Comparison of serum levels of seven cytokines in premature newborns undergoing different ventilatory procedures: high frequence oscillatory ventilation or synchronized intermittent mandatory ventilation

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ABSTRACT. ***Objective.*** **The severity of pulmonary dysfunction and subsequent development of chronic lung disease (CLD) in preterm neonates depends on several factors, among them oxygen administration. The aim of this report is to compare the effects of high-frequency, oscillatory ventilation (HFOV)** ***versus*** **synchronized, intermittent, mandatory ventilation (sIMV) on serum cytokine levels (IL-6, IL-8, IL-10, MCP-1, PDGF-BB, VEGF and TGF-beta1) and ventilator indices during the first week of life. Moreover, CLD development and several other outcomes were compared between the two groups.** ***Design.*** **Randomized clinical trial.** ***Setting.*** **Third level NICU.** ***Patients.*** **40 preterm neonates with a gestational age between 24 and 29 weeks were randomly (20 per group) assigned to one of the two, above-mentioned ventilation strategies within 30 minutes of birth.** ***Measurements and results.*** **At 1, 3 and 5 days, neonates were monitored by means of ventilator indices and levels of seven pro-inflammatory or anti-inflammatory (pro-fibrotic) cytokines in serum. No clinical or biochemical differences were observed at baseline. The neonates assigned to HFOV benefited from early and sustained improvement in gas exchange, with earlier extubation and lower incidence of CLD, as compared to the neonates assigned to sIMV treatment, and showed a significant reduction of serum IL-6, IL-8 and IL-10 over time only when the HFOV treatment was administered. In addition, at days 3 and 5, the IL-6 levels were significantly lower in the HFOV group as compared to sIMV patients.** ***Conclusions.*** **The results of this randomized clinical trial support the hypothesis that early use of HFOV, combined with an optimum volume strategy, has a beneficial effect, reducing serum levels of pro-inflammatory cytokines and consequently the acute phase leading to lung injury.**

Keywords: IL-10, IL-6, IL-8, serum, premature newborns, ventilatory procedures

In contrast with the large bulk of data regarding cytokine concentrations in bronchoalveolar lavage fluid (BALF) of premature newborns in relationship to ventilatory treatments [1-7], very little information is available regarding the levels of inflammatory or anti-inflammatory cytokines in plasma or serum [8, 9].

Recently, our group published data regarding the levels of various cytokines in epithelial lining fluids (ELF) of preterm newborns, randomly assigned to high frequency, oscillatory ventilation (HFOV) or synchronized, intermittent, mandatory ventilation (sIMV) during the first week of life [1].

Our study showed that ELF transforming growth factor-beta 1 (TGF-β1) levels were significantly increased in sIMV patients as compared to the HFOV patients. In addition, the HFOV strategy was associated with better respiratory function and lower CLD prevalence [1].

The present study, to evaluate the possible systemic inflammatory modifications, determined the serum levels of the seven cytokines already assayed in the same patients included in the above mentioned report [1].

Recent data from animal models demonstrated that, at the mRNA level, pro-inflammatory cytokines were reduced in the HFOV-treated as compared with the conventional mechanical ventilation group [3]. More recent data, in adult humans with acute lung injury, showed that lower tidal volume ventilation was associated with a decrease in plasma cytokines [9, 13, 14]. Therefore, our hypothesis was that an early HFOV strategy may also be associated, in preterm infants, with lower serum levels of pro-inflammatory cytokines and a reduced risk of the develop-

ment of CLD. As the control, the behavior of some anti-inflammatory cytokines was also observed.
The levels of the following cytokines were determined: interleukin (IL)-6, IL-8, IL-10, monocyte chemoattractant protein (MCP)-1, platelet derived growth factor (PDGF)-BB, vascular endothelial growth factor (VEGF) and TGF-β1. The cytokine selection was based upon knowledge of their specific functions. In fact, it has been suggested that different ventilation strategies may contribute to the development of chronic lung disease (CLD), depending on inflammatory processes [15]. Therefore, we evaluated three, representative, inflammatory cytokines (IL-6 and chemokines IL-8 and MCP-1), and two of the anti-inflammatory modulators (IL-10 and TGF-β1). In addition, because of the data observed in the previous study [1], we also analyzed PDGF-BB and VEGF as representative cytokines (together with TGF-β1) of the angiogenic, profibrotic group.

MATERIALS AND METHODS

This randomized clinical trial was carried out in our neonatal intensive care unit (NICU) over a period of 2.5 years, ending in January 2003.

Power analysis

A power analysis, based on serum cytokine levels, could not be performed due to the lack of sufficient data. Alternatively, based on our data for 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 days 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 [16], 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) [16], with 99% power at the 95% significance level, a total of 24 patients were required (see our previous study [1]). Considering that, in our clinical practice, 40% of infants with a gestational age < 30 weeks are successfully extubated within the 1st week of life, a total number of 42 patients was enrolled.

Patients

Neonates with a birth weight ranging between 500 and 1500 grams and gestational age between 24 and 29 weeks were studied. They were eligible when endotracheal intubation was required at birth, following current practice as described in the neonatal resuscitation program guidelines [17], and when on-going intensive care was required.
Premature infants with major congenital malformations or prenatal infections (positive blood and/or BALF cultures at birth) were excluded from the study, to avoid biases due to induction of inflammatory cytokines associated with the infection. The study protocol and consent forms were approved by the Ethics Committee of the Department of Pediatrics.
Several procedural characteristics have already been published and may be found in detail in our previous report [1]. Randomization to HFOV and sIMV was achieved by random number allocation and was carried out upon admission to the NICU, within the first 30 min after birth, by opening opaque, numbered, sealed envelopes. All newborns were treated with conventional ventilation before enrolment, while being transferred from the delivery room to the neonatal intensive care unit.
During the study period, 42 patients met the entry criteria. Two neonates, one per each study group, with late-diagnosed congenital pneumonia were subsequently excluded.

Ventilation strategies

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

HFOV

HFOV was performed using the Draeger Babylog 8000 plus (Draeger, Lubeck, Germany) with an "optimum volume strategy" defined as: initial use of a mean airway pressure (MAP) of 2 cm H_2O higher than on conventional mechanical ventilation, and initial weaning of the fraction of inspired oxygen (FiO_2) before MAP. The ventilation started at a MAP of 10 cm H_2O, 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 an adequate oxygenation, and when the FiO_2 was greater than 0.25, the MAP was increased by 0.5 to 1.0 cm H_2O every 10 to 15 minutes until it was possible to decrease the FiO_2. The FiO_2 was reduced to 0.25 before the MAP was decreased, provided that lungs were not hyperinflated (a condition ascertained by the flattening of the diaphragm below the margin of the ninth rib on chest radiography). The oxygenation was managed by adjusting MAP and FiO2; PaCO2 was managed by the adjustment of the oscillatory amplitude and ventilator frequency, when required. If pulmonary air leak developed, the strategy was changed to one of low volume and high FiO_2 with the reduction of MAP to a lower level, even if this strategy resulted in an increase in FiO_2 to the range of 0.6-0.8. Extubation was attempted when the neonate's condition remained stable for at least six hours while receiving minimal ventilation: $FiO_2 \leq 0.25$, MAP < 6 cm H_2O and an amplitude below 30%. Babies were extubated on nasal continuous positive airway pressure of 4-5 cm H_2O (nasal prongs Argyle, Sherwood Medical, St. Louis, MO, USA).

sIMV

Neonates in this group received sIMV with a Draeger Babylog 8000 plus (Draeger Co, Lubeck, Germany). This ventilator provides flow-triggered sIMV and continuous tidal volume monitoring at 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 cm H_2O, depending on the FiO_2 and lung inflation. Inspiration times of 0.30 to 0.40 second were used, with rates not exceeding 60 breaths per minute. Peak inspiratory pressure

(PIP) was lowered as the first step in improving infants. The policy of weaning during the recovery stage of their illness consisted of the reduction of PIP and ventilator rate until the peak pressure of 18 cm H_2O and the ventilator rate of 15 breaths per minute were achieved. The FiO_2 was reduced until 0.25 when the arterial oxygen tension exceeded 70 mmHg (9.3 kPa). PEEP and inspiratory time were maintained at 4 cm H_2O and at 0.4 seconds respectively. When these values were maintained for at least 6 hours, the ventilator rate was reduced by two to four breaths per minute every two hours to a minimum rate of 5 breaths per minute, and then the babies were extubated to nasal continuous positive airway pressure (4-5 cm H_2O). Extubation failure was defined, for both groups, as no longer than 72 hours, with clinical deterioration requiring re-intubation. No infant needed to be ventilated again within the next 72 hours following extubation. Two infants in sIMV group died with the endotracheal tube in place.

Medical treatment

Surfactant (a pig-derived natural surfactant, Curosurf, Chiesi Farmaceutici, Parma, Italy) was administered at a dose of 200 mg/kg. A second dose of 100 mg/kg was used for babies receiving sIMV, when the inspired oxygen concentration was greater than 30% and for those receiving HFOV if MAP was greater than 10 cm H_2O. All neonates studied were given intravenous ibuprofen as the lysine salt (Arfen, Lysafarma, Erba-Como, Italy) as prophylaxis for patent ductus arteriosus (PDA) according to our previous experience. All babies were initially treated with antibiotics (ampicillin and amikacyn) for the first seven days of life, to prevent lung colonization or infection. Caffeine citrate (caffeine citrate 10 mg/mL-Monico, Venice, Italy) was administered intravenously at a dose of 5 mg/kg per day after a loading dose of 20 mg/kg, from birth to the 33rd week of post-conceptual age to prevent apnoeic spells. No significant differences were found in medical treatments administered to the two groups of patients.

Cytokine determinations

After initial stabilization, and after surfactant therapy had been administered, serum samples were obtained at the end of the first day of life and on postnatal days 3 and 5, unless there was early extubation. Commercially available enzyme linked immunosorbent assay kits (IL-6, IL-8, IL-10, MCP-1, PDGF-BB, VEGF, TGF-beta1; R&D Systems Europe Ltd. 4-10, The Quadrant, Barton Lane, Abingdon, Oxon, UK), were used, following manufacturer's instructions. The detection limits (pg/mL) for IL-6 was = 0.04, IL-8 = 3.0, IL-10 = 0.5, VEGF = 2.5, PDGF-BB = 15, TGF-beta1 = 7, MCP-1 = 5.0. No children showed serum values below the detection levels.

Table 1
Patient characteristics of the groups analyzed. Values expressed as mean ± SD and n (%)

Characteristics	HFOV (20)	SIMV (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/total	9 (45)	11 (55)	0.75
Caesarean section	15 (75)	18 (90)	0.41
Antenatal steroids			
a) Complete[a]	11 (55)	12 (60)	1.0
b) Partial/incomplete[b]	9 (45)	6 (30)	0.51
c) None	0	2 (10)	0.48
Rupture of membranes ≥ 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
Requirement of 2nd surfactant dose	2 (13)	7 (47)	0.10
Mean age of extubation (days)	3 (1-26)	7 (1-33)	0.11
Deaths	1 (5)	2 (10)	1.0
Ductus arteriosus surgically ligated	0 (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
O_2 dependence at 36 weeks (%)	2:19 (10.5)	8:18 (44.4)	0.048
Pneumothorax (%)	2 (10)	1 (5)	1.0
Cumulative morbidity (sum of the last 10 rows)	25	30	-

[a] Number of subjects treated with a completed course of prenatal betamethasone (two doses administered more than 24 hours, but no more than seven days before delivery).
[b] Number of subjects treated with a partial course (one dose administered more than 24 hours but no more than seven days before delivery) and number of subjects treated with incomplete (any steroid administered less than 24 hours or more than seven days before delivery). T test or Chi-square when appropriate.

Ventilatory indices

The values for MAP, P/F (PaO_2/FiO_2 ratio), OI [oxygenation index (MAP x FiO_2/ PaO_2 x 100)], $PaCO_2$ and pH were established at the time of serum sampling.

Statistical analysis

Categorical variables were compared 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. Cytokine levels, due to the limited number of subjects and non-Gaussian distribution, were expressed as median and ranges.

RESULTS

Table 1 reports the main characteristics of all 40 patients enrolled (20 cases treated with HFOV and 20 with sIMV). No significant differences in the clinical characteristics of the patients included in the study were found, indicating that the two groups of patients were superimposable.
The number of subjects requiring surfactant and with bacteriemia was exactly the same in the two groups. A slight, but significantly reduced prevalence of CLD was observed in the HFOV-treated group.

Table 2 suggests also that ventilatory indicators were very similar at a baseline level, indicating a similar degree of respiratory failure. As expected, before surfactant administration, the neonates assigned to HFOV received ventilation with higher MAP and lower FiO_2. In the remaining study period, MAP levels did not show significant differences between the two groups, while FiO_2 remained significant, even when the significance level was sequentially lower. MAP values were comparable at extubation time in the two groups. The mean P/F ratio was always significantly higher in infants receiving HFOV as compared to those receiving sIMV *(table 2)*. Overall, the mean OI values were always lower in infants assigned to HFOV and the differences were statistically significant on days 1 and 3. Mean $PaCO_2$ and pH values were similar in both groups *(table 2)*.

The distributions of the serum levels of the seven cytokines evaluated are shown in *table 3*, stratified for ventilation strategy and analyzed over time. Only 16 patients undergoing HFOV and 18 undergoing sIMV were followed over time. In fact, six patients were extubated within the first 24 hours of life, prior to serum sample collection.

The extubation of the babies, when the criteria were reached, determined the end of the blood sampling and therefore the reduction of the patient number over time.

Table 2
Ventilatory indices, arterial blood gas values, pH and a/A ratio over time

Base line	**HFOV (20)**	**sIMV (20)**	**p value**
MAP[a] (cm H_2O)	8.2 ± 0.8	8.3 ± 1.1	0.74
FiO_2[b]	0.49 ± 0.24	0.54 ± 0.22	0.49
Pre-Surfactant	N. 15	N. 15	
MAP (cm H_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 (cm H_2O)	7.2 ± 1.6	7.9 ± 3.3	0.45
FiO_2	0.22 ± 0.01	0.30 ± 0.12	**0.002**
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 (cm H_2O)	6.6 ± 1.1	8.8 ± 4.5	0.10
FiO_2	0.23 ± 0.03	0.36 ± 0.20	**0.03**
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	**1.0**
Day 5	n = 6	n = 10	
MAP (cm H_2O)	6.2 ± 1.0	9.4 ± 5.9	0.21
FiO_2	0.23 ± 0.02	0.34 ± 0.11	**0.02**
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

Only intubated patients were considered. Plus-minus values are means ± SD. Unpaired *t* test used for comparison.
[a] MAP: mean airways pressure.
[b] FiO_2: fraction of inspired oxygen; P/F: PaO_2/FiO_2 ratio; OI: oxygenation index (MAPxFiO_2/ PaO_2 x100); $PaCO_2$: partial pressure of arterial carbon dioxide.

Table 3
Serum cytokine levels during the study period stratified for treatment

SERUM	HFOV	Median	range	SIMV	Median	range	p value
IL-6[a]	16	26	3-179	18	38.5	4-1046	0.83
Day 1							
Day 3	13	7	1-47	15	20	4–144	**< 0.05**
Day 5	6	6	2-44	10	14	5-145	**< 0.05**
		p = 0.005			p = 0.44		
IL-8[b]	16	262	39-853	18	209	22-653	0.88
Day 1							
Day 3	13	155	34-398	15	140	33-749	0.93
Day 5	6	104	47-284	10	128	40-559	0.75
		p < 0.05			p = 0.55		
IL-10[c]	16	12.9	1.4-41	18	7.8	1.3-58	0.31
Day 1							
Day 3	13	3.7	1.6-37	15	4.9	0.9-46	0.63
Day 5	6	3.2	0.7-11	10	4.0	2.0-31	0.47
		p = 0.02			p = 0.72		
MCP[c]	16	1438	175-8792	18	992	369-2587	0.20
Day 1							
Day 3	13	1565	1005-2749	15	1247	299-6005	0.51
Day 5	6	1521	657-4720	10	1129	606-3212	0.31
		p = 0.92			p = 0.49		
PDGF	16	2313	237- 7500	18	1534	571-7123	0.48
Day 1							
Day 3	13	1249	543- 6425	15	1475	393-2065	0.63
Day 5	6	908	328-10000	10	1454	237-8517	0.22
		p = 0.19			p = 0.21		
VEGF	16	43	3-350	18	28	10-754	0.54
Day 1							
Day 3	13	37	9-327	15	25	3-150	0.35
Day 5	6	24	6-450	10	31	15-395	0.75
		p = 0.77			p = 0.49		
TGF[d]	16	3130	200-15261	18	5366	500-16851	0.41
Day 1							
Day 3	13	2749	350-11898	15	4015	767- 7480	0.23
Day 5	6	4601	173-17138	10	4086	202-12088	0.79
		p = 0.66			p = 0.89		

Concentrations of serum cytokines are expressed as pg/mL. Left side P is calculated between the two groups (HFOV and sIMV) in the same day. P reported under the levels of each cytokine is calculated considering the three day variations in the same group. Groups were compared within the same day by non-parametric Mann-Whitney test, while the different days of the same group were compared by non-parametric Kruskall-Wallis variance analysis.

[a] IL-6 = interleukin-6.

[b] IL-8 = interleukin-8.[c] IL-10 = interleukin-10.

[c] MCP = monocyte chemoattractant protein-1; ° PDGF = platelet derived growth factor-BB; VEGF = vascular endothelial growth factor.

[d] TGF = transforming growth factor-β1.

This procedure conditioned not only the number of babies followed, but also the severity of the clinical conditions of the newborns included in each group. In fact, the extubated infants showed better respiratory markers than those followed for more prolonged periods.

The data reported in *table 3* may be summarized as follows:

– There were no initial significant differences between the two groups;

– Blood levels of IL-6 showed significant differences at days 3 and 5 between HFOV and sIMV treatments, and HFOV showed the lowest levels. No significant differences were observed for the remaining mediators;

– A significant reduction of IL-6, IL-8 and IL-10 over time was only observed in the HFOV group, suggesting that these babies had reduced inflammation. The behaviour of IL-10 is not surprising, since generally, IL-10 levels are correlated with an inflammation rate (anti-inflammatory activity).

A further analysis has been performed to evaluate possible correlations of the serum levels of the seven cytokines.

The results showed that IL-6, IL-8, IL-10 and MCP-1 correlated significantly and p was always significant: $p < 0.01$ for all couples of values). Finally, further positive, significant correlations were observed between PDGF-BB, VEGF and TGF-beta1 values ($p < 0.01$).

DISCUSSION

Very few data are available in the literature concerning the levels of cytokines determined in serum from preterm newborns correlated with different ventilatory treatments. In fact, it has been suggested that different ventilation strategies may influence the development of CLD and death depending on the inflammatory processes [15]. Recent data have been published regarding the role of some cytokines in CLD induction in adult patients only [15].

Ventilation strategies used for premature newborns may represent a major cause of lung injury. Oxygen may, in fact, cause an inflammatory reaction in the lungs, through a complex network of mediators, including pro-inflammatory and anti-inflammatory cytokines [10-12, 18].

The degree of such a response is important in the induction of chronic lung disease (CLD) over time [5, 6, 19]. As the first hour after birth seems to be crucial to the well-being of the infant [8], great efforts are being made to improve ventilation strategies, employing less aggressive methods for administering oxygen to premature neonates. Barotrauma, volutrauma and oxygen toxicity during intermittent, positive pressure ventilation are, in fact, thought to be pathogenic factors for CLD [9, 10, 12, 17].

In a previous publication, on the same group of patients, we found that ELF levels of TGF-beta1, a profibrotic (TH3)-cytokine, were higher in sIMV-treated infants, suggesting a higher fibrotic induction and more frequent development of CLD [1].

Gitto *et al*. reported that serum pro-inflammatory cytokine concentrations were correlated with the duration of mechanical ventilation and oxygen exposure [8].

Our results showed that serum IL-6 values were significantly lower at the third and fifth day of ventilation in HFOV infants as compared with the sIMV group. In addition, a significant, over time reduction in IL-6, IL-8 and IL-10 levels was observed in HFOV patients only, suggesting that this procedure, in the context of the treatment program described, may induce less important flogistic processes. These findings are evident notwithstanding the fact that the selection of the patients, upon extubation criteria, should have favoured the presence of more seriously ill patients at day 5.

No differences were noted for serum levels of TGF-beta1, VEGF and PDGF-BB, while previous data showed that ELF TGF-beta1 levels were increased in sIMV treatment, suggesting a more pronounced risk of pulmonary fibrosis [1, 6, 13].

In addition, confirming data previously seen in other fields [1, 6, 7, 20, 21], significant correlations of pro-inflammatory cytokines (IL-6, IL-8 and MCP-1) plus IL-10, as well as significant correlations for the pro-fibrotic VEGF, PDGF-BB and TGF-beta1 values were registered, as expected for the inflammatory cytokine network, suggesting an orchestrated synthesis of these molecules.

This report suggests that the proinflammatory cytokines are more closely associated, at the peripheral level, with sIMV treatment, and therefore may be the expression of activated molecular mechanisms which lead to the development of CLD. In this trial, although the primary outcome was the possible difference in cytokine values, development of CLD was distributed differently between the two treatment groups: in fact, there was an increase in its prevalence in sIMV infants. Other studies have failed to show this significant difference probably because of several factors: a) 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; b) differences in surfactant administration; c) the maturity of the babies enrolled; d) the machine used to deliver HFOV; e) some aspects of our medical treatment (*i.e.*, antibiotic and ibuprofen prophylaxis); f) the time to extubation of our HFOV-treated neonates was much shorter when compared to other published trials; and, finally, g) the different optimum volume strategy adopted.

Other secondary, clinical outcomes are reported in *table 1*: no significant differences were noted, although cumulative morbidity is greater in sIMV.

In conclusion, our results show that significant differences were found between the two different ventilation groups, suggesting that the HFOV strategy is associated with lower proinflammatory cytokine levels, hence with a possible lower induction of inflammatory processes [12-14]. The common behavior, in the HFOV group, of pro-inflammatory IL-6, IL-8, and anti-inflammatory IL-10 and the correlation identified, highlight the concept of a cytokine network, fundamentally directed towards regulating the cellular homeostasis of the organism.

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REFERENCES

1. Vento G, Matassa PG, Ameglio F, *et al.* HFOV in premature neonates: effects on pulmonary mechanics and epithelial lining fluid cytokines. A randomized controlled trial. *Intensive Care Med* 2005; 31: 463.
2. Yoder BA, Siler-Khodr T, Winter VT, *et al.* 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: 1867.
3. von der Hardt K, Kandler MA, Fink L, *et al.* High frequency oscillatory ventilation suppresses inflammatory response in lung tissue and microdissected alveolar macrophages in surfactant depleted piglets. *Pediatr Res* 2004; 55: 339.
4. Thome U, Gotze-Speer B, Speer CP, *et al.* Comparison of pulmonary inflammatory mediators in preterm infants treated with intermittent positive pressure ventilation or high frequency oscillatory ventilation. *Pediatr Res* 1998; 44: 330.
5. Jankov RP, Tanswell K. Growth factors, postnatal lung growth and bronchopulmonary dysplasia. *Paediatr Respir Rev* 2004; 5: S265.
6. Vento G, Matassa PG, Ameglio F, *et al.* Effects of early dexamethasone on pulmonary fibrogenic mediators and respiratory mechanics in preterm infants. *Eur Cytokine Netw* 2002; 13: 207.
7. Vento G, Matassa PG, Capoluongo E, *et al.* Glucocorticoids in preterm infants and discrepancies of Vascular Endothelial Growth Factor. *Am J Respir Crit Care Med* 2003; 168: 501.
8. Gitto E, Reiter RJ, Amodio A, *et al.* Early indicators of chronic lung disease in preterm infants with respiratory distress syndrome and their inhibition by melatonin. *J Pineal Res* 2004; 36: 250.
9. Parsons PE, Eisner MD, Thompson BT, *et al.* Lower tidal volume ventilation and plasma cytokine markers of inflammation in patients with acute lung injury. *Crit Care Med* 2005; 33: 1.
10. Abman SH, Groothuis JR. Pathophysiology and treatment of bronchopulmonary dysplasia: current issues. *Pediatr Clin North Am* 1994; 41: 277.

11. Ellsbury DL, Acarregui MJ, McGuinness GA, *et al.* Controversy surrounding the use of home oxygen for premature infants with bronchopulmonary dysplasia. *J Perinatol* 2004; 24: 36.

12. Ricard JD, Dreyfuss D, Saumon G. Ventilator-induced lung injury. *Eur Respir J* 2003; 42: 2S.

13. Bustani P, Kotecha S. Role of cytokines in hyperoxia mediated inflammation in the developing lung. *Front Biosci* 2003; 8: 694S.

14. Kumar VH, Ryan RM. Growth factors in the fetal and neonatal lung. *Front Biosci* 2004; 1: 464.

15. Laberge S, El Bassam S. Cytokines, structural cells of the lungs and airway inflammation. *Paediatr Respir Rev* 2004; 5: S41.

16. Tortorolo L, Vento G, Matassa PG, *et al.* Early changes of pulmonary mechanics to predict the severity of bronchopulmonary dysplasia in ventilated preterm infants. *J Matern Fetal Neonatal Med* 2002; 12: 332.

17. Niermeyer S, Kattwinkel J, Van Reempts P, *et al.* International Guidelines for Neonatal Resuscitation: An Excerpt From the Guidelines 2000 for Cardiopulmonary Resuscitation and Emergency Cardiovascular Care. *Internat Consensus Sci Pediatr* 2000; 106: 1.

18. Collard KJ, Godeck S, Holley JE, *et al.* Pulmonary antioxidant concentrations and oxidative damage in ventilated premature babies. *Arch Dis Child Fetal Neonatal* 2004; 89: F412.

19. Pierce MR, Bancalari E. The role of inflammation on the pathogenesis of brunchopulmonary dysplasia. *Ped Pulmonol* 1995; 9: 371.

20. Bonifati C, Ameglio F. Cytokines in psoriasis. *Int J Dermatol* 1999; 38: 241.

21. d'Auria L, Cordiali-Fei P, Ameglio F. Cytokines and bullous pemphigoid. *Eur Cytokine Netw* 1999; 10: 123.