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HFOV in premature neonates: effects on pulmonary mechanics and epithelial lining fluid cytokines. A randomized controlled trial

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Abstract *Objective:* Ventilation strategies for preterm neonates may influence the severity of pulmonary dysfunction and later development of chronic lung disease. The objective of this report is to compare the effects of high-frequency oscillatory ventilation (HFOV) versus synchronized intermittent mandatory ventilation (sIMV) from the points of views of biochemical and functional variables. *Design:* Randomized controlled trial. *Setting:* Third level NICU. *Patients and participants:* Forty preterm neonates with a gestational age of 24–29 weeks were randomly assigned to one of the two above-mentioned ventilation strategies within 30 min from birth. *Measurements and results:* At 1, 3, 5, and 7 days, the babies were monitored by means of ventilator indices, pulmonary function, and eight pro-inflammatory or anti-inflammatory cytokines measured in bronchoalveolar lavage fluid. The neonates assigned to the HFOV procedure benefited from early and sustained improvement in pulmonary mechanics and gas exchange—significantly higher dynamic respiratory compliance values, significantly lower expiratory airway resistance and oxygenation index values—with earlier extubation as compared to the neonates assigned to sIMV treatment, and showed significantly lower transforming growth factor-β1 concentrations in bronchoalveolar lavage fluid. *Conclusions:* The results of this randomized clinical trial support the hypothesis that early and exclusive use of HFOV, combined with optimum volume strategy, has a beneficial effect during the acute phase of lung injury.

Keywords High-frequency oscillatory ventilation · Synchronized intermittent mandatory ventilation · Pulmonary function tests · Cytokines · Chronic lung disease

Introduction

The pathogenesis of chronic lung disease (CLD) is multifaceted with a major causal element represented by the premature state itself [1]. Perinatal infections have been associated with a higher incidence of CLD but, undoubtedly, mechanical ventilation with excessive tidal volume breaths [2], as well as oxygen therapy with its high fraction of inspired oxygen, constitute an important triggering factor for airway inflammation [3, 4, 5, 6].

High-frequency oscillatory ventilation (HFOV), especially when optimum volume strategy is used, has been suggested as a means to reduce lung injury, as compared to conventional mechanical ventilation (CMV), by significantly decreasing CLD in preterm neonates [7, 8, 9, 10].

It has been demonstrated in animal models with acute lung injury that HFOV improves lung function, mechanics, and histopathology with reduced inflammatory mediators as compared to CMV [11, 12]. This study is a randomized trial designed to examine the effect of early, optimum volume strategy HFOV compared with synchronized intermittent mandatory ventilation (sIMV) on pulmonary mechanics in premature neonates with a gestational age <30 weeks, and to correlate them with the results of pro- and anti-inflammatory cytokine quantification in bronchoalveolar lavage fluid (BALF). Specifically, we hypothesized that early HFOV should improve gas exchange, enhance pulmonary mechanics, and reduce lung concentrations of pro-inflammatory and/or pro-fibrotic cytokines at various time points during the acute phase of lung injury.

Our preliminary data showed significantly lower transforming growth factor-β1 (TGF-β1) levels in epithelial lining fluid (ELF) and improved lung mechanics in HFOV, compared to CMV treated patients [13].

Materials and methods

This randomized clinical trial was carried out in our neonatal intensive care unit (NICU) over a 2.5-year period ending in January 2003. Neonates with a birth weight between 500 g and 1,500 g and gestational age between 24 weeks and 29 weeks were studied. They were eligible when inborn, when endotracheal intubation was required at birth, and on-going intensive care was required. Babies with major congenital malformations or prenatal infection were excluded from the study. The study protocol and consent forms were approved by the Ethics Committee of the Department of Paediatrics.

Based on our data of dynamic respiratory compliance (unpublished data) in a previous cohort of 30 preterm neonates with gestational age <30 weeks studied on day 1 (12–14 h after surfactant therapy), and on day 3, 5, and 7 (0.49±0.11 ml·cmH_2O·kg, 0.46±0.19 ml·cmH_2O·kg, 0.54±0.16 ml·cmH_2O·kg and 0.61±0.14 ml·cmH_2O·kg, respectively), and on our previous experience of pulmonary mechanics measurements for the prediction of CLD [14], to detect a difference in Cdyn between the two modes of ventilation from the mean values previously found during the first week of life to 0.88 ml·cmH_2O·kg (level reached by neonates with uncomplicated RDS after the 3rd day of life) [14], with 99% power at the 95% significance level, a total of 24 patients were required. Considering that, in our clinical practice, 40% of infants with gestational age <30 weeks are successfully extubated within the 1st week of life, a total number of 42 patients was enrolled.

Randomisation to HFOV and sIMV was obtained by random number allocation and was carried out upon admission in the NICU, within the first 30 min after birth, by opening opaque numbered sealed envelopes. Twenty-one neonates were studied for each ventilator strategy group. All babies were treated with conventional ventilation before enrolment, during the transfer from the delivery room to the NICU.

Ventilation strategies

The goals of respiratory management were to maintain blood gas values with pH 7.30–7.45, $PaCO_2$ 45–55 mmHg (5.9–7.2 kPa) and PaO_2 50–70 mmHg (6.6–9.3 kPa) with oxygen saturation 90–94%.

HFOV

HFOV was performed with Draeger Babylog 8000 plus (Draeger, Lubeck, Germany) with "optimum volume strategy" defined as: initial use of a mean airway pressure (MAP) of 2 cmH_2O higher than on CMV and initial weaning of fraction of inspired oxygen (FiO_2) before MAP. Ventilation was started at a MAP of 10 cmH_2O and a frequency of 10 Hz, and the amplitude, set at 30% at the beginning, was increased, if necessary, until the infant's chest was seen to be "bouncing". The FiO_2 was initially set to ensure adequate oxygenation, and when the FiO_2 was greater than 0.25, the MAP was increased by 0.5 cmH_2O to 1.0 cmH_2O every 10–15 min until it was possible to decrease the FiO_2. Extubation was attempted when the neonate's condition remained stable for at least 6 h while receiving minimal ventilation: $FiO_2 \leq 0.25$, MAP <6 cmH_2O, and an amplitude below 30%.

sIMV

Neonates in this group received sIMV with Draeger Babylog 8000 plus, which provides flow-triggered sIMV and continuous tidal volume monitoring at the connection of the endotracheal tube. Expiratory tidal volumes of 4–6 ml/kg were allowed, positive end-expiratory pressure (PEEP) was set at 4–5 cmH_2O, depending on the FiO_2 and lung inflation. Inspiration times of 0.30–0.40 s were used, with rates not exceeding 60 breaths per minute. Peak inspiratory pressure (PIP) was lowered as the first step in improving the babies' condition. The policy of weaning during the recovery stage of their illness consisted of the reduction of PIP and ventilator rate until a peak pressure of 18 cmH_2O and the ventilator rate of 15 breaths per minute were achieved. Babies of both groups were extubated on nasal continuous positive airway pressure of 4–5 cmH_2O (nasal prongs Argyle, Sherwood Medical, St. Louis, Mo., USA).

Extubation failure was defined as shorter than 72 h with clinical deterioration requiring re-intubation. Neonates with extubation failure were placed back on their originally assigned ventilation strategy.

Medical treatment

After initial stabilization, and after surfactant therapy had been administered, bronchoalveolar lavage was performed at the end of the first day of life (in the first 24 h of life), and on postnatal days 3,

5, 7, unless there was early extubation, as previously described [15].

In the HFOV group, when oxygenation deteriorated after this procedure, MAP was temporarily increased (1–2 cmH_2O) until a stabilization of oxygenation could be observed (usually 30–60 s). At this point, MAP was lowered again to the preaspiration level. The amount of fluid recovered varied between 60% and 80% of the initial lavage fluid instilled.

Cytokine concentrations in BALF were determined using commercially available enzyme linked immunosorbent assay kits [interleukin (IL)-6, IL-8, IL-10, leukemia inhibitory factor (LIF), monocyte chemo-attractant protein-1 (MCP-1), platelet derived growth factor-BB (PDGF-BB), vascular endothelial growth factor (VEGF), and TGF-β1; R&D Systems Europe 4–10, The Quadrant, Barton Lane, Abingdon, Oxon, UK]. BALF concentration of the cytokines was recalculated as epithelial lining fluid (ELF) concentration, using a dilution factor obtained by means of the urea method [15, 16, 17, 18, 19]: ELF= BALF analyte concentration × (serum urea/BALF urea).

Pulmonary mechanics were measured during mechanical ventilation, as reported in our previous studies [14, 15], at the end of the first day of life (in the first 24 h of life), always 12–14 h after surfactant, and at postnatal days 3, 5 and 7, except in the case of early extubation, in HFOV and sIMV babies, both groups breathing in the same modality during the test. HFOV patients were then changed to sIMV for 5 min before assessment to ensure appropriate tidal volume avoiding the risk of carry-over effects from preceding ventilation support, then immediately switched back to HFOV after pulmonary function measurements were completed. A standardized technique of pulmonary mechanics measurement was used in an attempt to minimize methodological variability. In both groups the inspiratory time was 0.4 s, ventilator rate was set to 60 breaths per minute to overcome spontaneous breaths and to fully adapt the babies to the mechanical ventilator, while PEEP was reduced to 2 cmH_2O so as to avoid gas trapping. PIP was similar to that used for clinical ventilation support in the sIMV Group and was 15–18 cmH_2O in HFOV Group. In both groups tidal volumes during pulmonary mechanics evaluation were maintained within the defined range (4–6 ml/kg).

No sedation, muscle relaxants or esophageal balloon were used. Dynamic respiratory compliance (Cdyn), total airway resistance, and expiratory airway resistance were measured using a computer program (linear regression analysis, based on the equation of motion) [20]. The time delay between BALF procedure and lung function measurements was similar for all patients (at least 2 h).

The values for MAP, P/F (PaO_2/FiO_2 ratio), OI [oxygenation index ($MAP \times FiO_2/ PaO_2 \times 100$)], $PaCO_2$ and pH were extracted at the time of pulmonary function measurements. Although this study was not powered to detect differences in clinical outcomes, we also collected them: we evaluated incidence of CLD (O_2 dependence at 36 weeks of postmenstrual age), air leak, severe intracranial hemorrhage (grade III or IV), periventricular leucomalacia, necrotizing enterocolitis, retinopathy of prematurity (stage >2), ductus arteriosus surgically ligated, and sepsis.

Statistical analysis

Categorical variables were compared by using a two-tailed Fisher's exact test. Both parametric and non-parametric tests were used as necessary. The statistical software used included Instat (GraphPad PRISM Version 3.02) and Epi-Info 2000. A P value <0.05 was considered statistically significant.

Table 1 Patient characteristics of the groups analyzed. Values expressed as mean±SD and no. (%).

Characteristic	HFOV (*n* 20)	sIMV (*n* 20)	P value
Gestational age (weeks)	27.1±1.4	27.4±1.2	0.47
Birth weight (grams)	882±157	936±285	0.46
Appropriate for gestational age	16 (80)	15 (75)	1.0
Male	9 (45)	11 (55)	0.75
Caesarean section	15 (75)	18 (90)	0.41
Antenatal steroids[a]			
a) Complete	11 (55)	12 (60)	1.0
b) Partial/incomplete	9 (45)	6 (30)	0.51
c) None	0	2 (10)	0.48
Rupture of membranes $\geq$ 12 hours	9 (45)	7 (35)	0.74
Median Apgar score (range)			
One-minute	3 (1–7)	3 (2–7)	0.80
Five-minute	7 (6–9)	7 (4–9)	0.90
Surfactant given	15 (75)	15 (75)	1.0

[a] A completed course of prenatal betamethasone was defined as two doses administered more than 24 h but no more than 7 days before delivery; a partial course as one dose administered more than 24 h but no more than 7 days before delivery; and incomplete as any steroid administered less than 24 h or more than 7 days before delivery

Table 2 Clinical outcomes. Values expressed as patients' number and percentage (%).

Outcome	HFOV (*n* 20)	sIMV (*n* 20)	p value
Ductus arteriosus surgically ligated	0	1 (5)	1.0
Bacteriemia or fungemia	7 (35)	7 (35)	1.0
Intracranial hemorrhage grade III or IV	3 (15)	2 (10)	1.0
Periventricular leucomalacia	1 (5)	1 (5)	1.0
Necrotizing enterocolitis	2 (10)	0	0.48
Isolated intestinal perforation	1 (5)	0	1.0
Retinopathy of prematurity (stage >2)	6 (30)	8 (40)	0.74
Survival to discharge	19 (95)	18 (90)	1.0

Results

During the study period 42 patients met the entry criteria. Two neonates, one for each study group, with late-diagnosed congenital pneumonia (positive BALF culture at birth), were subsequently excluded. Analyses were performed on the remaining 40 neonates, 20 assigned to HFOV and 20 assigned to sIMV.

Relevant clinical information on the study groups is provided in Table 1. Fifteen patients in both HFOV and sIMV group met the criteria to receive surfactant administration.

Table 2 and Table 3 show both early and late clinical outcomes of the study groups. Only two HFOV-treated

Table 3 Short- and long-term respiratory outcomes. Values expressed as patients number and percentage (%). Plus-minus values are means±SD.

Outcome	HFOV (*n* 20)	sIMV (*n* 20)	*P* value
Requirement of 2nd dose of surfactant	2 (13)	7 (47)	0.10
Pneumothorax	2 (10)	1 (5)	1.0
Pulmonary interstitial emphysema	2 (10)	3 (15)	1.0
Successful extubation[a]	20	18	0.48
Median age at extubation—days (range)	3 (1–26)	7 (1–33)	0.11
O_2 dependence at 36 wks (%)[b]	2:19 (10.5)	8:18 (44.4)	0.048
Mechanical ventilation (h)[c]	310±313	656±981	0.15
O_2 therapy (h)[c]	760±473	1445±1297	0.03

[a] Two infants in sIMV group died with the endotracheal tube in place
[b] Data were not available for patients died before 36 weeks of post-conceptual age
[c] Data are referred to survivors babies only. Seven infants in HFOV group (35%) and twelve infants in sIMV group (60%) received late systemic corticosteroids

patients required a second administration of surfactant as compared to seven babies in the sIMV group. The acute complications of mechanical ventilation were manifested in three cases of pneumothorax and five cases of air-leak without any difference between study groups. The age at successful extubation was lower for the babies assigned to HFOV than for those assigned to sIMV, but the difference was not statistically significant. More neonates in the HFOV group were alive without requiring supplemental oxygen at 36 weeks of post-conceptual age. Three babies died before discharge: one in the HFOV group at 29 days of age due to septic shock and two in the sIMV group at 5 days and 10 days of life, due to severe respiratory insufficiency with intracranial hemorrhage III° and sepsis, respectively.

At the time of enrollment, the severity of the lung disease was similar in both groups, as demonstrated by MAP and FiO_2 values at base line (Table 4). As expected, prior to surfactant administration, the neonates assigned to HFOV received ventilation with a higher MAP (P=0.0009) and lower FiO_2 (P<0.0001) (Table 4). In the remainder of the study period, MAP levels were lower in patients assigned to HFOV as compared to those assigned to sIMV, although this difference did not reach statistical significance. The mean P/F ratio was always significantly higher, except on the 7th day, in babies receiving HFOV as compared to those receiving sIMV (Table 4). Overall, the mean OI values were always lower in infants assigned to HFOV and the differences were statistically significant on day 1 and 3. Mean $PaCO_2$ and pH values were similar in both groups (Table 4).

Four patients in HFOV group and two in sIMV group were extubated within the first 24 h of life, prior to pulmonary function tests and BALF collection. Comparisons of respiratory system mechanics are shown in Table 5. Specific dynamic respiratory compliance was significantly higher in the HFOV group compared to the sIMV group at all time points measured during the first week of life, except for day 7. Expiratory airway resistance showed significantly lower values in HFOV-treated patients as compared to sIMV-treated neonates during the first week of life, except at day 7. No significant differences between the groups were found in total airway resistance, even if the trend was similar.

Table 4 Ventilator indices, partial pressure of arterial carbon dioxide and pH during the study period. Only intubated patients were considered. Plus-minus values are means±SD. Unpaired *t*-test used for comparison. [*MAP* mean airways pressure, FiO_2 fraction of inspired oxygen, *P/F* PaO_2/FiO_2 ratio, *OI* oxygenation index (MAP x FiO_2/ PaO_2 × 100), $PaCO_2$ partial pressure of arterial carbon dioxide].

	HFOV	sIMV	*P* Value
Base line	*n* 20	*n* 20	
MAP (cmH_2O)	8.2±0.8	8.3±1.1	0.74
FiO_2	0.49±0.24	0.54±0.22	0.49
Pre-Surfactant	*n* 15	*n* 15	
MAP (cmH_2O)	13.2±1.6	10.5±2.3	0.0009
FiO_2	0.29±0.05	0.67±0.24	<0.0001
Day 1	*n* 20	*n* 20	
MAP (cmH_2O)	7.2±1.6	7.9±3.3	0.45
P/F	323±54	236±79	0.0002
OI	2.3±0.6	4.3±3.6	0.02
$PaCO_2$ (mmHg)	43±6	41±9	0.47
pH	7.33±0.04	7.36±0.09	0.18
Day 3	*n* 13	*n* 15	
MAP (cmH_2O)	6.6±1.1	8.8±4.5	0.10
P/F	309±67	209±82	0.001
OI	2.3±0.8	5.9±5.8	0.03
$PaCO_2$ (mmHg)	43±7	48±8	0.11
pH	7.30±0.04	7.30±0.02	1
Day 5	*n* 6	*n* 10	
MAP (cmH_2O)	6.2±1.0	9.4±5.9	0.21
P/F	297±21	223±62	0.01
OI	2.1±0.4	5.1±4.8	0.15
$PaCO_2$ (mmHg)	49±4	47±4	0.40
pH	7.31±0.05	7.30±0.03	0.62
Day 7	*n* 3	*n* 10	
MAP (cmH_2O)	6.3±0.6	9.0±5.3	0.41
P/F	256±71	227±93	0.63
OI	2.5±0.6	5.1±5.6	0.44
$PaCO_2$ (mmHg)	45±11	50±6	0.34
pH	7.33±0.05	7.30±0.03	0.21

One BALF specimen collected on day 1, one on day 3, and two on day 7 from patients assigned to sIMV as well

Table 5 Pulmonary function test during the study period. Mann Whitney U-test used for comparison. [*Cdyn* dynamic respiratory compliance (ml/cmH_2O/kg), *Re* expiratory airway resistance (cmH_2O·l·s), *Rrs* total airway resistance (cmH_2O·l·s)]

	HFOV			sIMV			*P* value
	n	Median	Range	*n*	Median	Range	
Cdyn							
Day 1	16	0.74	0.42–1.4	18	0.57	0.19–0.88	0.01
Day 3	13	0.82	0.46–1.10	15	0.48	0.18–0.85	0.001
Day 5	6	0.66	0.43–0.95	10	0.48	0.27–0.61	0.04
Day 7	3	0.65	0.48–1.30	10	0.52	0.20–0.78	0.28
Re							
Day 1	16	160	80–226	18	193	118–400	0.02
Day 3	13	170	60–216	15	224	144–319	0.01
Day 5	6	178	148–220	10	225	163–416	0.04
Day 7	3	179	144–207	10	214	144–505	0.46
Rrs							
Day 1	16	99	58–162	18	117	59–304	0.19
Day 3	13	101	70–194	15	131	88–283	0.12
Day 5	6	114	92–124	10	145	75–231	0.08
Day 7	3	116	111–177	10	136	63–297	1

as one sample collected on day 3 from a patient assigned to HFOV, were excluded for visible blood staining. All BALF specimens taken into consideration for final analysis showed no bacterial or fungal growth.

Median values and ranges for assayed cytokines are shown in Table 6. On the whole, there was wide variability within each study group, at each time point evaluated and across time points.

IL-6 and IL-8 concentrations observed in BALF of HFOV and sIMV neonates presented no significant differences between treatments, nor over time, showing increasing initial and lowering late levels. Different results were observed for MCP-1 ELF concentrations, where the initial increase, particularly significant in HFOV patients, was followed by a slow decrease over time in both treatments.

Among anti-inflammatory cytokines ELF IL-10 medians showed no significant trend, while LIF values seem to have a different trend with high levels in the first days and low levels at 7th day in the HFOV group as compared to sIMV group, who showed a progressive increase over time.

No significant variations occurred in ELFs for either PDGF-BB or VEGF, but concentrations showed an increasing trend over time, more evident in the sIMV group. Finally, the concentration of TGF-β1 was characterized by significant ELF differences between the two groups at the 3rd, 5th and 7th day, due to an increase in concentrations for sIMV patients over time against a reduction in HFOV subjects.

A positive correlation was found between TGF-β1 and PDGF-BB, as well as with VEGF (r varying between 0.36 and 0.44; $P<0.01$), indicating that these modulators change their levels concomitantly.

Discussion

The results of this randomized clinical trial support the hypothesis that early and exclusive use of HFOV, combined with initial lung volume recruitment, has a beneficial effect during the acute phase of lung injury. HFOV-treated neonates benefited, in fact, from early and sustained improvement in pulmonary mechanics and gas exchange, with earlier extubation as compared to the neonates assigned to sIMV treatment, and showed significantly lower ELF TGF-β1 concentrations. The novelty of the study is a synthetic view of lung mechanics and gas exchange, correlated with the results of pro- and anti-inflammatory cytokine quantification. Unfortunately, attrition—particularly in the HFOV group attributable to earlier successful extubation—limited the number of subjects available for analysis, reducing the ability to interpret the results in the late observation period.

A significant difference was found in Cdyn and expiratory resistance values from day one to five, always in favour of the HFOV babies. Although Cdyn cannot be seen as the gold standard for comparing two groups of patients because it is too much dependent from the ventilator setting, several steps were taken to eliminate errors. The inspiratory time of analyzed breaths was 0.4 s, which is enough to reach a plateau pressure at the airway. For comparison of lung mechanics one expect to be in the same area of the pressure-volume (P/V) curve where different infants were ventilated. The only way to prevent being in the lower inflection point is by using sufficient PEEP and at the same level for all infants (as we did). It is also important to avoid over-inflation reaching the flat upper portion of the P/V curve by limiting tidal volume (not necessarily PIP). We cannot use the same PIP for all infants because it may create larger tidal volumes in those with higher compliance. To support our claim of improvement in lung mechanics in HFOV patients, it is remarkable that they were extubated earlier than sIMV

Table 6 ELF cytokine levels during the study period. Concentrations of ELF cytokines are expressed as (pg/ml). (*IL-6* interleukin-6, *IL-8* interleukin-8, *IL-10* interleukin-10, *MCP* monocyte chemoattractant protein-1, *LIF* leukemia inhibitory factor, *PDGF* platelet derived growth factor-BB, *VEGF* vascular endothelial growth factor, *TGF* transforming growth factor-β1)

ELF	HFOV			sIMV			*P* value
	n	Median	Range	*n*	Median	Range	
IL-6							
Day 1	16	3056	347–44575	17	8420	409–26896	0.33
Day 3	12	5718	900–57825	14	8024	1558–68529	0.50
Day 5	6	4621	2955–19650	10	2780	764–22747	0.33
Day 7	3	2520	1233–3758	8	4185	817–29041	0.43
IL-8							
Day 1	16	35034	3879–463791	17	39999	2935–1200000	0.94
Day 3	12	118075	20530–427312	14	113689	15088–1400000	0.92
Day 5	6	160235	81111–405600	10	51743	14988–659100	0.12
Day 7	3	82423	29340–216000	8	56778	10166–327810	0.84
MCP							
Day 1	16	13838	1691–35741	17	20632	3097–44271	0.08
Day 3	12	80059	20035–222013	14	54092	8684–618181	0.21
Day 5	6	159124	14028–274073	10	41364	15679–322878	0.12
Day 7	3	154000	107734–224280	8	38725	15966–737703	0.60
IL-10							
Day 1	16	111	6–527	17	99	9–1104	0.69
Day 3	12	75	23–1283	14	57	8–689	0.30
Day 5	6	103	15–206	10	118	14–246	0.85
Day 7	3	41	40–44	8	72	16–267	0.12
LIF							
Day 1	16	124	40–175	17	57	17–155	0.10
Day 3	12	257	25–1500	14	177	12–773	0.48
Day 5	6	321	141–597	10	149	12–555	0.10
Day 7	3	80	60–101	8	202	52–1057	0.84
PDGF							
Day 1	16	954	207–6882	17	877	85–8397	0.77
Day 3	12	3254	853–13333	14	2714	747–30536	0.53
Day 5	6	2062	545–23424	10	4576	1588–14567	0.30
Day 7	3	2400	554–4831	8	3449	1814–18971	0.50
VEGF							
Day 1	16	1439	178–11875	17	3019	124–25338	0.17
Day 3	12	3292	215–27208	14	4863	825–46000	0.41
Day 5	6	4973	1889–33615	10	7482	1210–43054	0.56
Day 7	3	3090	1253 - 4997	8	7723	1671–25279	0.12
TGF							
Day 1	16	1176	179–17490	17	1671	42–14596	0.91
Day 3	12	1169	253–11128	14	6226	41–22844	0.04
Day 5	6	566	259–6500	10	3454	566–20373	0.03
Day 7	3	630	149–1144	8	4714	1957–25809	0.04

babies. The improved oxygenation in the HFOV group, as demonstrated by ventilator indices, can be the result of more homogenous lung inflation and/or of blood flow diversion to the better-aerated lung fields. This indicates that atelectatic lung units were more adequately opened by optimal volume HFOV strategy than by sIMV. After surfactant therapy, greater improvement in gas exchange and higher values of Cdyn observed on day 1, in HFOV-treated neonates compared to sIMV group, suggest a recruitment of new alveolar units more than stabilization and distension of small airways and alveolar spaces alone. These results also suggest that lung volumes were managed better with an early optimal volume HFOV strategy than with sIMV strategy, when the PEEP is limited to 4–5 cmH_2O. Moreover the static airway inflation during HFOV versus intermittent airway expansion during sIMV could minimize biochemical and pathological factors associated with increased airway resistance.

The large initial difference in pre-surfactant FiO_2 between the two groups was not seen in other trials and could be related to the aggressive recruitment strategy, by working from the beginning at a high distending pressure to achieve a target $FiO_2 \leq 0.25$ (even if not in all patients), in an attempt to increase maximally the gas-exchanging surface. This resulted in a rapid improvement in oxygenation (Table 4). Recent trials [7, 8, 21, 22, 23] used a higher target FiO_2 (range 0.30–0.40) for not further increasing MAP, because of adverse effects on short-term neurological outcomes observed in some studies, by using an aggressive high volume strategy. Thereafter, the MAP values in our HFOV infants were substantially lower than those in the sIMV group, probably because of a lower degree of lung damage.

Prior evaluation of sequential pulmonary mechanics during the acute phase of respiratory disease failed to demonstrate any substantial differences in neonates ran-

domly assigned to HFOV versus those assigned to CMV [24], but neonates in both groups received conventional ventilation for a long period (<12 h) before randomisation and were administered brief but frequent episodes of manual ventilation, which has been known to cause alterations in pulmonary mechanics.

Significant changes were observed in TGF-β1 concentrations, higher in sIMV patients as compared to HFOV neonates. It can be speculated that low TGF-β1 levels (a pro-fibrotic cytokine) may be a signal of a mild lung injury induced by HFOV treatment. In fact, increased levels of TGF-β1 were found during the first days of life in BALF samples from premature babies who later developed CLD [15, 25, 26]. Although no significant differences were found between the two treatments regarding PDGF-BB and VEGF, the significant correlation between these cytokines and TGF-β1 indicate that these profibrotic cytokines change their levels concomitantly. More data concerning other profibrotic cytokines could confirm our hypothesis, that HFOV is associated with low lung fibrotic injury.

We failed to demonstrate different ELF IL-6, IL-8, and MCP-1 levels between study groups, suggesting a similar inflammatory response to ventilator injury. ELF IL-10 concentrations remained low in both study groups, while LIF, whose anti-inflammatory action appears to be a consequence of TNF-α down-regulation [27, 28], showed a different trend suggesting a possible lung protective role during HFOV rather than sIMV.

Thome et al. [29] failed to find any significant differences between HFOV and CMV neonates in cytokine production or protein leak. More recently, Yoder et al. [11], using an immature baboon model for neonatal CLD, found a significant increase in BALF concentrations of IL-8 in a low tidal volume-positive pressure ventilation group, which peaked on approximately day 10, and remained consistently high thereafter. It is quite possible that we found no significant difference in IL-8 concentrations because the study was conducted during the first week of life, and many babies, especially in the HFOV group, were extubated early.

In conclusion, our data suggest that early HFOV, with optimum volume strategy, could be more useful than sIMV in improving lung function and in reducing early lung ventilator injury in preterm babies who need to be ventilated soon after birth. An additional analysis examining the cytokine levels of both treatment groups, subdivided into babies developing CLD or not showed a nonsignificant increasing trend of the fibrogenic cytokines in the first group together with a significant increase of MCP-1 in the same group of patients (data not shown), according to previously published data [15, 25, 26, 30]. No significant differences were observed between the three dead babies and all the others, possibly because the death causes were not CLD-related.

Some discordances in results concerning pulmonary outcome between patients treated with HFOV or CMV in the studies reported in the Cochrane Review [9] and in this paper, may be explained by several factors: the interval between birth and initiation of HFOV was very short in our study (within 30 min) when compared with other trials, and we are aware that delayed HFOV may limit the benefits of this approach. Other factors include differences in surfactant administration, the maturity of the babies enrolled, the machine used to deliver HFOV, some aspects of our medical treatment (i.e., antibiotic and ibuprofen prophylaxis), the extubation time of our HFOV-treated neonates—much earlier when compared to other published trials—and, finally, the different optimum volume strategy adopted.

A major limitation of this study is the small number of babies studied, but our results, consistent with those reported by Yoder et al. [11] in immature baboons, should encourage neonatologists to study the mechanisms of early lung injury to identify the best ventilator strategy for preterm neonates.

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