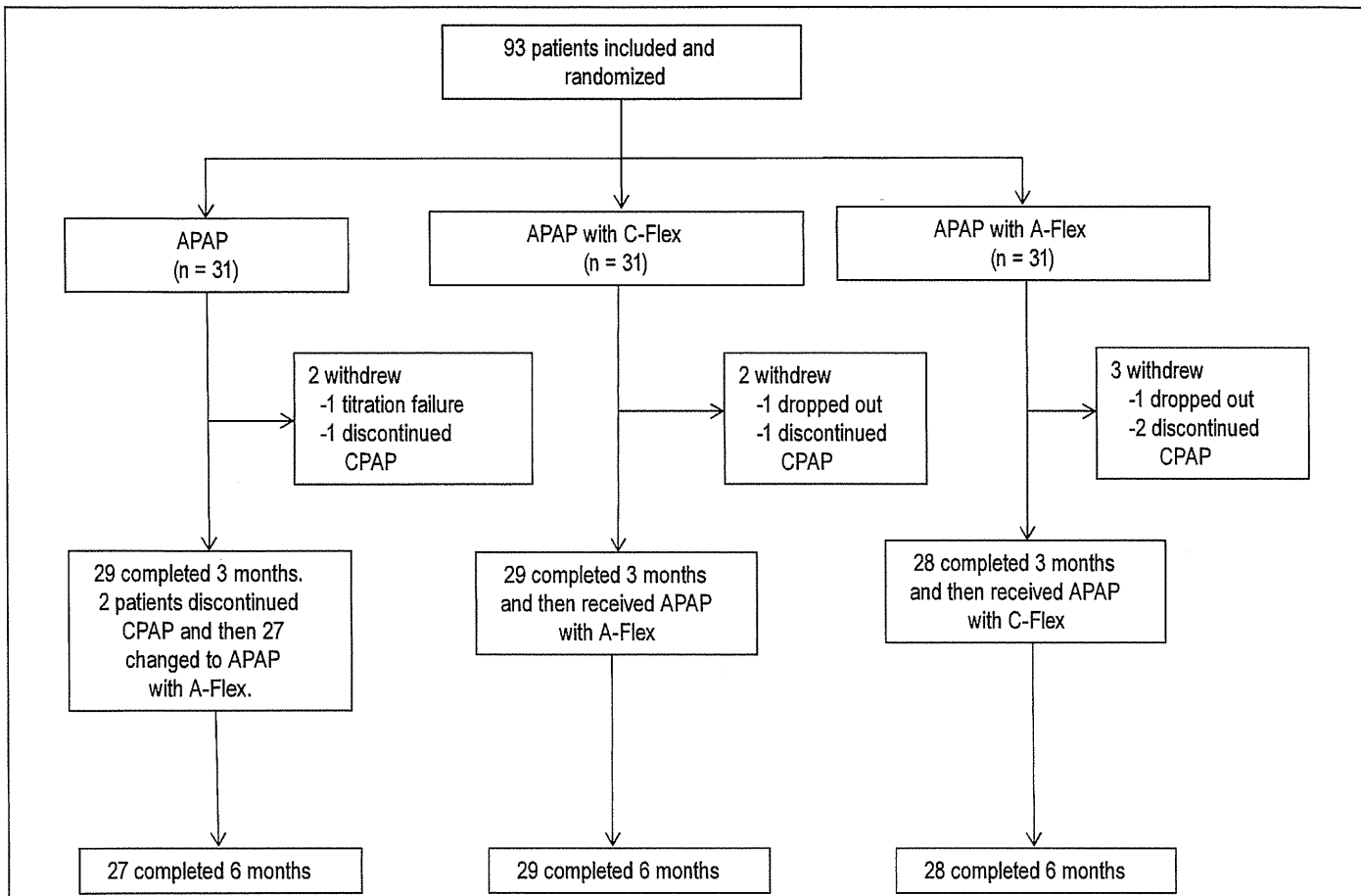

Figure 2—Flow chart of the study population. APAP, auto-adjusting positive airway pressure; CPAP, continuous positive airway pressure.

Randomization

This study was randomized via blinded envelope prior to the beginning of the study.

Power Analysis

Based on a previous study,[7] differences of 1 h/night of PAP adherence between each group were decided to be clinically significant, and standard deviation (SD) of 1.5 h/night was expected. The sample size was set to achieve 80% power at a 5% significance level. The calculated sample size in each group was 36 patients.

Statistical Analysis

Data analysis was conducted using a statistical software program (Statview, version 5.0; SAS Institute Inc., Cary, NC, USA). Data were expressed as mean ± SD or absolute numbers and percentages in each study group. The patients' demographic characteristics, polysomnographic data, responses to three questionnaires at baseline and after 3 mo of CPAP treatment, and adherence after 3 mo of CPAP treatment were compared among the three groups using a one-way analysis of variance. When a significant difference was observed, we used the Bonferroni/Dunn method to identify where the differences were significant. For categorical variables, the χ^2 test was used. Within the group, comparisons of adherence (3 mo versus 6 mo) or of results from the three questionnaires (baseline versus 3 mo, 3 mo versus 6 mo) were analyzed using a paired *t* test. In all analyses, $P < 0.05$ was considered statistically significant.

RESULTS

Baseline Assessments

Figure 2 shows the study flow chart. Ninety-three patients in whom OSA was newly diagnosed were randomly assigned to receive 3 mo of APAP (n = 31), APAP with C-Flex (n = 31), or APAP with A-Flex (n = 31). During the first 3 mo, two patients withdrew from the APAP group (one discontinued PAP, one had Cheyne-Stokes breathing during PAP titration), two patients withdrew from the APAP with C-Flex group (one dropped out, one discontinued PAP), and three patients withdrew from the APAP with A-Flex group (one dropped out, two discontinued PAP). Data on patients who dropped out during the first 3 mo were excluded from the analysis.

Tables 1 and 2 summarize baseline characteristics and polysomnographic data, respectively, on study participants. Patients were predominantly male, middle-aged, and had moderate to severe OSA. Neither baseline characteristics nor polysomnographic data differed among the three groups.

Effects of CPAP Treatment on PSG Variables

Table 3 shows the polysomnographic data on the PAP night. Compared with the baseline PSG, significant changes in polysomnographic variables such as the AHI and arousal index were

Table 1—Baseline characteristics of study participants[a]

	All (n = 86)	APAP (n = 29)	APAP with C-Flex (n = 29)	APAP with A-Flex (n = 28)	P value
Age (y)	59.3 ± 10.8	56.9 ± 8.8	61.0 ± 10.7	59.8 ± 12.6	0.33
Sex (male/female)	72/14	25/4	24/5	23/5	0.90
BMI (kg/m^2)	27.1 ± 4.7	27.4 ± 5.5	27.4 ± 4.2	26.6 ± 4.4	0.77
Living alone	11 (12.8)	6 (20.7)	3 (10.3)	2 (7.1)	0.29
Hypertension	50 (58.1)	14 (48.3)	20 (69.0)	16 (57.1)	0.28
Dyslipidemia	42 (48.8)	14 (48.3)	13 (44.8)	15 (53.6)	0.80
Diabetes mellitus	27 (31.4)	11 (37.9)	7 (24.1)	9 (32.1)	0.52
ESS	9.2 ± 4.4	9.5 ± 4.6	9.2 ± 4.5	8.9 ± 4.3	0.88

[a]Mean ± SD or number (%). APAP, auto-adjusting positive airway pressure; BMI, body mass index; ESS, Epworth Sleepiness Scale.

Table 2—Baseline polysomnographic data on study participants[a]

	APAP (n = 29)	APAP with C-Flex (n = 29)	APAP with A-Flex (n = 28)	P value
Sleep efficiency, %	75.1 ± 11.9	72.2 ± 12.7	70.6 ± 15.0	0.43
S1, %	26.6 ± 13.7	26.8 ± 14.2	27.2 ± 14.2	0.98
S2, %	52.0 ± 11.8	54.2 ± 13.5	52.7 ± 11.0	0.78
S3/4, %	6.4 ± 8.9	4.4 ± 6.2	6.3 ± 8.6	0.57
REM, %	15.0 ± 5.0	14.6 ± 5.0	13.9 ± 6.7	0.73
Arousal, events/h	34.1 ± 12.4	36.3 ± 14.7	36.2 ± 14.2	0.79
Central apnea, events/h	2.9 ± 5.0	2.3 ± 3.7	3.3 ± 5.2	0.69
AHI, events/h	40.8 ± 12.4	43.3 ± 15.2	45.7 ± 15.9	0.46
Mini SpO_2 (%)	77.0 ± 7.4	75.2 ± 12.8	78.4 ± 6.5	0.44
SpO_2 < 90% (% TST)	16.2 ± 14.9	14.9 ± 16.6	18.4 ± 23.2	0.77

[a]Mean ± SD or number (%). AHI, apnea-hypopnea index; APAP, auto-adjusting positive airway pressure; REM, rapid eye movement; SpO_2, percutaneous oxygen saturation; TST, total sleep time.

Table 3—Polysomnographic data on study participants upon CPAP titration[a]

	APAP (n = 29)	APAP with C-Flex (n = 29)	APAP with A-Flex (n = 28)	P value
Sleep efficiency, %	77.2 ± 11.4	76.5 ± 7.9	72.0 ± 11.5	0.14
S1, %	15.7 ± 7.7	16.2 ± 9.9	16.9 ± 9.5	0.89
S2, %	57.3 ± 8.0	60.7 ± 11.8	57.1 ± 9.3	0.29
S3/4, %	6.9 ± 8.3	4.4 ± 5.3	6.6 ± 7.3	0.34
REM, %	20.1 ± 5.0	18.7 ± 6.1	19.5 ± 5.8	0.63
Arousal, events/h	20.2 ± 9.0	19.7 ± 9.4	20.7 ± 9.7	0.93
Central apnea, events/h	1.4 ± 1.6	1.4 ± 1.8	2.5 ± 2.9	0.09
AHI, events/h	4.0 ± 3.7	3.8 ± 4.7	6.0 ± 4.5	0.11
Mini SpO_2 (%)	90.2 ± 3.9	87.5 ± 9.4	88.8 ± 4.5	0.27
SpO_2 < 90% (% TST)	0.2 ± 0.6	2.1 ± 6.2	1.2 ± 4.8	0.30

[a]Mean ± SD or number (%). AHI, apnea-hypopnea index; APAP, auto-adjusting positive airway pressure; CPAP, continuous positive airway pressure; REM, rapid eye movement; SpO_2, percutaneous oxygen saturation; TST, total sleep time.

noted in all three groups. There were no significant differences among the three groups in the polysomnographic data on CPAP titration. Also, the number of patients who used hypnotic agents during CPAP titration did not differ significantly among the three groups (APAP with C-Flex: 7 of 29, 24.4% versus APAP: 11 of 29, 37.9% versus APAP with A-Flex: 9 of 28, 32.1%; P = 0.52).

After 3 mo of treatment, the 90th percentile PAP, leak, and mean AHI were downloaded and recorded from the memory card located in the PAP device. There were no significant differences among the three groups in terms of residual AHI (APAP with C-Flex: 3.4 ± 2.2 events/h versus APAP: 3.5 ± 2.0 h/night versus APAP with A-Flex: 4.6 ± 2.9 h/night; P = 0.11), 90th percentile PAP (APAP with C-Flex: 8.4 ± 1.7 cm H_2O versus APAP: 8.2 ± 1.3 cm H_2O versus APAP with A-Flex: 8.8 ± 1.7 cm H_2O; P = 0.32), or 90th percentile leak (APAP with C-Flex: 45.7 ± 7.0 L/min versus APAP: 49.7 ± 13.1 L/min versus APAP with A-Flex: 46.5 ± 11.5 L/min; P = 0.34).

Primary Outcome: Adherence Over 3 Mo

Figure 3 shows a comparison among the three groups with regard to adherence to CPAP treatment over a 3-mo period. Median adherence was significantly greater in the APAP with C-Flex group, especially compared with the APAP group (APAP with C-Flex, n = 29: 5.19 ± 1.84 h/night versus APAP, n = 292 9: 3.96 ± 1.66 h/night versus APAP with A-Flex, n = 28: 4.27 ± 2.12 h/night; P = 0.04). In the *post hoc* analysis, adherence to APAP with C-Flex was significantly greater in comparison with APAP (P = 0.01). In addition, there were significant differences among the groups in percentage of days PAP was used (APAP with C-Flex: 91.9 ± 10.6% versus APAP: 79.4 ± 21.8% versus APAP with A-Flex: 82.5 ± 22.9%; P = 0.04) but not in percentage of days PAP used > 4 h (APAP with C-Flex: 66.6 ± 29.8% versus APAP: 54.8 ± 27.9% versus APAP with A-Flex: 57.6 ± 32.7%; P = 0.30). *Post hoc* testing revealed that the percentage of days PAP was used in the APAP with C-Flex group was significantly higher than in the APAP group (P = 0.02).

Secondary Outcomes: ESS, PSQI, and SAQLI After 3 Mo of CPAP Treatment

Table 4 details the effect of CPAP treatment on ESS, PSQI, and SAQLI in the three groups. For the entire group, ESS ($P = 0.004$) and domain A ($P = 0.04$), domain C ($P = 0.006$), and domain D ($P < 0.0001$) in the SAQLI questionnaire were significantly improved after 3 mo of PAP. Differences in responses to these three questionnaires were not statistically significant among the three groups at baseline, but after 3 mo of PAP treatment differences were noted. ESS and PSQI scores were significantly improved using APAP with C-Flex (ESS, 9.2 ± 4.5 to 7.3 ± 3.8, $P = 0.01$; PSQI, 7.2 ± 3.6 to 6.1 ± 2.8, $P = 0.04$), whereas significant improvements with APAP and APAP with A-Flex were not observed. In the SAQLI questionnaire, two domains were significantly improved and the other two domains trended toward improvement in the APAP with C-Flex group. On the other hand, the APAP and APAP with A-Flex groups had a significant improvement only in one domain of the SAQLI. However, as to the changes in the values for ESS, PSQI, and SAQLI among the three groups between before and after 3 mo of CPAP, the APAP with C-Flex group tended to have greater improvement in PSQI ($P = 0.08$) than the other two groups, whereas differences in the ESS and SAQLI were not significantly different among the three groups (Table 5).

Crossover in CPAP Mode

After 3 mo, two patients discontinued PAP treatment in the APAP group. The change in the CPAP mode did not have a significant effect on PAP adherence in any group (from APAP to APAP with A-Flex, n = 27, from 4.15 ± 1.51 h/night to 4.12 ± 1.16 h/night, $P = 0.89$; from APAP with C-Flex to APAP with A-Flex, n = 29, from 5.19 ± 1.84 h/night to 4.95 ± 1.94 h/night, $P = 0.17$; from APAP with A-Flex to APAP with C-Flex, n = 28, from 4.27 ± 2.12 h/night to 4.15 ± 1.99 h/night, $P = 0.56$). Although the group of patients moving from APAP to APAP with A-Flex tended to show improvement in domain B of the SAQLI ($P = 0.07$), ESS, PSQI, and SAQLI scores did not significantly change in any group (results not shown).

In a subgroup analysis of poor compliers (< 4 h/night of PAP use), there was a significant increase in PAP adherence in the patients moving from APAP to APAP with A-Flex (n = 10, from 2.55 ± 0.76 h/night to 3.20 ± 0.85 h/night, $P = 0.01$) (Figure 4). In contrast, adherence did not change between 3 and 6 mo in the other groups.

DISCUSSION

The current study is the first to show a significant superiority of APAP with C-Flex on treatment adherence during the first 3 mo of use compared with APAP. Also, only patients using APAP with C-Flex had a significant improvement in subjective sleepiness and sleep quality. These results suggest that APAP with C-Flex may be a superior CPAP modality for the initial treatment of moderate to severe OSA.

To date, it has not been shown that PAP adherence can be improved by using different pressure applications of PAP. A meta-analysis of a number of published studies comparing

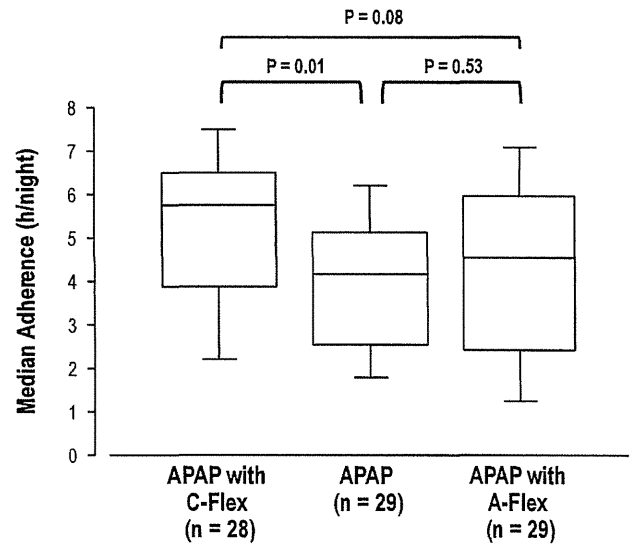

Figure 3—CPAP adherence with APAP with C-Flex, APAP, and APAP with A-Flex after 3 mo of treatment. Data are shown as a bow-whisker plot with median and 25th percentile of CPAP adherence. APAP, auto-adjusting positive airway pressure.

Table 4—Analysis of health-related quality of life and sleep related questionnaires[a]

	APAP (n = 29)			C-Flex (n = 29)			A-Flex (n = 28)		
	BL	3 mo	P value	BL	3 mo	P value	BL	3 mo	P value
ESS	9.5 ± 4.6	8.2 ± 4.0	0.12	9.2 ± 4.5	7.3 ± 3.8[b]	0.01	8.9 ± 4.3	8.1 ± 4.3	0.34
PSQI	6.0 ± 2.5	6.1 ± 2.9	0.72	7.2 ± 3.6	6.1 ± 2.8[b]	0.04	6.3 ± 3.2	5.8 ± 2.3	0.21
SAQLI									
Domain A	5.6 ± 1.1	5.7 ± 1.2	0.56	5.2 ± 1.3	5.5 ± 1.1[c]	0.08	5.4 ± 1.1	5.6 ± 1.3	0.37
Domain B	5.6 ± 0.9	5.7 ± 1.0	0.70	5.3 ± 1.1	5.7 ± 1.0[b]	0.02	5.8 ± 1.1	5.8 ± 0.8	0.89
Domain C	5.1 ± 0.9	5.3 ± 1.1	0.11	4.9 ± 1.0	5.2 ± 1.0[c]	0.06	5.1 ± 0.9	5.3 ± 0.9	0.26
Domain D	2.7 ± 1.1	3.7 ± 1.4[b]	0.0009	2.6 ± 1.2	3.5 ± 1.6[b]	0.001	2.3 ± 1.2	3.8 ± 1.7[b]	< 0.0001
Domain E		4.7 ± 1.2			4.3 ± 1.1			4.6 ± 1.2	
Total	4.8 ± 0.7	4.7 ± 1.2	0.71	4.5 ± 0.8	4.5 ± 1.1	0.83	4.7 ± 0.7	4.8 ± 1.1	0.65

[a]Mean ± SD. [b]$P < 0.05$ versus BL. [c]$P < 0.10$ versus BL. APAP, auto-adjusting positive airway pressure; BL, baseline; ESS, Epworth Sleepiness Scale; PSQI, Pittsburgh Sleep Quality Index; SAQLI, Calgary Sleep Apnea Quality of Life Index. Domain A, daily functioning; Domain B, social interactions; Domain C, emotional functioning; Domain D, symptoms; Domain E, treatment-related symptoms.

APAP with fixed CPAP revealed that there was no difference between the two modalities regarding subjective sleepiness and adherence to PAP.[4,18] The difficulty of exhaling against a fixed positive pressure is a common complaint when using CPAP, so C-Flex was developed, which reduces pressure at the beginning of exhalation and increases pressure to a level determined to be therapeutic for the latter part of exhalation. Aloia et al.[7] demonstrated that use of a C-Flex device provided a significant improvement in CPAP adherence (1.7 h/night of additional use after a 12-wk follow-up period compared with a fixed PAP therapy). However, this study was not a randomized trial. Thereafter, Marshall et al.[19] reported that in a 4-wk randomized study there was a trend toward greater CPAP adherence with C-Flex compared with fixed PAP therapy (C-Flex, 4.7 ± 2.9 h/night versus fixed PAP, 3.0 ± 2.1 h/night), but the difference was not statistically significant because of the small sample size. A meta-analysis also found that C-Flex does not provide any significant benefit over fixed PAP in terms of treatment adherence.[20]

In the current study, we confirmed that APAP with C-Flex was associated with significantly greater CPAP adherence than APAP. Many studies suggested that APAP reduces the mean PAP requirements, which are thought to influence CPAP adherence.[4-6] C-Flex is designed to flexibly deliver pressure on a breath-by-breath basis by adjusting pressure within exhalation, which may be a more important variable for treatment adherence than the overall PAP level. This C-Flex technology may be responsible for the significant superiority of treatment adherence with APAP with C-Flex compared with APAP. Our study also showed that adherence in the APAP with A-Flex group did not improve after changing to the use of APAP with

Table 5—Changes in values of ESS, PSQI, and SAQRI among the three groups from before and after 3 mo of CPAP[a]

	APAP (n = 29)	APAP with C-Flex (n = 29)	APAP with A-Flex (n = 28)	P value
ESS	-1.3 ± 4.3	-2.2 ± 3.9	-0.8 ± 4.3	0.45
PSQI	0.2 ± 2.5	-1.3 ± 2.9	-0.5 ± 2.1	0.08
SAQLI				
Domain A	0.1 ± 0.8	0.1 ± 1.7	0.2 ± 1.0	0.97
Domain B	0.1 ± 0.7	0.1 ± 1.5	0.03 ± 1.1	0.94
Domain C	0.2 ± 0.6	0.1 ± 1.2	0.2 ± 0.7	0.89
Domain D	1.0 ± 1.4	0.8 ± 1.4	1.5 ± 1.7	0.26
Domain E	–	–	–	–
Total	-0.1 ± 0.9	-0.1 ± 1.3	0.1 ± 1.0	0.73

[a]Mean ± SD. APAP, auto-adjusting positive airway pressure; ESS, Epworth Sleepiness Scale; PSQI, Pittsburgh Sleep Quality Index; SAQLI, Calgary Sleep Apnea Quality of Life Index.

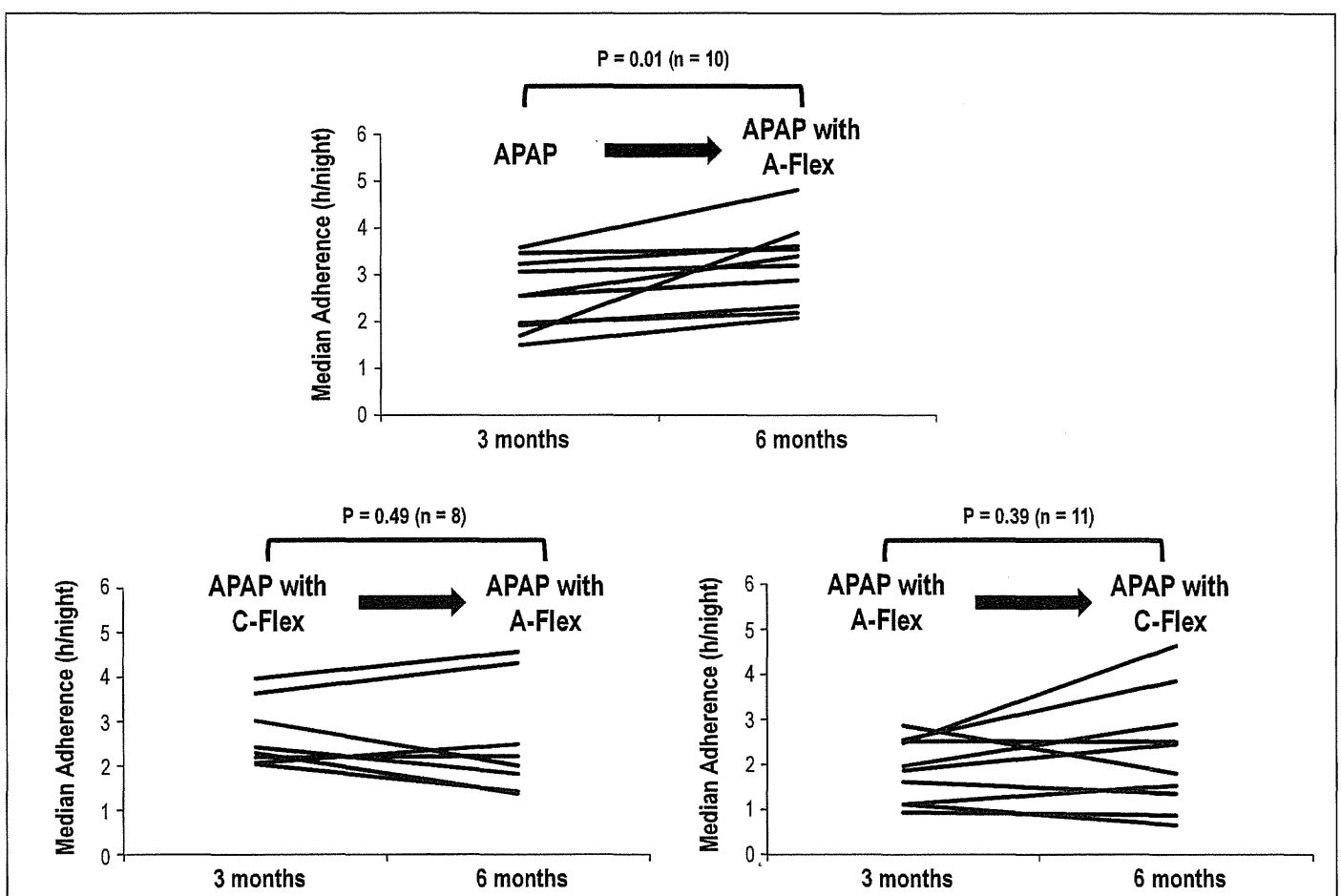

Figure 4—Analysis of poor compliers (< 4 h/night of CPAP use) between 3 and 6 mo. Individual data are presented.

C-Flex. CPAP adherence within the first 3 mo has been described as a strong predictor of long-term CPAP use.[21]

CPAP adherence is a key issue in OSA, as greater improvements in daytime sleepiness, blood pressure, and QOL occur with greater CPAP use.[22-25] A number of different variables independent of the CPAP mode can influence CPAP adherence. Older age, the use of lipid-lowering medications, the use of sedative/hypnotic agents on CPAP titration, a higher AHI, and greater daytime sleepiness were all factors shown to increase CPAP adherence.[21,26-30] In contrast, a lower body mass index (BMI) ($\leq$ 30 kg/m^2) and psychosocial factors such as living alone have been shown to be associated with poor CPAP adherence.[31,32] In the current study, there was no significant difference in these variables among the three groups. Also, all study patients received the same education on CPAP treatment. In addition, no humidification was used in any participant and their interfaces for CPAP treatment did not change during the study period. Therefore, we think that the CPAP mode was the main driver of the results of our study.

Pépin et al.[10] reported that after changing the CPAP mode from a fixed PAP to C-Flex, CPAP adherence was significantly improved in patients with low adherence (< 4 h/night of CPAP use) during the initial 3 mo, whereas this improvement did not occur in patients who were already using CPAP for > 4 h/night. These results suggest that C-Flex is an effective modality for improving adherence. Compared with patients in previous reports,[8-11] our patients had relatively low CPAP adherence (mean ± SD: 4.5 ± 1.9 h/night). Several reasons could account for this finding. As described previously, our study population had a low BMI (27.1 ± 4.7) and mild to moderate daytime sleepiness (mean ESS:,9.2 ± 4.4). It has already been described that a lower BMI and less daytime sleepiness were associated with poor CPAP adherence.[21,31] In addition, poor sleep quality and QOL were only minimally abnormal in our patients compared with previous reports.[20,32,33] Improving sleep-related QOL after CPAP in patients already having comparatively high sleep quality and QOL would seem to be difficult. Wells et al.[34] demonstrated that patients who experienced greater improvements in daily functioning had higher levels of CPAP adherence. Therefore, the absence of improvement in subjective symptoms makes it unlikely that high levels of CPAP adherence would be achieved. Taken together, our patients had several traits that could be associated with poor CPAP compliance. This might explain why C-Flex had a significant effect on CPAP adherence in the current study.

We also demonstrated that the use of APAP with A-Flex significantly improved some QOL factors and that CPAP adherence in the APAP with A-Flex group was not greater than in the APAP group. On the other hand, a significant improvement in CPAP adherence was observed in the subgroup of poor compliers who were moved from APAP to APAP with A-Flex. This result suggests that A-Flex may be an alternative CPAP mode for improving adherence in patients with poor compliance. However, further studies using randomized prospective designs are needed to confirm this result. In addition, a future study that investigates the change in PAP adherence after a switch from APAP to APAP with C-Flex is warranted.

This study had some limitations. First, our study population was Asian, and their BMI was comparatively low. Therefore, it might be difficult to apply the results of the current study to all patients with OSA. Second, this was not a double-blind but a single-blind (patients blinded) randomized study. The investigators might be influenced in their assessments by knowing the treatment received. However, the same education on PAP therapy and follow-up methods after randomization were conducted for all patients. Thus, we think this possible limitation had a minimal effect on the study outcomes.

In conclusion, this prospective, randomized study demonstrated that APAP with C-Flex led to significantly greater adherence than APAP. APAP with C-Flex is a potentially superior CPAP mode among the three tested. Although we did not observe significant changes in PAP adherence after the switch from APAP to APAP with C-Flex, A-Flex seemed to be an effective CPAP approach in patients with poor adherence to APAP.

ACKNOWLEDGMENTS

The authors thank Dr. Takegami for her kind comments and suggestions for statistical analysis, along with Mses. Toki, Tamura, and Kimura for their support of this clinical research. Work for this study was performed at the Sleep unit of Kyoto University Hospital. This work was supported by grants from the Japanese Ministry of Education, Culture, Sports, Science and Technology (nos. 20590921 and 22590860), Respiratory Failure Research Group and Health Science Research Grants (Comprehensive Research on Life-Style Related Diseases Including Cardiovascular Diseases and Diabetes Mellitus) from the Ministry of Health, Labor and Welfare of Japan, and the Japan Vascular Disease Research Foundation.

DISCLOSURE STATEMENT

This was not an industry supported study. Drs. Hitomi, Yoshimura, Oga, and Chin belong to departments that have received support from Philips-Respironics, Teijin Pharma, and Fukuda Denshi. The other authors have indicated no financial conflicts of interest.

REFERENCES

1. Marin JM, Carrizo SJ, Vicente E, Agusti AG. Long-term cardiovascular outcomes in men with obstructive sleep apnoea-hypopnoea with or without treatment with continuous positive airway pressure: an observational study. Lancet 2005; 365:1046-53.
2. Jenkinson C, Davies RJ, Mullins R, Stradling JR. Comparison of therapeutic and subtherapeutic nasal continuous positive airway pressure for obstructive sleep apnoea: a randomised prospective parallel trial. Lancet 1999;353:2100-5.
3. Montserrat JM, Ferrer M, Hernandez L, et al. Effectiveness of CPAP treatment in daytime function in sleep apnea syndrome: a randomized controlled study with an optimized placebo. Am J Respir Crit Care Med 2001;164:608-13.
4. Ayas NT, Patel SR, Malhotra A, et al. Auto-titrating versus standard continuous positive airway pressure for the treatment of obstructive sleep apnea: results of a meta-analysis. Sleep 2004;27:249-53.
5. Nolan GM, Doherty LS, Mc Nicholas WT. Auto-adjusting versus fixed positive pressure therapy in mild to moderate obstructive sleep apnoea. Sleep 2007; 30:189-94.
6. Hukins C. Comparative study of autotitrating and fixed-pressure CPAP in the home: a randomized, single-blind crossover trial. Sleep 2004;27:1512-7.
7. Aloia MS, Stanchina M, Arnedt JT, Malhotra A, Millman RP. Treatment adherence and outcomes in flexible vs standard continuous positive airway pressure therapy. Chest 2005;127:2085-93.
8. Nilius G, Happel A, Domanski U, Ruhle KH. Pressure-relief continuous positive airway pressure vs constant continuous positive airway pressure: a comparison of efficacy and compliance. Chest 2006;130:1018-24.

9. Dolan DC, Okonkwo R, Gfullner F, Hansbrough JR, Strobel RJ, Rosenthal L. Longitudinal comparison study of pressure relief (C-Flex) vs. CPAP in OSA patients. Sleep Breath 2009;13:73-7.
10. Pépin JL, Muir JF, Gentina T, et al. Pressure reduction during exhalation in sleep apnea patients treated by continuous positive airway pressure. Chest 2009; 136:490-7.
11. Bakker J, Campbell A, Neill A. Randomized controlled trial comparing flexible and continuous positive airway pressure delivery: effects on compliance, objective and subjective sleepiness and vigilance. Sleep 2010;33:523-9.
12. Johns MW. A new method for measuring daytime sleepiness: the Epworth sleepiness scale. Sleep 1991;14:540-5.
13. Buysse DJ, Reynolds CF 3rd, Monk TH, Berman SR, Kupfer DJ. The Pittsburgh Sleep Quality Index: a new instrument for psychiatric practice and research. Psychiatry Res 1989;28:193-213.
14. Flemons WW, Reimer MA. Development of a disease-specific health-related quality of life questionnaire for sleep apnea. Am J Respir Crit Care Med 1998; 158:494-503.
15. Flemons WW, Reimer MA. Measurement properties of the calgary sleep apnea quality of life index. Am J Respir Crit Care Med 2002;165:159-64.
16. Rechtschaffen A, Kales A. A manual of standardized terminology, techniques and scoring system for sleep stages of human subjects. Washington, DC: National Institutes of Health, 1968.
17. Iber C, Ancoli-Israel S, Chesson A, Quan S. The AASM manual for the scoring of sleep and associated events: rules, terminology and technical specifications. Westchester, Illinois: American Academy of Sleep Medicine, 2007.
18. Smith I, Lasserson TJ. Pressure modification for improving usage of continuous positive airway pressure machines in adults with obstructive sleep apnoea. Cochrane Database Syst Rev 2009:CD003531.
19. Marshall NS, Neill AM, Campbell AJ. Randomised trial of compliance with flexible (C-Flex) and standard continuous positive airway pressure for severe obstructive sleep apnea. Sleep Breath 2008;12:393-6.
20. Bakker JP, Marshall NS. Flexible pressure delivery modification of continuous positive airway pressure for obstructive sleep apnea does not improve compliance with therapy: systematic review and meta-analysis. Chest 2011;139:1322-30.
21. McArdle N, Devereux G, Heidarnejad H, Engleman HM, Mackay TW, Douglas NJ. Long-term use of CPAP therapy for sleep apnea/hypopnea syndrome. Am J Respir Crit Care Med 1999;159:1108-14.
22. Faccenda JF, Mackay TW, Boon NA, Douglas NJ. Randomized placebo-controlled trial of continuous positive airway pressure on blood pressure in the sleep apnea-hypopnea syndrome. Am J Respir Crit Care Med 2001;163:344-8.
23. Weaver TE, Maislin G, Dinges DF, et al. Relationship between hours of CPAP use and achieving normal levels of sleepiness and daily functioning. Sleep 2007; 30:711-9.
24. Stradling JR, Davies RJ. Is more NCPAP better? Sleep 2000;23:S150-3.
25. Zimmerman ME, Arnedt JT, Stanchina M, Millman RP, Aloia MS. Normalization of memory performance and positive airway pressure adherence in memory-impaired patients with obstructive sleep apnea. Chest 2006;130:1772-8.
26. Collen J, Lettieri C, Kelly W, Roop S. Clinical and polysomnographic predictors of short-term continuous positive airway pressure compliance. Chest 2009;135:704-9.
27. Platt AB, Kuna ST, Field SH, et al. Adherence to sleep apnea therapy and use of lipid-lowering drugs: a study of the healthy-user effect. Chest 2010;137:102-8.
28. Lettieri CJ, Collen JF, Eliasson AH, Quast TM. Sedative use during continuous positive airway pressure titration improves subsequent compliance: a randomized, double-blind, placebo-controlled trial. Chest 2009;136:1263-8.
29. Lettieri CJ, Shah AA, Holley AB, Kelly WF, Chang AS, Roop SA. CPAP promotion and prognosis-The Army Sleep Apnea Program Trial: effects of a short course of eszopiclone on continuous positive airway pressure adherence: a randomized trial. Ann Intern Med 2009;151:696-702.
30. Drake CL, Day R, Hudgel D, et al. Sleep during titration predicts continuous positive airway pressure compliance. Sleep 2003;26:308-11.
31. Pelletier-Fleury N, Rakotonanahary D, Fleury B. The age and other factors in the evaluation of compliance with nasal continuous positive airway pressure for obstructive sleep apnea syndrome: a Cox's proportional hazard analysis. Sleep Med 2001;2:225-32.
32. Lewis KE, Seale L, Bartle IE, Watkins AJ, Ebden P. Early predictors of CPAP use for the treatment of obstructive sleep apnea. Sleep 2004;27:134-8.
33. Siccoli MM, Pepperell JC, Kohler M, Craig SE, Davies RJ, Stradling JR. Effects of continuous positive airway pressure on quality of life in patients with moderate to severe obstructive sleep apnea: data from a randomized controlled trial. Sleep 2008; 31:1551-8.
34. Wells RD, Freedland KE, Carney RM, Duntley SP, Stepanski EJ. Adherence, reports of benefits, and depression among patients treated with continuous positive airway pressure. Psychosom Med 2007;69:449-54.

LIVER TRANSPLANTATION 18:1217-1225, 2012

ORIGINAL ARTICLE

Use of Noninvasive Ventilation for Pediatric Patients After Liver Transplantation: Decrease in the Need for Reintubation

Kimihiko Murase,[1] Yuichi Chihara,[1] Kenichi Takahashi,[5] Shinya Okamoto,[6] Hajime Segawa,[2] Kazuhiko Fukuda,[2] Koichi Tanaka,[7] Shinji Uemoto,[3] Michiaki Mishima,[1] and Kazuo Chin[4]

Departments of [1]Respiratory Medicine, [2]Anesthesiology, [3]Hepatobiliary-Pancreatic Surgery and Transplantation, and [4]Respiratory Care and Sleep Control Medicine, Graduate School of Medicine, Kyoto University, Kyoto, Japan; [5]Department of Respiratory Medicine, Red Cross Otsu Hospital, Otsu, Japan; [6]Department of Pediatric Surgery, Kyoto University Hospital, Kyoto, Japan; and [7]Foundation for Biomedical Research and Innovation, Kobe, Japan

Noninvasive ventilation (NIV) refers to ventilation delivered through a noninvasive interface (a nasal or face mask) rather than an invasive interface (an endotracheal tube or tracheostomy). The role of NIV in preventing reintubation after abdominal surgery in pediatric patients is uncertain. Therefore, we evaluated the role of NIV for this purpose in pediatric patients after liver transplantation. We successfully started using NIV for respiratory complications (RCs) in pediatric patients undergoing liver transplantation in 1999. For this report, we screened all medical records of patients under the age of 12 years who underwent liver transplantation between 2001 and 2009, and we retrieved data for cases at high risk of extubation failure. We retrospectively compared the clinical outcomes of patients who received NIV during their intensive care unit (ICU) stay and patients who did not. Data for 94 cases (92 patients) were included in this analysis. NIV was used in 47 patients during their ICU stay. The rate of reintubation for RCs was significantly lower in NIV patients versus non-NIV patients [3/47 (6.4%) versus 11/47 (23.4%), $P = 0.02$]. Furthermore, the discharge rate from the ICU was significantly better for NIV patients versus non-NIV patients. The use of NIV after extubation prevented the worsening of atelectasis and stabilized respiratory conditions in this cohort. No major changes in operative procedures or other treatments during the examined period were found. In conclusion, NIV is acceptable and promising for the respiratory management of pediatric patients undergoing liver transplantation. Its use may stabilize respiratory conditions and decrease the need for reintubation in pediatric liver transplant patients, and it may also facilitate an early ICU discharge. *Liver Transpl 18:1217-1225, 2012.* © 2012 AASLD.

Received February 21, 2012; accepted May 31, 2012.

Orthotopic liver transplantation (OLT) has become the standard of care for many pediatric patients with fatal liver diseases. In pediatric OLT patients, respiratory complications (RCs) are among the most frequently encountered early postoperative complications, and they have been reported as important causes of postoperative morbidity and mortality.[1,2]

Abbreviations: ABG, arterial blood gas; CMV, conventional mechanical ventilation; CXR, chest X-ray; EPAP, expiratory positive airway pressure; FiO_2, fraction of inspired oxygen; ICU, intensive care unit; IPAP, inspiratory positive airway pressure; NIV, noninvasive ventilation; OLT, orthotopic liver transplantation; $PaCO_2$, partial pressure of carbon dioxide; PaO_2, partial pressure of arterial oxygen; PS, pressure support; RC, respiratory complication; RCT, randomized controlled study; SaO_2, arterial oxygen saturation.

This work was supported by grants from the Japanese Ministry of Education, Culture, Sports, Science, and Technology, the Respiratory Failure Research Group (Japanese Ministry of Health, Labor, and Welfare), and the Japan Vascular Disease Research Foundation.

Address reprint requests to Kazuo Chin, M.D., Ph.D., Department of Respiratory Care and Sleep Control Medicine, Graduate School of Medicine, Kyoto University, 54 Shogoin Kawahara-Cho, Sakyo, Kyoto, Japan 606-8507. Telephone: 81-75-751-3852; FAX: 81-75-751-3854; E-mail: chink@kuhp.kyoto-u.ac.jp

DOI 10.1002/lt.23491
View this article online at wileyonlinelibrary.com.
LIVER TRANSPLANTATION.DOI 10.1002/lt. *Published on behalf of the American Association for the Study of Liver Diseases*

Noninvasive ventilation (NIV) refers to ventilation delivered through a noninvasive interface (a nasal or face mask) rather than an invasive interface (an endotracheal tube or tracheostomy). NIV is effective for respiratory insufficiency in various situations. In particular, the use of NIV to wean patients from conventional mechanical ventilation (CMV) by early extubation or to prevent reintubation has attracted much attention lately.[3-5] However, NIV has been used for this purpose only to a very small extent in pediatric populations. Although data supporting this technique in children have emerged during the last few years, experience with children has typically been restricted to case series and has mainly involved the long-term use of NIV.[6] We successfully started using NIV for RCs in OLT pediatric patients in 1999.[7] We have subsequently had many more OLT cases, and some have presented difficulties such as RCs. Therefore, we have retrospectively examined patient data to elucidate the benefits of NIV use for preventing reintubation after surgery in pediatric OLT patients, and we have compared the clinical outcomes of NIV patients and non-NIV patients.

PATIENTS AND METHODS

Subjects

We screened all medical records of patients less than 12 years old who underwent OLT between 2001 and 2009, and we identified patients at high risk for extubation failure after the operation as follows. First, we excluded patients from the analysis for the following reasons: (1) death or transfer to another hospital without extubation, (2) reintubation after extubation within the previous 3 months, (3) accidental extubation, and (4) the performance of OLT concurrently with other organ transplants. Thereafter, on the basis of previous reports on extubation failure, every patient who fulfilled at least 1 of the following criteria at extubation was included in the analysis: (1) CMV use for more than 1 week, (2) a partial pressure of arterial oxygen (PaO_2)/fraction of inspired oxygen (FiO_2) ratio < 250 mm Hg, (3) a partial pressure of carbon dioxide ($PaCO_2$) > 45 mm Hg, and (4) atelectasis of more than 1 lobe.[8-10] The data collection and analysis were performed only by authors of this article, and the use of these data was approved by the institutional review board of kyoto university hospital.

Criteria for Extubation After Operation

Details for the treatments (except for respiratory management) were reported previously by our group.[11] Although decisions about respiratory management were based on clinical judgment, the following factors were considered to be indications for extubation: (1) clinical stability and no major abdominal complications from the operation, (2) no markedly disturbed consciousness, (3) well-maintained spontaneous breathing, and (4) a PaO_2/FiO_2 ratio > 200 mm Hg under minimal ventilator support [eg, pressure support (PS) < 5 cm H_2O and expiratory positive airway pressure (EPAP) < 4 cm H_2O]. Because immunosuppressants were given to all patients, we were eager to perform extubation as early as possible to prevent ventilator-associated pneumonia. After extubation, chest X-rays (CXRs) and arterial blood gas (ABG) data were evaluated repeatedly according to the patient's respiratory condition.

NIV Technique and Oxygen Supplementation

In patients whose respiratory condition was deteriorating after extubation, the use of NIV (VPAP, ResMed, North Ryde, Australia) was considered (Fig. 1). Although decisions about the implementation of NIV were also dependent on clinical judgment, patients who fulfilled at least 1 of the following criteria were considered to be candidates for NIV application: (1) arterial oxygen saturation (SaO_2) $< 90\%$ despite maximal oxygen administration (oxygen flow rate = 15 L/minute with a simple mask or a mask with a reservoir bag), (2) hypercapnia ($PaCO_2 > 45$ mm Hg), (3) pulmonary edema, (4) worsening atelectasis, and (5) respiratory distress [eg, tachypnea and/or respiratory muscle fatigue]. PS ventilation was delivered with a nasal or face mask. After the mask was secured, the levels of PS and EPAP and the amount of oxygen were progressively increased according to the judgment of the attending physician until (1) SaO_2 was $>95\%$ and (2) the respiratory rate was decreased and/or thoracic and abdominal paradoxical movement was reduced. After NIV was introduced, the patient's condition was assessed repeatedly, and adjustments were made to NIV and oxygen settings. When NIV was initially applied, the goal was to continue its use as long as the patient could tolerate it or it appeared to no longer be necessary. When we administered only oxygen, the amount of oxygen was adjusted to keep $SaO_2 > 95\%$. Sedative medications were not given during NIV use.

Criteria for Reintubation

Patients whose respiratory conditions deteriorated despite NIV treatment or oxygen therapy or who were intolerant of NIV treatment were reintubated and managed with CMV. The predetermined criteria for reintubation were as follows: (1) a failure to maintain an SaO_2 level $> 90\%$ with adjusted NIV and/or maximal oxygen settings, (2) the development of copious tracheal secretions that could not be expectorated, (3) an increase in $PaCO_2$ accompanied by a pH ≤ 7.30 even after adjusted metabolic acidosis (suggesting rapid worsening of respiratory acidosis), (4) impaired consciousness necessitating endotracheal intubation to protect the upper airway (eg, seizure or hepatic coma), and (5) severe hemodynamic instability (defined as a systolic blood pressure < 70 mm Hg) even with inotropic drugs. If the patient was reintubated for any of the first 3 items, we counted the event as reintubation due to an RC.

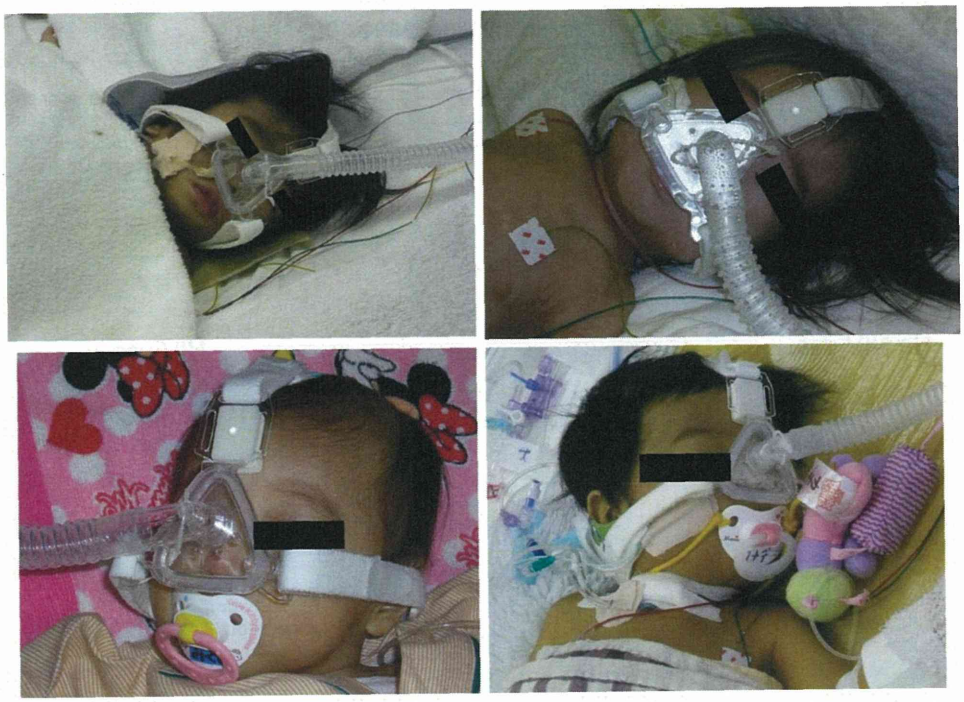

Figure 1. Pictures of pediatric patients receiving NIV in this study.

Data Analysis

We compared the clinical outcomes of the NIV and non-NIV cohorts during their intensive care unit (ICU) stays. The evaluation of outcomes was based on the following factors: reintubation in the ICU, the length of stay in the ICU and in the hospital after extubation, and survival. In each case, the timing and duration of NIV use, the initial NIV settings, the reason for reintubation, the reason for NIV use, and the cause of death were clarified. Sequential ABG data and CXR findings for patients receiving NIV were recorded before and after NIV use at 12- to 24-hour intervals. The CXR findings were reviewed by 2 respiratory physicians (K.M and Y.C.) who were blinded to clinical information.

The outcomes and background demographic data for the 2 cohorts were compared with the unpaired t test or the Mann-Whitney U test for continuous variables and with the chi-square test for categorical variables. A comparison of the ICU discharge rates for the 2 cohorts was made with Kaplan-Meier curves and a log-rank test. If a patient died or was reintubated for reasons other than respiratory problems, we did not include any further data on that patient in our analysis. Changes in ABG data were assessed with the Wilcoxon signed-rank test. Two-tailed P values < 0.05 were considered statistically significant. All statistical analyses were performed with JMP 7.0.2 statistical software (SAS Institute, Inc., Cary, NC).

RESULTS

Between 2001 and 2009, pediatric OLT was performed 251 times. After the records were screened for those cases, we found that 94 cases fulfilled the criteria for this study, and they were included in the analysis (Fig. 2). Two patients underwent retransplantation during this period. This cohort included 9 patients managed with NIV who were also included in our previous case series.[7,12] NIV was used for 47 cases during their ICU stay (Fig. 2). We compared the perioperative clinical backgrounds of NIV patients and non-NIV patients. No significant differences between the 2 cohorts were found with respect to patient characteristics, preoperative disease severity, operative conditions, or the administration of immunosuppressants (Table 1). At extubation, the vital signs and the ABG data did not differ significantly between the 2 cohorts. However, the number of patients with atelectasis was greater in the NIV cohort, and the hemoglobin level was lower (Table 2). No major changes in operative procedures were identified during the examined period.

Seven of the 47 NIV patients (14.9%) required reintubation, and 3 were reintubated because of RCs. On the other hand, 15 of the 47 non-NIV patients (31.9%) required reintubation, and 11 of these patients were reintubated because of RCs. The rate of reintubation due to RCs was significantly lower for the NIV patients ($P = 0.02$). No statistically significant differences were found between these 2 cohorts with respect to the rate of reintubation under all circumstances, the length of the hospital stay, or survival (Table 3). During the 9-year study period, the annual rate of NIV use for the cases in our analysis increased from 8.3% in 2001 to more than 80% in 2009. On the other hand, the annual rate of reintubation due to RCs decreased from 25% in 2001 to 0% in 2009 (Fig. 3).

The major reasons for NIV use were worsening atelectasis and the development of respiratory distress. Detailed reasons for NIV use, the time from extubation to NIV use, and the initial NIV settings are shown

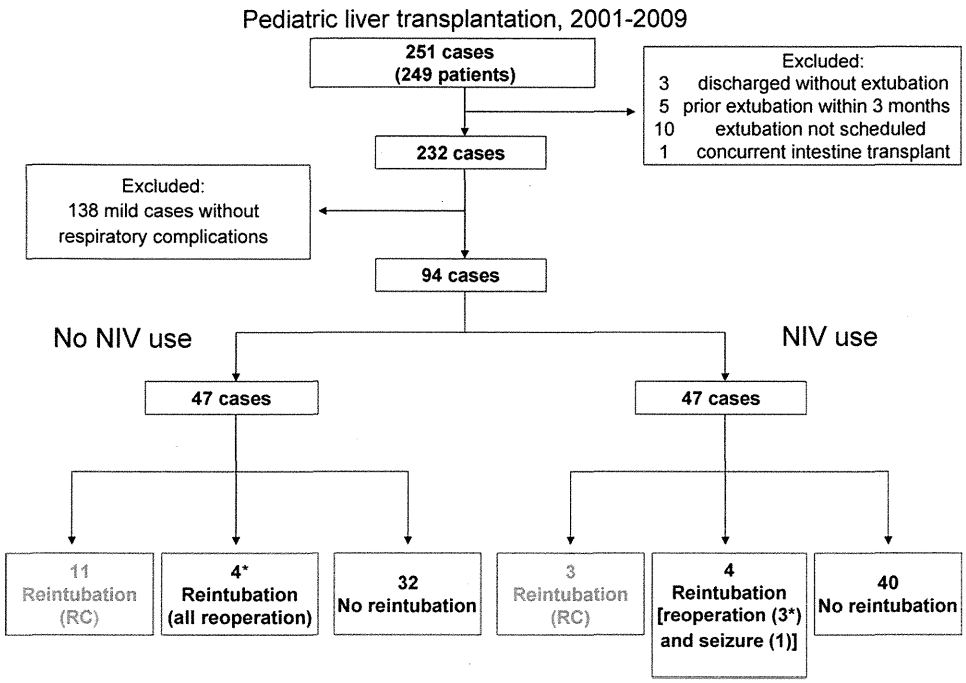

Figure 2. Patient selection and respiratory management of the study patients during their ICU stay. *Seven patients (4 in the non-NIV group and 3 in the NIV group) were reintubated because of reoperation for abdominal complications such as intra-abdominal bleeding, hepatic artery thrombosis, and bile leakage.

TABLE 1. Patient Characteristics and Perioperative Conditions

	No NIV Use (n = 47)	NIV Use (n = 47)	*P* Value
Background			
Male sex [n (%)]	20 (42.6)	20 (42.6)	>0.89
Age (months)*	10 (7-63)	10 (7-87)	0.87
Age < 1 year [n (%)]	21 (44.7)	25 (53.2)	0.54
Height (cm)*	72 (63-105)	67 (63-109)	0.60
Body weight (kg)*	8.5 (6.8-18.9)	7.5 (5.9-17)	0.16
Etiology [n (%)]			
Biliary atresia	31 (66.0)	29 (61.7)	0.09
Fulminant hepatitis	3 (6.4)	1 (2.1)	
Wilson's disease	2 (4.3)	0	
Hepatoblastoma	3 (6.4)	0	
Congenital portosystemic shunt	0	4 (8.5)	
Retransplantation	3 (6.4)	7 (14.9)	
Other	5 (10.6)	6 (12.8)	
Preoperative factors*			
Total bilirubin (mg/dL)	9.7 (1.9-18.6)	11.9 (4.3-17.4)	0.75
Albumin (g/dL)	3.4 (3.0-3.9)	3.3 (2.9-3.8)	0.32
Prothrombin time (seconds)	14.3 (12.8-18.5)	14.6 (13.1-16.7)	0.85
Time from onset to transplantation (months)	8 (3-38)	8 (4-54)	0.82
Pediatric End-Stage Liver Disease score	18.8 (1.3-26.7)	16.7 (7.5-26.2)	0.95
Operative factors			
Incompatible donor blood type [n (%)]	6 (12.8)	11 (23.4)	0.18
Blood loss/body weight (mL/g)*	0.09 (0.03-0.20)	0.13 (0.06-0.21)	0.27
Graft liver/body weight (%)*	2.57 (1.57-3.16)	2.82 (1.76-3.46)	0.49
Immunosuppressants [n (%)]			
Tacrolimus	47 (100)	47 (100)	>0.88
Steroid	47 (100)	47 (100)	>0.88
Mycophenolate mofetil	9 (19.1)	10 (21.3)	0.80

*The data are expressed as medians and first and third quartiles.

in Table 4. Because it was occasionally difficult for the spontaneous-timed mode of our device to trigger a patient's breathing and to achieve sufficient ventilator support for tachypneic breathing, we basically applied the timed mode for patients less than 1 year old.[7]

TABLE 2. Condition of the Patients at Extubation

	No NIV Use (n = 47)	NIV Use (n = 47)	*P* Value
Time from operation to extubation (days)*	1 (1-2)	1 (1-3)	0.11
Vital signs at extubation			
Systolic blood pressure (mm Hg)	102.8 ± 15.7	99.2 ± 15.5	0.25
Diastolic blood pressure (mm Hg)	49.5 ± 10.3	53.7 ± 13.6	0.40
Heart rate (beats/minute)	105.7 ± 23.9	111.1 ± 21.7	0.23
Body temperature (°C)	36.9 ± 0.8	37.0 ± 0.7	0.41
Respiratory rate (breaths/minute)	29.3 ± 11.6	26.5 ± 6.7	0.70
ABG data at extubation			
PaO_2/FiO_2 ratio (mm Hg)	282.7 ± 114.5	281.8 ± 103.8	0.99
$PaCO_2$ (mm Hg)	41.4 ± 8.2	40.0 ± 6.0	0.89
pH	7.40 ± 0.06	7.39 ± 0.05	0.19
Imaging findings for atelectasis [n (%)]	23 (48.9)	35 (74.5)	0.01
Blood examination data on extubation			
White blood cells ($\times 10^3/\mu L$)	9.7 ± 4.7	10.7 ± 5.0	0.33
Hemoglobin (g/dL)	9.9 ± 1.5	8.9 ± 1.8	<0.01
Platelets ($\times 10^4/\mu L$)	10.2 ± 7.6	9.1 ± 6.1	0.64
Total bilirubin (mg/dL)	5.2 ± 5.4	5.9 ± 4.7	0.20
Direct bilirubin (mg/dL)	2.6 ± 5.1	3.0 ± 3.6	0.40
Blood urea nitrogen (mg/dL)	9.7 ± 6.5	11.1 ± 7.9	0.27
Creatinine (mg/dL)	0.19 ± 0.15	0.17 ± 0.13	0.56
Albumin (g/dL)	3.03 ± 0.55	2.96 ± 0.53	0.45
C-reactive protein (mg/dL)	3.6 ± 2.7	4.1 ± 3.6	0.23

NOTE: The data are expressed as means and standard deviations unless otherwise noted.
*The data are expressed as medians and first and third quartiles.

TABLE 3. Clinical Outcomes

	No NIV Use (n = 47)	NIV Use (n = 47)	*P* Value
Reintubation in ICU due to RCs [n (%)]	11 (23.4)	3 (6.4)	0.02
Reintubation in ICU [n (%)]	15 (31.9)	7 (14.9)	0.05
Death in hospital [n (%)]	5 (10.6)	5 (10.6)	>0.98
Hospital stay (days)*	80.7 ± 10.3	75.8 ± 10.3	0.74

*The data are expressed as means and standard deviations.

The reasons for reintubation and death are shown in Table 5. All but 1 of the reintubations and 43 of the 47 NIV introductions (91.5%) in the ICU were performed within 72 hours of extubation. Sequential ABG data for the 47 NIV patients showed significant improvements in PaO_2, $PaCO_2$, and pH after NIV use (Fig. 4). Fourteen of the 21 patients who were administered NIV because of worsening atelectasis showed improvements in atelectasis (as shown by CXRs) after NIV use. Changes in CXRs for typical cases are shown in Fig. 5. Furthermore, the discharge rate from the ICU was significantly better for NIV patients versus non-NIV patients (Fig. 6).

All patients tolerated NIV, and no severe complications from NIV use occurred. Nasal bridge ulcers and gastric distension were prevented by soft silicone seals and nasogastric tubes, respectively.

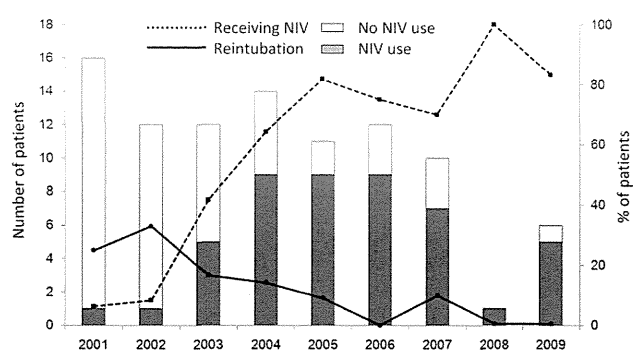

Figure 3. Rates of NIV use and reintubation and numbers of patients included in the analysis by year during the 9-year study period. The gray columns represent the annual number of patients who received NIV, and the white columns represent the annual number of patients who did not. The dashed line represents the percentage of patients treated with NIV with respect to the total number of patients included in the analysis. The solid line represents the percentage of patients who required reintubation because of RCs with respect to the total number of patients included in the analysis.

DISCUSSION

Studies of postextubation NIV use in children are scarce. According to our literature survey, no studies