Journal of Perinatology (2011) 31, 104–111

www.nature.com/jp

ORIGINAL ARTICLE

Comparable effect of conventional ventilation versus early high-frequency oscillation on serum CC16 and IL-6 levels in preterm neonates

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Objective: Clara cell 16 kD protein (CC16) and interleukin (IL)-6 have been used as peripheral blood biomarkers of alveolar leakage and inflammation, respectively. Thus, their measurement in the bloodstream could be used to assess ventilator-induced lung injury. The objective of this study was to evaluate the effect of optimized synchronized intermittent mandatory ventilation (SIMV) and high-frequency oscillatory ventilation (HFOV) on circulating CC16 and IL-6 levels when used as the initial ventilation modes in preterm neonates.

Study Design: Single center, prospective, randomized clinical study in preterm neonates (gestational age $\leqslant$30 weeks) requiring mechanical ventilation within the first 2 h of life. Serum CC16 and IL-6 were measured on establishment of the assigned ventilation mode after admission, at days 3 and 14 of life as well as at 36 weeks postmenstrual age. Demographic-perinatal data and clinical parameters were also recorded.

Result: Of the 30 neonates studied, 24 (gestational age 27.1 $\pm$ 1.7 weeks, birth weight 942 $\pm$ 214 g) were finally analyzed, equally assigned into the SIMV and HFOV groups. Both groups had comparable demographic-perinatal characteristics and clinical parameters. Serum CC16 and IL-6 altered significantly over time (repeated-measures analysis of variance, both $P<0.001$). However, changes were not affected by the ventilation mode. *Post hoc* analysis showed a significant decrease in CC16 and IL-6 from birth up to 36 weeks postmenstrual age in both groups.

Conclusion: In preterm neonates, SIMV and HFOV are associated with comparable circulating CC16 and IL-6 levels. These findings suggest a similar alveolar leakage and systemic inflammation with any of the ventilation modes evaluated when their usage is optimized.
Journal of Perinatology (2011) **31,** 104–111; doi:10.1038/jp.2010.78; published online 29 July 2010

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Received 15 December 2009; revised 9 March 2010; accepted 1 April 2010; published online 29 July 2010

Keywords: biomarker; mechanical ventilation; cytokines; lung injury; respiratory distress syndrome

Introduction

Conventional mechanical ventilation has been associated with several adverse effects, especially in very low birth weight infants, in which bronchopulmonary dysplasia (BPD) remains a significant long-term pulmonary morbidity.[1] High-frequency oscillatory ventilation (HFOV) is a ventilatory technique found to induce less lung damage in experimental models of surfactant-deficient animals.[2,3] However, after >15 randomized control trials published, it is not clear yet whether HFOV is more advantageous than conventional ventilation in reducing lung injury when used as the initial ventilation mode in preterm neonates with respiratory distress syndrome (RDS).[4,5]

Inflammatory markers in bronchoalveolar lavage has been used extensively to evaluate early lung damage in ventilated preterm neonates and ensuing progression to BPD.[6] Nevertheless, although the lavage fluid technique may not be used after weaning off the ventilator, there are very few data regarding lung injury biomarkers in the circulation of neonates involving mainly pro- and anti-inflammatory cytokines.[7,8]

Recently, Clara cell protein (CC16) has been used as peripheral blood biomarker of various lung diseases in adults,[9–11] children[12] and neonates.[13,14] CC16 is a lung-specific protein produced by the tracheobronchial epithelium, especially in the terminal bronchioles in which nonciliated Clara cells are localized and is believed to raise in the blood stream of subjects with pathological situations characterized by increased permeability of the alveolar–capillary barrier.[15] On this basis, we advanced the hypothesis that measurement of CC16 in the peripheral blood could detect changes of the alveolar integrity occurring with different ventilatory modes (HFOV vs pressure synchronized intermittent mandatory ventilation (SIMV)). At the same time, CC16 was tested against interleukin (IL)-6 as it is a commonly

used marker of inflammation and/or injury,[16,17] but also because IL-6 is a pro-inflammatory cytokine while CC16 has significant anti-inflammatory properties,[15] and thus, their measurement might reflect different biological actions.

The objective of this study was to evaluate the influence of SIMV and HFOV on CC16 and IL-6 circulating levels, when the two ventilation modalities are optimally applied as initial modes of respiratory support in very immature preterm neonates.

Methods

This prospective, randomized clinical study was performed in a single, tertiary-level neonatal intensive care unit (NICU). The study protocol was approved by the human research review board of our institution. An informed written consent was obtained from all parents before the preterm delivery during consultation regarding postpartum management of their baby.

Study population

Subjects were eligible if they were inborn, preterm neonates (gestational age $\leqslant 30$ weeks) with respiratory failure requiring artificial ventilation via an endotracheal tube within the first 2 h of life. Neonates were intubated either in the delivery room when establishment of adequate spontaneous respiration failed in spite of the adequate face mask ventilation using a self-inflating bag or in the NICU. After intubation and up to NICU admission, positive pressure ventilation was provided through a T-piece (Neopuff, Fisher & Paykel Healthcare, Auckland, New Zealand), using peak inspiratory pressure needed to elevate the chest-wall while positive end-expiratory pressure was set at 4 cmH_2O. Prophylactic surfactant administration in the delivery room was not performed. Spontaneously breathing neonates in the delivery room were put on nasopharyngeal continuous positive airway pressure through Neopuff, which was maintained during transport. In the NICU, nasopharyngeal continuous positive airway pressure was applied through the SLE 5000 (SLE Limited, London, UK) or the VIP Gold (VIASYS Healthcare, Yorba Linda, CA, USA) ventilator circuit. Indications for mechanical ventilation in the NICU setting included clinical symptoms of worsening respiratory distress while on nasopharyngeal continuous positive airway pressure associated with hypoxemia (partial pressure of arterial oxygen (PaO_2) <50 mm Hg with a fraction of inspired oxygen (FiO_2) >0.5), respiratory or mixed acidosis (pH <7.2, partial pressure of arterial carbon dioxide ($PaCO_2$) >50 mm Hg) as well as recurrent apnea and bradycardia episodes. Eligible neonates were randomly assigned into the SIMV and HFOV groups, respectively, if already intubated on admission to the NICU or when mechanical ventilation was commenced afterward. Randomization was based on admission order. Exogenous surfactant (Curosurf, Chiesi, Italy, 200 mg kg^{-1} per dose) was given therapeutically with clinical and radiographic documentation of RDS and for two further doses (100 mg kg^{-1} per dose) at 12-h intervals as needed.

Exclusion criteria

Exclusion criteria were suspected clinical chorioamnionitis (intrapartum maternal fever, abdominal pain, foul-smelling amniotic fluid or vaginal discharge, laboratory indications of infection to the mother), severe acute perinatal asphyxia (fetal bradycardia, 5 min Apgar scores $\leqslant 3$, severe acidosis on admission; base deficit $\geqslant -12$ mEq l^{-1}) possible (clinical and laboratory evidence) or confirmed (positive blood culture) early-onset sepsis as well as congenital infections and anomalies. In addition, failure to obtain a second blood sample because of early death and lack of parental consent were causes of exclusion from the study. Switch from SIMV to HFOV and vice versa was not allowed in treatment failure, and thus, crossed over neonates were also excluded. However, HFOV-treated neonates were allowed to continue on SIMV until final extubation when HFOV was considered not suitable (for example, reintubation for apneas without evidence of pulmonary disease, established severe BPD). In this case, neonates remained in the HFOV group during statistical analysis.

Ventilatory management

SIMV and HFOV modes were applied using the SLE 5000 ventilator. Mechanical ventilation settings were adjusted to maintain pH >7.2, PaO_2 45 to 70 mm Hg and $PaCO_2$ 40 to 50 mm Hg on arterial blood gases obtained 15 to 20 min after each change of the ventilator setting. Preductal arterial saturation measured by pulse oxymetry (SpO_2) was kept between 88 and 93%. In general, optimized mechanical ventilation was attempted using lower pressure, high rate and shorter inspiratory time in the SIMV, and optimal lung volume strategy in the HFOV mode. These ventilation strategies are described below and are thought to have a protective effect on the immature lung.[4,18]

Synchronized intermittent mandatory ventilation

Pressure-limited SIMV was initially applied as follows: SIMV breath rate 60 min^{-1} with rates not exceeding 60 per minute, inspiratory time 0.30 to 0.4 sec, peak inspiratory pressure as needed to achieve adequate chest expansion (typically 14 to 20 cmH_2O), positive end-expiratory pressure 4 to 5 cmH_2O and FiO_2 as required for target oxygen levels. Flow trigger sensitivity was set at the maximum level. Weaning was performed by decreasing peak inspiratory pressure, SIMV rate and FiO_2. Extubation was attempted at low SIMV setting (breath rate 10 to 15 min^{-1}, peak inspiratory pressure 12 to 14 cmH_2O, positive end-expiratory pressure 3 to 4 cmH_2O and FiO_2 <0.3) when adequate oxygenation and ventilation were maintained.

HFOV strategy

Conventional ventilation in the NICU was not tried before HFOV. Mean airway pressure (Paw) was set initially at 8 to 10 cmH_2O, increasing stepwise by 1 cmH_2O until oxygen saturation no longer

increased to achieve lung recruitment. At this point, Paw was decreased by 1 cmH_2O to avoid possible hyperinflation. Amplitude (ΔP) was adjusted based on visible abdominal vibrations, frequency was 10 Hz and the I:E ratio was fixed at 1:1. FiO_2 was selected according to target SpO_2. Lung volume was considered optimal when the right hemidiaphragm was between eighth and ninth rib. In air leak syndromes, lower lung volume (seventh to eighth rib) was accepted. Weaning from HFOV was attempted with (a) Paw <6 cm H_2O, (b) FiO_2 <0.3, (c) delta pressure (ΔP) <20 and (d) sufficient respirations of the neonate.

After extubation, with both ventilation modes, infants were put on nasopharyngeal continuous positive airway pressure. Caffeine citrate was started before extubation. General supportive treatment was at the discretion of the attending neonatologist in line with the protocols applied in our department.

Data collection

In all neonates, the demographic-perinatal characteristics as well as arterial blood gas (pH, PaO_2 and $PaCO_2$) and pulmonary data were recorded. The severity of acute respiratory failure and the degree of respiratory support were assessed using the alveolar–arterial difference of oxygen ($AaDO_2 = (FiO_2 \times 713) - PaCO_2 - PaO_2$) and oxygenation index ((mean airway pressure*$FiO_2 \times 100$)/PaO_2) when applicable. Diagnosis of the cause of respiratory failure was based on the X-ray findings. Outcome parameters recorded included prematurity-associated complications (interstitial pulmonary emphysema-pneumothorax, treatment for patent ductus arteriosus, late-onset sepsis, necrotizing enterocolitis, intraventricular hemorrhage grade 3 to 4 according to Papile classification[19] and development of BPD defined as the need for oxygen, nasal continuous positive airway pressure or mechanical ventilation at 36 weeks postmenstrual age[20]) and survival to discharge.

Blood sampling and serum CC16 and IL-6 assay

Serum CC16 and IL-6 were measured as soon as the assigned ventilation mode was established (T0), and before the first dose of surfactant in those treated, at days 3 (T1) and 14 of life (T2) as well as at 36 weeks postmenstrual age in those still in hospital or before discharge (T3). Sera were stored at $-80\,^{\circ}C$ until measurement of the specific biomarkers was performed by an investigator blinded to the study design. The serum human CC16 and IL-6 concentrations were measured using commercially available enzyme-linked immunosorbent assays (BioVendor GmbH, Germany and R&D Systems, Human IL-6 Quantikine HS ELISA Kit, Minneapolis, MN, USA, respectively). Each sample was run in duplicate and the mean concentration was calculated. The lower limit of detection of IL-6 and CC16 was 0.7 pg ml^{-1} and 0.02 ng ml^{-1}, respectively. The intra- and inter-assay coefficients of variation of CC16 were 7.0 and 6.0%, respectively, and of IL-6 were 3.3 and 7.4%, respectively.

Statistical analysis

Numerical data are expressed as means $\pm$ s.d. The normality assumption was assessed using the Kolmogorov–Smirnov test. Repeated-measures analysis of variance was used to evaluate the effect of time and ventilation mode on serum CC16 and IL-6 levels. Dunnett's multiple comparisons test was performed to compare values at T0 with those at T1, T2 and T3. Fisher's exact test was used as a test for independence between categorical variables. Pearson's correlation was used to describe relationships between continuous variables. A P-value <0.05 was considered statistically significant. Statistical analysis was performed using Statistica 8.0 (StatSoft, Tulsa, OK, USA) and MedCalc software (Version 11.1.1, MedCalc Software, Mariakerke, Belgium).

Results

Consent was obtained in 30 singleton and 4 twin pregnancies, when obstetric criteria were fulfilled, whereas parents refused to consent in 6 cases. However, only 30 out of 38 neonates were enrolled in the study, as 8 were either not intubated or required mechanical ventilation after the first 2 h of life. Two neonates were subsequently excluded because of death before the second blood sampling, one because of early-onset sepsis and three because of crossover from SIMV to HFOV. No neonate was excluded because of severe acute asphyxia, congenital infection or anomalies. Thus, finally, 24 preterm neonates were included in the statistical analysis, 12 assigned into each group (Figure 1). As shown in Table 1, the two groups were comparable regarding the demographic and perinatal characteristics. Time of the first blood sampling (T0) did not differ between the two groups (58 ± 25 vs 75 ± 28 min for the SIMV and HFOV group, respectively, $P = 0.189$). Data regarding the survival and the need for ventilatory support as well as values of $AaDO_2$, oxygenation index and blood gases at the different time points are presented in Table 2. Exogenous surfactant was administered as early treatment for RDS in 10 (83.3%) and 11 (91.6%) neonates in the SIMV and HFOV group, respectively ($P = 1$). Respiratory failure was attributed to nonspecific lung immaturity in the remaining three neonates. During the first 14 days of the study, none of the neonates assigned into the HFOV group was put on SIMV. However, after T2, two neonates in the HFOV group developing apneas secondary to sepsis and prematurity, respectively, and one with BPD were managed with SIMV (Figure 1).

Serum CC16 and IL-6 levels changed significantly as a function of time in the two groups (repeated-measures analysis of variance, both $P<0.001$). However, these changes were not affected by the application of SIMV or HFOV (repeated-measures analysis of variance, $P = 0.834$ and $P = 0.075$ for CC16 and IL-6, respectively) (Figures 2 and 3). Similarly, the overall levels of CC16 and IL-6 did not differ between groups ($P = 0.559$ and $P = 0.658$ for the SIMV and the HFOV group, respectively. Dunnett's multiple

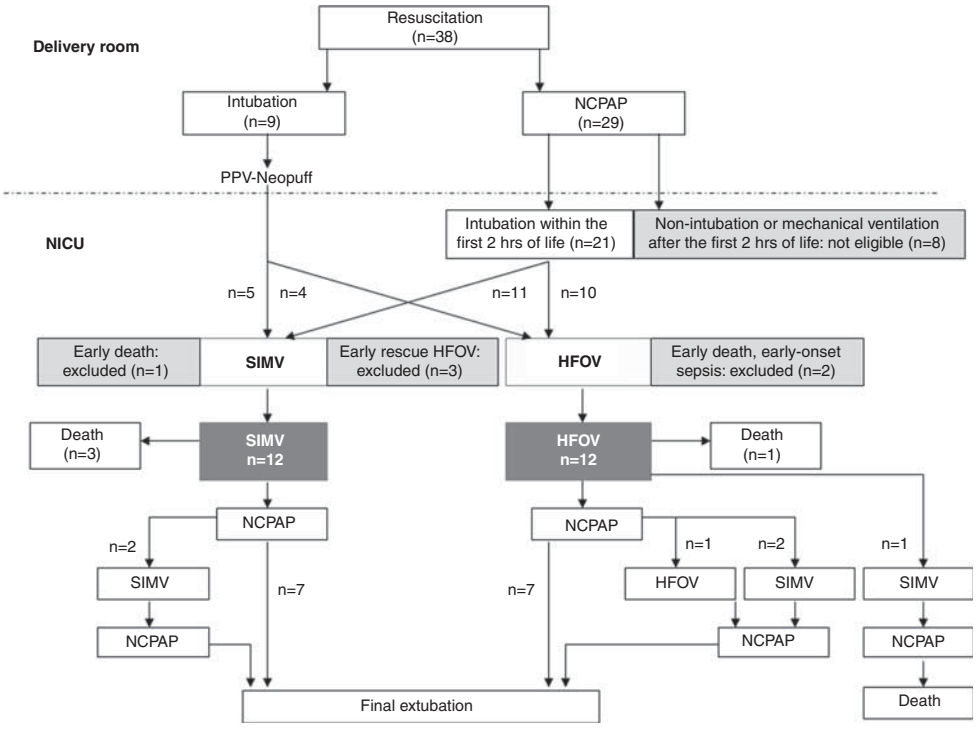

Figure 1 Study flow chart. HFOV, high-frequency oscillatory ventilation; NCPAP, nasal continuous positive airway pressure; NICU, neonatal intensive care unit; PPV, positive pressure ventilation; SIMV, synchronized intermittent mandatory ventilation.

Table 1 Demographic and perinatal characteristics

	SIMV group (n = *12*)	*HFOV group* (n = *12*)	P-*value*
Birth weight (g)[a]	909 ± 217	959 ± 182	0.549
Gestational age (weeks)[a]	27 ± 1.8	27.1 ± 1.5	0.813
Male gender (*n*, %)	6 (50)	5 (41.6)	1
Intrauterine growth retardation (*n*, %)	2 (16.6)	2 (16.6)	1
Preeclampsia/eclampsia (*n*, %)	1 (8.3)	2 (16.6)	1
Premature rupture of membranes >18 h (*n*, %)	4 (33.3)	5 (41.6)	1
Maternal antibiotics (*n*, %)	7 (58.3)	5 (41.6)	0.684
Prenatal steroids (*n*, %)	10 (83.3)	9 (75)	1
Caesarian section (*n*, %)	8 (66.6)	9 (75)	1
Apgar score			
1 min[a]	4.8 ± 1.4	5.1 ± 1.8	0.630
5 min[a]	7.3 ± 1.2	7.3 ± 1	0.606
Intubation in delivery room (*n*, %)	2 (16.6)	3 (25)	1

Abbreviations: HFOV, high-frequency oscillatory ventilation; SIMV, synchronized intermittent mandatory ventilation.
[a]Numerical data are expressed as mean ± s.d.

comparisons test showed that within the SIMV group CC16 at T0 differed significantly as compared with CC16 at T1, T2 and T3, whereas differences in CC16 within the HFOV group were significant only between T0 vs T1 and T3. In addition, significant differences were noted with respect to IL-6 within the SIMV group between T0 vs T1 and T3, as well as within the HFOV group between T0 and the rest time points. No correlation was found between serum CC16 and IL-6 levels at any time point evaluated.

The incidence of prematurity-associated complications and survival are shown in Table 3. However, statistical comparisons of these parameters were not performed because of the small number of patients in the study. Three neonates in the SIMV group died of sepsis-necrotizing enterocolitis and severe intraventricular hemorrhage ($n = 1$), refractory hypotension ($n = 1$) and pulmonary hemorrhage ($n = 1$). In the HFOV group, two deaths occurred, one secondary to severe intraventricular hemorrhage

Table 2 Data related to oxygenation and blood gas by timepoint and ventilation mode

Time point	*Groups*	*Alive neonates* (n)	*Ventilated neonates* (n)	*$AaDO_2$*	*MAP/Paw*[a]	*Oxygenation index*[b]	*pH*	*$PaCO_2$*
T0	SIMV	12	12	195 ± 168	6.5 ± 1.4[a]	4.6 ± 2.9[a]	7.311 ± 0.710	44.7 ± 8.8
	HFOV	12	12	238 ± 145	9.3 ± 2.4	8.6 ± 4.1	7.293 ± 0.102	44.9 ± 12.9
T1	SIMV	12	7	77 ± 46	5.5 ± 2.9	2.1 ± 1.2	7.288 ± 0.093	40.9 ± 8.1
	HFOV	12	7	164 ± 156	7.2 ± 2.6	7.9 ± 9.7	7.295 ± 0.082	41.5 ± 11.7
T2	SIMV	11	3	92 ± 47	6.7 ± 1.5	4.1 ± 2.1	7.373 ± 0.091	41.5 ± 13.5
	HFOV	11	4	124 ± 95	7 ± 2.1	4.1 ± 1.1	7.348 ± 0.088	44.3 ± 12.1
T3	SIMV	9	0	74 ± 78	NA	NA	7.408 ± 0.0640	44.6 ± 12.9
	HFOV	11	0	91 ± 79	NA	NA	7.449 ± 0.059	41.4 ± 9.9

Abbreviations: $AaDO_2$, alveolar-arterial difference of oxygen; HFOV, high-frequency oscillatory ventilation; MAP, mean airway pressure in conventional ventilation; NA, non applicable; $PaCO_2$, partial pressure of arterial carbon dioxide; Paw, mean airway pressure in HFOV; SIMV, synchronized intermittent mandatory ventilation.
[a]SIMV vs HFOV: $P<0.05$.
[b]Applicable to ventilated neonates.
Data are expressed as mean ± s.d.

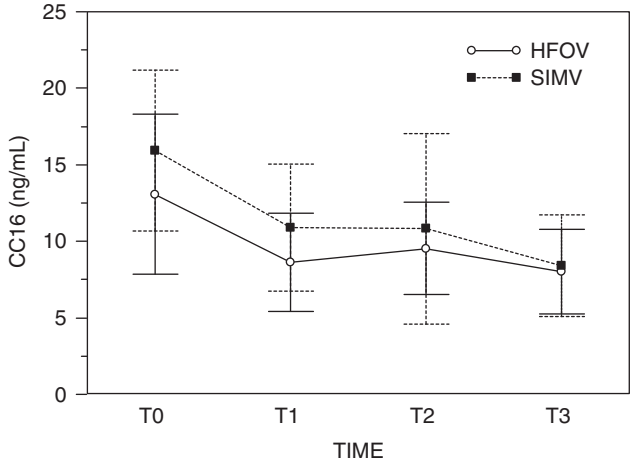

Figure 2 Serum CC16 levels (means ± 95% confidence intervals for means) at the different time points. HFOV, high-frequency oscillatory ventilation; SIMV, synchronized intermittent mandatory ventilation.

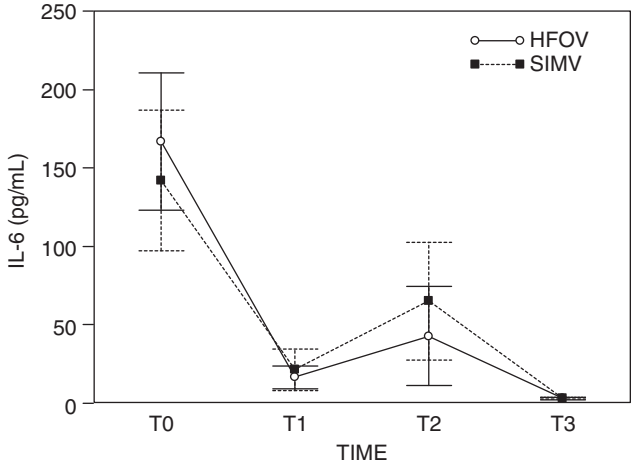

Figure 3 Serum IL-6 levels (means ± 95% confidence intervals for means) at the different time points. HFOV, high-frequency oscillatory ventilation; SIMV, synchronized intermittent mandatory ventilation.

Table 3 Neonatal complications and outcome

	SIMV group (n = *12*)	*HFOV group* (n = *12*)
Interstitial pulmonary emphysema	1 (8.3)	1 (8.3)
Pneumothorax	0 (0)	0 (0)
Indomethacin for patent ductus arteriosus (*n*, %)	3 (25)	4 (30)
Late-onset sepsis (*n*, %)	4 (33.3)	6 (50)
Necrotizing enterocolitis	1 (8.3)	1 (8.3)
Intraventricular hemorrhage 3–4 (*n*, %)	1 (8.3)	1 (8.3)
Bronchopulmonary dysplasia (*n*, %)	3/9 (30)	4/11 (36.3)
Survival to discharge (*n*, %)	9 (75)	10 (83.3)

Abbreviations: HFOV, high-frequency oscillatory ventilation; SIMV, synchronized intermittent mandatory ventilation.

and one in the infantile period (after T3) because of complications of BPD. Total duration of mechanical ventilation in survivors did not differ between groups (11 ± 17.5 vs 14.1 ± 10.9 days for the SIMV and HFOV group, respectively, $P = 0.654$). Of the surviving neonates, 2 out of 9 (22.2%) and 3 out of 10 (30%) of those treated with SIMV and HFOV, respectively, were given postnatally a short course of dexamethasone at low dose to facilitate extubation because of BPD. One survivor in the HFOV group also needed inhaled nitric oxide for refractory respiratory failure.

Discussion

In this randomized clinical study, we found that preterm neonates, in which SIMV or HFOV was applied as the initial ventilation mode after birth, have comparable circulating CC16 and IL-6 concentrations throughout their NICU stay. These findings suggest similar systemic alveolar leakage (CC16) and inflammation (IL-6) when the use of these ventilatory techniques is optimized.

Disruption of the alveolar membrane with mechanical ventilation enhances leakage of CC16 from the alveoli into the bloodstream as documented in experimental models of acute lung injury,[21] adults ventilated for acute respiratory failure[9,11] and preterm neonates with RDS.[14] Nevertheless, although all types of mechanical ventilation cause structural disruption of the alveoli,[22] damage of the alveolar–capillary barrier and protein leakage was found to be less with HFOV, at least in animal studies.[23] This was not confirmed in this clinical study rejecting our initial hypothesis, because SIMV and HFOV were associated with comparable CC16 levels in the bloodstream.

In this study, levels of CC16 immediately after birth with both ventilation modes were higher than those reported previously by the same group of investigators in preterm neonates with mild respiratory distress escaping mechanical ventilation.[14] Similarly, Loughran-Fowlds *et al*.[13] reported increased serum CC16 in mechanically ventilated preterm neonates, speculating that high blood concentrations of the specific protein at birth may represent a protective mechanism during acute postnatal lung adaptation. CC16 is a protein with favorable action against hyperoxic injury, surfactant degradation and inflammation of the lung.[15] In support of this view, decreased cord blood CC16 in preterm neonates was associated with BPD development.[24] However, subclinical chorioamnionitis may also have affected serum levels of CC16. In animals[21] and humans,[25] lipopolysaccharide-induced, acute lung inflammation increases leakage of CC16 from lung into the blood, whereas neonates with postnatal infections have elevated CC16 in the bronchoalveolar lavage.[26] Interestingly, in a recent study, Thomas *et al*.[27] reported lower CC16 in the tracheobronchial aspirates of extremely premature neonates with histological evidence of systemic inflammation compared with the control group, although no difference could be documented in the umbilical cord levels of the protein. This impairment of CC16-mediated lung protection against inflammation is another likely explanation for the development of BPD in the presence of chorioamnionitis.[28]

Different ventilation strategies significantly affect lung and systemic cytokines.[29] In this regard, theoretically 'less injurious for the immature lung' HFOV could be associated with reduced release of several inflammatory mediators in the blood of neonates requiring artificial ventilation. Indeed, Jaarsma *et al*.[30] reported attenuated systemic activation of inflammation in preterm lambs with RDS ventilated with HFOV relative to conventional ventilation. In addition, Capoluongo *et al*.[7] found significantly lower circulating levels of IL-6 on days 3 and 5 of life, when an optimal HFOV strategy was applied as compared with SIMV for the management RDS in preterm neonates. In the latter investigation, changes of the cytokine levels supporting less lung inflammation with HFOV were also associated with significantly less BPD. In our study, serum IL-6 levels were evaluated from the immediate post-delivery period and up to 36 weeks postmenstrual age, when moderate–severe BPD was documented,[20] but no differences could be detected across groups at any time point. This finding implies similar activation of the inflammatory cascade in the bloodstream if the use of SIMV and HFOV is optimized. Although measurements in the bronchoalveolar lavage fluid were not performed, our results are in agreement with other clinical studies, in which concentrations of albumin, IL-8 and leukotriene B4 in the tracheal aspirates were found to be similar between preterm neonates ventilated conventionally and by using HFOV.[6] Moreover, in a model of neonatal lung injury, IL-6 in tracheal aspirates was significantly higher in the HFOV-treated animals only at 24 h of age but not during the following 28 days.[3]

Systemic inflammatory response either in the fetus (chorioamnionitis)[28] or in the neonate (nosocomial sepsis)[31] influences circulating IL-6 greatly. In this study, subclinical chorioamnionitis cannot be ruled out, but comparable IL-6 levels are suggestive of a similar baseline inflammation in both groups. The lack of any significant association between CC16 and IL-6, however, indicates that circulating IL-6, contrary to CC16, may have been primarily affected by the systemic inflammation and less by the lung injury after artificial ventilation. Actually, five neonates in each group had IL-6 values at T0 above the cutoff point of $200\,pg\,ml^{-1}$ suggested by Chiesa *et al*.[17] to distinguish infected neonates at birth. Hence, IL-6 in the blood may not be appropriate for the evaluation of acute lung damage, when perinatal infection involving preterm delivery is a concern. The fact that, in adults with acute lung injury, elevated serum IL-6 before mechanical ventilation does not increase further if a lung-protective ventilation strategy is used,[32] also supports this assumption.

In the clinical setting, results of this study are consistent with other relatively recent trials in premature neonates comparing SIMV with early HFOV, which failed to document any decrease in BPD, in spite of the optimized respiratory treatment.[33] Improvement in the use of conventional ventilation under the concept of lung-protective strategies most probably has contributed significantly to this effect.[4] Experimental data showed similar lung injury and cytokine release in the bloodstream when the lung units are recruited using surfactant and adequate positive end-expiratory pressure (conventional ventilation) or an optimal lung volume strategy (HFOV).[34]

A limitation of this investigation was the small size of the study population. Power analysis revealed that an unfeasible number of subjects for a single-center study would be needed to achieve statistical significance in the biomarkers studied. Therefore, results, including clinical outcomes, should be interpreted with caution. An additional limitation was the lack of histological examination of the placenta to detect subclinical choriomnionitis. Furthermore, CC16 and IL-6 were not evaluated in the bronchoalveolar lavage fluid. Such measurements could offer important insights into the effect of ventilation modes on these proteins at the lung level, possibly justifying novel therapeutic agents, such as the

intratracheal administration of recombinant human Clara cell protein.[35] Finally, other biomarkers, which may be more appropriate for the evaluation of lung injury, should be examined, especially in association with multiple cytokine interactions.

In conclusion, respiratory support of preterm neonates using optimized SIMV or HFOV results in comparable CC16 and IL-6 concentrations in the bloodstream. These findings are suggestive of a similar alveolar leakage and systemic inflammation with any of the ventilation modes evaluated, at least as reflected by the circulating CC16 and IL-6, respectively. Association of these peripheral blood biomarkers with different ventilation modes and pulmonary outcomes should be evaluated in larger multicenter clinical studies.

Conflict of interest

The authors declare no conflict of interest.

Acknowledgments

We thank Dr Christos Nakas for his assistance in statistical analysis of this paper.

References

1 Smith VC, Zupancic JA, McCormick MC, Croen LA, Greene J, Escobar GJ *et al.* Trends in severe bronchopulmonary dysplasia rates between 1994 and 2002. *J Pediatr* 2005; **146**(4): 469–473.

2 Jackson JC, Truog WE, Standaert TA, Juul SE, Murphy JH, Chi EY *et al.* Effect of high frequency ventilation on the development of alveolar edema in premature monkeys at risk for hyaline membrane disease. *Am Rev Respir Dis* 1991; **143**(4): 865–871.

3 Yoder BA, Siler-Khodr T, Winter VT, Coalson JJ. High-frequency oscillatory ventilation: effects on lung function, mechanics, and airway cytokines in the immature baboon model for neonatal chronic lung disease. *Am J Respir Crit Care Med* 2000; **162**(5): 1867–1876.

4 Bollen CW, Uiterwaal CS, van Vught AJ. Meta-regression analysis of high-frequency ventilation vs conventional ventilation in infant respiratory distress syndrome. *Intensive Care Med* 2007; **33**(4): 680–688.

5 Cools F, Henderson-Smart DJ, Offringa M, Askie LM. Elective high frequency oscillatory ventilation versus conventional ventilation for acute pulmonary dysfunction in preterm infants. *Cochrane Database Syst Rev* 2009; (3): CD000104.

6 Thome U, Götze-Speer B, Speer CP, Pohlandt F. Comparison of pulmonary inflammatory mediators in preterm infants treated with intermittent positive pressure ventilation or high frequency oscillatory ventilation. *Pediatr Res* 1998; **44**(3): 330–337.

7 Capoluongo E, Vento G, Santonocito C, Matassa PG, Vaccarella C, Giardina B *et al.* Comparison of serum levels of seven cytokines in premature newborns undergoing different ventilatory procedures: high frequency oscillatory ventilation or synchronized intermittent mandatory ventilation. *Eur Cytokine Netw* 2005; **16**(3): 199–205.

8 Vento G, Capoluongo E, Matassa PG, Concolino P, Vendettuoli V, Vaccarella C *et al.* Serum levels of seven cytokines in premature ventilated newborns: correlations with old and new forms of bronchopulmonary dysplasia. *Intensive Care Med* 2006; **32**(5): 723–730.

9 Doyle IR, Hermans C, Bernard A, Nicholas TE, Bersten AD. Clearance of Clara cell secretory protein 16 (CC16) and surfactant proteins A and B from blood in acute respiratory failure. *Am J Respir Crit Care Med* 1998; **158**(5): 1528–1535.

10 Hermans C, Petrek M, Kolek V, Weynand B, Pieters T, Lambert M *et al.* Serum Clara cell protein (CC16), a marker of the integrity of the air-blood barrier in sarcoidosis. *Eur Respir J* 2001; **18**(3): 507–514.

11 Lesur O, Langevin S, Berthiaume Y, Legare M, Skrobik Y, Bellemare JF *et al.* Outcome value of Clara cell protein in serum of patients with acute respiratory distress syndrome. *Intensive Care Med* 2006; **32**(8): 1167–1174.

12 Martin AC, Laing IA, Khoo SK, Zhang G, Rueter K, Teoh L *et al.* Acute asthma in children: relationships among CD14 and CC16 genotypes, plasma levels, and severity. *Am J Respir Crit Care Med* 2006; **173**(6): 617–622.

13 Loughran-Fowlds A, Oei J, Wang H, Xu H, Wimalasundera N, Egan C *et al.* The influence of gestation and mechanical ventilation on serum clara cell secretory protein (CC10) concentrations in ventilated and nonventilated newborn infants. *Pediatr Res* 2006; **60**(1): 103–108.

14 Sarafidis K, Stathopoulou T, Diamanti E, Soubasi V, Agakidis C, Balaska A *et al.* Clara cell secretory protein (CC16) as a peripheral blood biomarker of lung injury in ventilated preterm neonates. *Eur J Pediatr* 2008; **167**(11): 1297–1303.

15 Hermans C, Bernard A. Lung epithelium-specific proteins: characteristics and potential applications as markers. *Am J Respir Crit Care Med* 1999; **159**(2): 646–678.

16 Miller LC, Isa S, LoPreste G, Schaller JG, Dinarello CA. Neonatal interleukin-1 beta, interleukin-6, and tumor necrosis factor: cord blood levels and cellular production. *J Pediatr* 1990; **117**(6): 961–965.

17 Chiesa C, Pellegrini G, Panero A, Osborn JF, Signore F, Assumma M *et al.* C-reactive protein, interleukin-6, and procalcitonin in the immediate postnatal period: influence of illness severity, risk status, antenatal and perinatal complications, and infection. *Clin Chem* 2003; **49**(1): 60–68.

18 Ambalavanan N, Carlo WA. Ventilatory strategies in the prevention and management of bronchopulmonary dysplasia. *Semin Perinatol* 2006; **30**(4): 192–199.

19 Papile L, Burstein J, Burstein R, Koffler H. Incidence and evolution of subependymal and intraventricular hemorrhage: a study of infants with birth weights less than 1500. *J Pediatr* 1978; **92**(4): 529–534.

20 Jobe AH, Bancalari E. Bronchopulmonary dysplasia. *Am J Respir Crit Care Med* 2001; **163**(7): 1723–1729.

21 Arsalane K, Broeckaert F, Knoops B, Wiedig M, Toubeau G, Bernard A. Clara cell specific protein (CC16) expression after acute lung inflammation induced by intratracheal lipopolysaccharide administration. *Am J Respir Crit Care Med* 2000; **161**(5): 1624–1630.

22 Dreyfuss D, Saumon G. Ventilator-induced lung injury: lessons from experimental studies. *Am J Respir Crit Care Med* 1998; **157**(1): 294–323.

23 Tamura M, Kawano T, Fitz-James I, Bryan AC. High-frequency oscillatory ventilation and pulmonary extravascular water. *Anesth Analg* 1985; **64**(11): 1041–1046.

24 Schrama AJ, Bernard A, Poorthuis BJ, Zwinderman AH, Berger HM, Walther FJ. Cord blood Clara cell protein CC16 predicts the development of bronchopulmonary dysplasia. *Eur J Pediatr* 2008; **167**(11): 1305–1312.

25 Michel O, Murdoch R, Bernard A. Inhaled LPS induces blood release of Clara cell specific protein (CC16) in human beings. *J Allergy Clin Immunol* 2005; **115**(6): 1143–1147.

26 Lassus P, Nevalainen TJ, Eskola JU, Andersson S. Clara-cell secretory protein in preterm infants' tracheal aspirates correlates with maturity and increases in infection. *Pediatr Pulmonol* 2000; **30**(6): 466–469.

27 Thomas W, Seidenspinner S, Kawczyńska-Leda N, Chmielnicka-Kopaczyk M, Marx A, Wirbelauer J *et al.* Clara cell secretory protein in tracheobronchial aspirates and umbilical cord serum of extremely premature infants with systemic inflammation. *Neonatology* 2010; **97**: 228–234.

28 Gotsch F, Romero R, Kusanovic JP, Mazaki-Tovi S, Pineles BL, Erez O *et al.* The fetal inflammatory response syndrome. *Clin Obstet Gynecol* 2007; **50**(3): 652–683.

29 Stüber F, Wrigge H, Schroeder S, Wetegrove S, Zinserling J, Hoeft A *et al.* Kinetic and reversibility of mechanical ventilation-associated pulmonary and systemic inflammatory response in patients with acute lung injury. *Intensive Care Med* 2002; **28**(7): 834–841.

30 Jaarsma AS, Braaksma MA, Geven WB, Van Oeveren W, Oetomo SB. Early activation of inflammation and clotting in the preterm lamb with neonatal RDS: comparison of conventional ventilation and high frequency oscillatory ventilation. *Pediatr Res* 2001; **50**(5): 650–657.

31 Ng PC, Cheng SH, Chui KM, Fok TF, Wong MY, Wong W *et al.* Diagnosis of late onset neonatal sepsis with cytokines, adhesion molecule, and C-reactive protein in preterm very low birth weight infants. *Arch Dis Child Fetal Neonatal Ed* 1997; **77**(3): F221–F227.

32 Cepkova M, Brady S, Sapru A, Matthay MA, Church G. Biological markers of lung injury before and after the institution of positive pressure ventilation in patients with acute lung injury. *Crit Care* 2006; **10**(5): R126.

33 Van Reempts P, Borstlap C, Laroche S, Van der Auwera JC. Early use of high frequency ventilation in the premature neonate. *Eur J Pediatr* 2003; **162**(4): 219–226.

34 Krishnan RK, Meyers PA, Worwa C, Goertz R, Schauer G, Mammel MC. Standardized lung recruitment during high frequency and conventional ventilation: similar pathophysiologic and inflammatory responses in an animal model of respiratory distress syndrome. *Intensive Care Med* 2004; **30**(6): 1195–1203.

35 Levine CR, Gewolb IH, Allen K, Welch RW, Melby JM, Pollack S *et al.* The safety, pharmacokinetics, and anti-inflammatory effects of intratracheal recombinant human Clara cell protein in premature infants with respiratory distress syndrome. *Pediatr Res* 2005; **58**(1): 15–21.