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Cardio-Respiratory Events and Inflammatory Response After Primary Immunization in Preterm Infants < 32 Weeks Gestational Age: A Randomized Controlled Study

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Abstract

Background: Inflammation may depress respiration in neonates. This study aimed to establish a link between post-immunization inflammation and cardio-respiratory events (CRE).

Methods: Randomized double-blind controlled study of infants born <32 weeks gestation receiving the 2 months vaccine which comprised diphtheria and tetanus toxoids and acellular pertussis adsorbed combined with inactivated poliomyelitis vaccines, and Haemophilus b conjugate and the pneumococcal conjugate 10-valent vaccines. Infants were randomized to ibuprofen treatment or a placebo group (n=28/group). C-reactive protein (CRP) and prostaglandins E2 (PgE2) levels were assessed before and after immunization. CRE were recorded for 72 hours. Heart rate variability was assessed by polysomnography.

Results: In the placebo group, immunization was associated with significantly increased CRP levels and an increase in CRE (8.6±11.1 before vs. 14.0±12.8 after) which did not reach statistical significance (p=0.08), and no change in PgE2. The increase in CRP was correlated with changes in CRE (r=0.4, p<0.05). In the ibuprofen group, immunization significantly increased CRP levels but was not associated with change in CRE (6.7±7.7 before vs. 6.8±9.7 after) and PgE2 levels. Comparing the groups, variation in CRE (ΔCRE before vs. after immunization) was significantly lower in the ibuprofen group (0.1±7.9 vs. 5.4±10.0 ΔCRE, p<0.05).

Conclusion: The first immunization of infants born < 32 weeks was associated with an increase in CRP. Ibuprofen treatment significantly attenuated the variation (Δ) in CRE following first immunization in these infants but the current study could not demonstrate an impact on CRP and

PgE2 levels. The impact of anti-inflammatory treatment on antigenicity must be evaluated before their clinical use aiming at reducing CRE after immunization in preterm infants.

Introduction

Immunization with the pentavalent vaccines Diphtheria-Tetanus-Acellular pertussis-Inactivated poliomyelitis-Haemophilus influenzae type b (DTPa-IPV-Hib) at two months of chronological age is known to be associated with cardio-respiratory events (CRE), such as apnea and bradycardia, in 11 to 47% of preterm infants [1-4]. Apnea has also been reported as temporally associated with the administration of 10-valent pneumococcal conjugate vaccine with concurrent routine vaccines in premature infants [5]. It has been proposed that the immature brainstem respiratory control of preterms and their periodic irregular breathing with potential detrimental apneas make them more vulnerable to the inflammatory reaction caused by immunization but this has not been demonstrated [6].

We hypothesized that post-immunization CRE are correlated with the inflammatory reaction and that inhibition of inflammation would reduce post-immunization CRE. The primary objective was to examine the impact of inflammatory response inhibition (via administration of the non-steroidal anti-inflammatory drug ibuprofen vs. placebo at the time of the vaccines administration) on the occurrence of CRE following the first dose of pentavalent vaccines in preterm infants born < 32 weeks gestation.

The secondary objective was to identify predictive factors of occurrence of CRE in preterm infants after immunization through the analysis of their heart rate variability.

Methods

Study design and participants

This randomized, double blinded, placebo-controlled study was conducted in the neonatal intensive care unit of Sainte-Justine University Hospital (CHU Sainte-Justine, Montreal, QC,

Canada) over a period of fourteen months (2010 - 2011). The study was approved by CHU Sainte-Justine institutional Ethics Committee for Clinical Studies. Written informed parental consent was obtained for all infants.

The study population was composed of preterm infants, born at < 32 weeks gestation, at a postnatal age > 7 weeks, on full enteral feed and eligible to receive the first dose of pentavalent vaccine. All infants with anomalies in cardiac conduction, congenital malformations, intraventricular haemorrhage grade 3 or 4 or periventricular leukomalacia were excluded. Infants who required assisted ventilation at enrolment, those that were critically ill or with unstable vital signs according to attending neonatologist were also excluded.

Immunization and ibuprofen/placebo administration

The vaccines administered were: diphtheria and tetanus toxoids and acellular pertussis vaccine adsorbed combined with inactivated poliomyelitis vaccine and Haemophilus b conjugate vaccine (Pediacel®; Sanofi-Pasteur) and the pneumococcal conjugate 10-valent vaccines (Synflorix®; GlaxoSmithKline). The two vaccines were administered intramuscularly in the anterolateral region of each thigh, 60 minutes after topical EMLA® cream (0.5 g in a thick layer).

On enrollment, patients were randomized into two groups: the study group received oral ibuprofen (Advil® Pediatric drops; Wyeth-Ayerst) 5 mg/kg/dose (Ibuprofen; n=28) and the control group received an oral placebo (Placebo; n=28) prepared by the CHU Sainte-Justine pharmacy. Patients were randomly assigned into the two groups using 40 blocks of 4 with a computer generated-randomization list extracted from the website randomization.com. The randomization was done by the central hospital pharmacy to keep the investigators blinded.

The drugs were administered in an opaque syringe 30 minutes prior to immunization, and then at

8 and 16 hours following the immunization for a total of 3 doses (Figure 1).

Assessment of cardio-respiratory events

Cardio-respiratory monitoring and recordings were performed in all patients continuously for 72 hours, beginning 24 hours before and continuing until 48 hours after immunization (Figure 1). Monitoring tracings were printed and CRE were extracted and compared to nurses' surveillance noted on a separate sheet. These analyses were performed by two different operators: a medical fellow (WBJ) and a research nurse. The results were discussed and an agreement reached between operators in any instances of discordance. The operators were blinded to the treatment received by the patients for the assessment and the interpretation of these recordings. The recorded CRE included: bradycardia (a 33% decrease in baseline heart rate for at least 4 seconds, or a heart rate ≤ 80 bpm), desaturations (10% decrease in baseline saturation), and apnea (respiratory pause of at least 20 seconds, or a respiratory pause of 15 seconds associated with a bradycardia).

Total CRE were expressed as the average number of events (desaturation + apneas + bradycardia) / 24 hours. Δ Total CRE / patient / 24 hours was defined as the difference between the average numbers of events / 24 hours observed before vs. after immunization for each patient. Biographical data, maternal and pregnancy data, and infant medical data (base line heart rate, temperature and ventilation duration) were also collected for each patient.

Assessment of heart rate variability

Two annotated polysomnographies were performed for all patients with an AURA PSG GRASS ambulatory and wireless system. Each polysomnography had a duration of 2.5 hours: the first was conducted on enrolment (the day before immunization), and the second was conducted 18 to

24 hours after immunization (Figure 1). The patients were settled comfortably in an environment with reduced tactile, auditory and luminous stimulation, and the cardiac electrodes, oximeter and abdominal respiration detector were placed. The polysomnographies were annotated by the research nurse or medical fellow (WBJ) for the total duration of the recordings, and analysis was performed by the team of Pr Pladys (Rennes, France). All the team involved with Pr Pladys in the interpretation of these polysomnographies was blinded to the treatment group.

Custom-built signal processing tools designed with Matlab software (v6.0.0.42a, release 12; The Mathworks, Inc.) were used as previously described [7, 8]. In summary, a 1-hour sequence was selected in a period of quiet sleep or indeterminate sleep. QRS complexes were automatically detected using specific filter coefficients adapted to the newborn. Times between consecutive R waves (RR) (which measures cardiac cycle length) were manually verified. Parameters were selected based on studies showing changes in infants with sepsis (inflammation) or after immunization and were studied as previously reported[7-9]. The variables studied were the mean cardiac cycle length (mean), the standard deviation (SD) which measures the magnitude of global heart rate variability, and the square root of the mean squared differences of successive RR intervals (rMSSD; short-term beat-to-beat variability which reflects parasympathetic control). Complexity and regularity of RR series were estimated using entropy (ApEn) measurements; RR fluctuations (Alpha1 and Alpha2) and short term and long term variability (SD1 and SD2 respectively) were measured. The power spectral densities in the low frequency (LF, 0.02-0.2 Hz, baroreflex origin) and in the high frequency ranges (HF, 0.2- 2Hz, respiratory origin) were calculated and the ratio LF/HF was used as an index of sympathetic-parasympathetic balance.

Assessment of plasma prostaglandin E2 and C reactive protein levels

C reactive protein (CRP) and prostaglandin E2 (PgE2) were measured as systemic markers of inflammation. PgE2 was also selected because clinical and experimental data indicate its key role in mediating apnea associated with inflammation in newborns[10, 11]. Blood samples for CRP levels (0.5 ml / sample in microtube with lithium heparin and gel barrier) were analyzed by immunoturbidimetry (Sainte-Justine University Hospital Biochemistry laboratory) within 30-60 minutes after sampling. Capillary blood samples (0.5 ml / sample) were collected in an EDTA-coated tube 30-60 minutes prior to the immunization and 18 hours after. 10µM indomethacin was added to each tube within 30 minutes of sampling in order to inhibit ongoing PG synthesis by platelets[12]. After centrifugation, plasma was frozen (-80ºC) until analysis. Plasma PgE2 concentration was determined by ELISA (PgE2 Parameter Assay kit; R and D systems, #KGE004B; intra- and inter-assay variability of 6.7% and 10.6% respectively).

All blood samples were taken following sucrose administration (10 drops dextrose 24% gently administered in the infant mouth 15-30 seconds prior to needle prick) as per routine practice in the NICU. Δ CRP and Δ PgE2 were defined, respectively, as the difference between CRP and PgE2 levels before and after immunization.

Statistical analysis

Statistical analysis was performed using SPSS (v20.0 for Windows). All variables were tested for normal distribution using the Shapiro-Wilk normality test. When normally distributed (parametric), data were presented as mean ± standard deviation, applying one-way analysis of variance (ANOVA) and Bonferroni post hoc test. Nonparametric data were analyzed using the Kruskall-Wallis with Dunn post-test. Heart rate variability parameters were analyzed by paired

or unpaired Student's t-test, or Wilcoxon w-test and Mann-Whitney u-test as appropriate. Correlations between non parametric data were analyzed using Spearmen test. Comparisons between treatment groups of the number of infants in whom CRE were more frequent, less frequent or unchanged after immunization was done with Chi-square contingency table. The two-sided significance value was set at 0.05.

The sample size calculation (26 patients required in each group) was based on a 40% incidence of CRE post vaccination [1-4] and with the hypothesis that the ibuprofen will decrease this incidence from 40% to 15% (α of 0.05 and a power of 80%)[1, 2, 4].

Results

Characteristics of study participants

362 preterm infants were screened and 56 completed the study (Figure 2). Of the infants who did not participate in the study, 212 were discharged before the first immunization (92 to home and 120 to level II nurseries), 37 died, 28 were still intubated at the time of enrolment, 14 did not meet the inclusion criteria, and parents refused for the remaining 15.

As shown in Table 1, there was no significant difference between Placebo and Ibuprofen groups with regard to newborn and pregnancy characteristics and in the infant status at the time of immunization.

Effect of immunization within each group

In the Placebo group (Table 2), plasma CRP (mg/l) levels increased significantly after immunization (0.7 ± 1.0 vs. 20.1 ± 12.7, $P < 0.0001$) whereas PgE2 (pg/ml) levels remained unchanged (384 ± 235 vs. 396 ± 270, $P = 0.9$). CRE was detected in 21 infants (75%) prior to immunization and in 24 (86%) after immunization. After immunization, the number of CRE / 24

hours increased in 19 infants (68% of the group), was stable in 4 (14%) and lower in 5 (18%). Overall, the number of CRE / patient / 24 hours was 8.6 ± 11.1 before immunization vs. 14.0 ± 12.8 after, p = 0.08. A significant correlation was observed between Δ CRP and Δ Total CRE / patient / 24h ($r = 0.4$, $p<0.05$).

In the Ibuprofen group (Table 3), plasma CRP levels increased significantly after immunization (1.1 ± 3.0 vs. 16.6 ± 9.7, $P<0.0001$), and PgE2 levels were not significantly changed (473 ± 285 vs. 355 ± 272, $P = 0.11$). CRE was detected in 22 infants (79%) prior to immunization and in 24 (86%) after immunization. After immunization, the number of CRE / 24 hours increased in 9 infants (32%), was stable in 7 (25%) and lower in 12 (43%). Overall, the total number of CRE / patient / 24 h was unchanged before vs. after immunization (6.7 ± 7.7 before vs. 6.8 ± 9.7 after, $P = 0.9$). In this group Δ CRP and Δ Total CRE / patient / 24h were not correlated.

In both groups, Δ PGE2 and Δ Total CRE / patient / 24h were not correlated. Overall, immunization did not modify the polysomnography heart rate variability measurements within each group.

Effect of Ibuprofen administration

Comparing the Ibuprofen to the Placebo group, the proportion of infants who showed an increase in the number of CRE / 24 hours after immunization (vs. unchanged or lower number of CRE) was significantly reduced in the Ibuprofen (32%) vs. Placebo (68%) group, $p < 0.01$. Before and after immunization, the variation (Δ) of total CRE / patient / 24h was significantly lower in the Ibuprofen vs. Placebo group (Δ CRE 0.1 ± 7.9 Ibuprofen vs. 5.4 ± 10.0 Placebo, $P < 0.05$) (Table 4). There was no significant difference in the variation in plasma PgE2 levels and in plasma CRP levels between groups. Exclusion of infants who were already receiving daily caffeine (10

mg/kg/day caffeine citrate) for apneas and bradycardias of prematurity (6 in the Placebo group and 2 in the Ibuprofen group) did not modify the results.

Discussion

The findings of this prospective randomized, double blinded, placebo-controlled study demonstrate that in infants born at < 32 weeks gestation, immunization at two months of age increased CRP levels, as reported by other studies [13, 14], whereas PgE2 levels remained unchanged. The magnitude of the CRP elevation was correlated with the changes in CRE in the control Placebo group. Infants in the Placebo group showed an increase in their number of CRE / 24h by 63% but this did not reach statistical significance ($p=0.08$) presumably because of a large inter-patient variability. However, the difference (Δ) in CRE / 24h pre- vs. post-immunization was significantly decreased by Ibuprofen treatment vs. Placebo. Further, the proportion of infants who had an increase in the number of CRE / 24 hours after immunization was significantly less in the Ibuprofen group.

Despite the fact that we did not observe a significant decrease in PgE2 levels during treatment, Ibuprofen treatment significantly attenuated the increase in CRE after immunization, and abolished the correlation between increase in CRP and in CRE after immunization. Both ibuprofen and acetaminophen have been previously reported to reduce fever, pain, agitation and local redness following immunization in 2 to 7 months old infants compared to placebo [13, 15, 16]. In the current study, we chose ibuprofen because of its reduction in PG biosynthesis through non-selective inhibition of the cyclooxygenase (COX) site of the prostaglandin H2 synthetase enzyme. In contrast, acetaminophen exerts its analgesic and antipyretic effects by inhibition of the peroxidase site of the prostaglandin H2 synthetase enzyme in the central nervous system but

has limited effects on systemic prostaglandin reduction[17].

The impact of the first dose of pentavalent vaccines on the incidence of CRE in preterm infants has been the subject of controversies in the literature. Many studies have reported an 11 to 47% increase in CRE in preterm infants after the two-month immunization [1-4], but others [18, 19] did not find significant change in CRE. Of note, the definition for CRE used in current study differs from to the definition of "apneas and bradycardias" as used in the neonatology clinical settings or in [3, 4, 18, 19]. The current study aimed at examining changes in cardiorespiratory regulation after immunization which is why we used criteria similar to those in our previous study in preterm infants [7], and most probably explains the relatively high occurrence of CRE in our population. A recent multicenter retrospective cohort study involving 13,926 preterm infants born at 28 weeks' gestation or less argue further for a potential increased risk of CRE after the first immunization [20]. In this study, the need for respiratory support increased from 6.6 per 1000 patient-days in the pre-immunization period to 14.0 per 1000 patient-days in the post-immunization period [20]. The mechanism underlying the proposed relationship between immunization and CRE is not as yet understood.

Based on human and animal studies, we aimed to establish a link between the induced inflammatory reaction to the immunization and CRE. In this regard, Hoch et al. [21] measured PgE-M (urinary metabolite of PgE2) levels in two groups of 18 preterm infants (mean GA 32 weeks) presenting with apneas (study group) or not (control group); they found a significant positive relationship between urinary PgE-M concentration and the number of central apneas at 2-4 weeks after birth. A relationship between central apneas and elevated serum levels of prostaglandins has also been reported in infants with congenital cardiac anomalies who require a

continuous infusion of PgE1 to maintain the patency of the ductus arteriosus [22].

Several animal studies suggest that prostaglandins of the E series play a role in the central respiratory control areas, including in experiments using pro-inflammatory stimuli[23, 24 10, 25-27]. In the current study, the increase in CRE observed post-immunization was not correlated to an increase in PgE2 serum levels; however, administration of ibuprofen prevented the increase in CRE. Comparing the Ibuprofen and Placebo groups before and after immunization, the difference in PgE2 variation was not found statistically significant, probably because of the large within-group standard deviation, even though the three-dose regimen of ibuprofen (5 mg per kg at 8-hour interval) falls within the anti-inflammatory pediatric dose ranges and similar dosages have been reported to effectively reduce PgE2 levels in premature infants treated for patent ductus arteriosus in the first days of life[28, 29]. Measured PgE2 levels could be impacted by an inter-patient variation in the time between sampling and the addition of indomethacin to the sample (which was done within 30 min for all samples). In addition, all post-immunization measurements were conducted at 18 hours, which potentially does not represent a similar time point in the inflammation kinetics of all patients. Further, because of the large inter individual variability in pharmacokinetics parameters of the active S (+)-ibuprofen enantiomere expected in this population of preterm infants[30], we cannot exclude the possibility that the drug concentrations were not sufficient to significantly reduce PgE2 levels in a proportion of infants, while sufficient to mitigate the CRE elevation response to immunization. We are not aware of other studies reporting measures of PgE2 after immunization in infants.

The absence of major changes in the basal control of heart rate (measured by heart rate variability) in preterm infants post-immunization confirms the results of our previous studies [7].

The increase in entropy observed in the Ibuprofen group has also been shown to occur after oral acetaminophen treatment following immunization [7], and is usually considered to be a sign of adequate adaptability of heart rate (i.e. to the changes induced by immunization).

Several studies have shown a relationship between low gestational age, low birth weight, chronic lung disease and the occurrence of CRE [2, 3, 31] suggesting a role of cardio-respiratory control immaturity. Our study was not set to confirm these results.

Previous studies examining the effect of immunization on CRE in preterm infants did not use pneumococcal vaccine in combination with diphtheria-tetanus-acellular pertussis immunization[18, 19]. Overall, the literature reports comparable or increased antibody response to combined vaccines, including with 10-valent pneumococcal conjugate vaccine or 7-valent pneumococcal conjugate vaccine[32]. Side effects in pediatric studies such as fever and irritability are reported as similar or slightly increased with combined vaccines, while remaining within clinically acceptable ranges [33]. Accordingly, one can postulate that the combination used in the current study could be associated with stronger inflammatory response. This is supported by Pourcyrous et al.[34] reporting that a marked increase in CRP was more often present in 2 months old preterm infants who received multiple vs. single vaccine.

The major strengths of this study are the randomized placebo-controlled double blinded study design, the use of polysomnographies in addition to printed monitoring interpreted by two different blinded analysts in order to objectively assess the CRE. Limitations: Considering that at the age of 2 months, a large number of infants born at < 32 weeks of gestation had been discharged home or transferred to level II nurseries, we can postulate that the remaining infants represent sicker neonates, which limits the generalizability of current results. Despite blinded

randomization, the infants in the Ibuprofen group were one week older (post-menstrual age) at the time of the study; this difference was not statistically significant but could impact cardio-respiratory maturity and dampen the difference between groups. On the other hand, 21% in the Placebo group were on caffeine vs. 7% in the Ibuprofen group (difference not statistically significant). One limitation of the study was that despite recruiting the number of patients required according to the power analysis, the large standard deviation –especially in PgE2 levels- may have prevented detection of some significant differences. Larger studies may be required to confirm the improved cardio-respiratory outcomes of preterm infants treated with ibuprofen following their first immunization. The potential benefit of reducing CRE after immunization in young susceptible infants must be weighed against the possible impact of COX inhibition on humoral immunity in response to immunization [35]. Indeed, in one study evaluating the effectiveness of acetaminophen prophylaxis at the time of vaccination of healthy infants on fever rates, there was a reduction in antibody response to several vaccine antigens [36]. The impact of acetaminophen or COX inhibition on immunogenicity of first immunization of infants born preterm was, to our knowledge, not reported.

In conclusion, in the current study, the first immunization of infants born < 32 weeks was associated with an increase in CRP. Ibuprofen treatment significantly attenuated the variation (Δ) in CRE following first immunization in these infants but the current study could not demonstrate an impact on CRP and PgE2 levels. More prospective research is needed in order to fully understand the relationship between inflammatory response to immunization and CRE in preterm infants.

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Figure Legends

Figure 1: Establishment of study population.

Figure 2: Study design and protocol.

Table 1: Study infants and pregnancy characteristics

	Placebo (n=28)	Ibuprofen (n=28)
Infant characteristics:		
Gestational age (wk)	27.2 ± 2	27.8 ± 1.8
Birth weight (g)	972 ± 307	1048 ± 274
Sex (Male)	13	14
Small for gestational age birth weight (<10th percentile)	8	7
Respiratory Distress Syndrome	27	26
Surfactant use	19	14
Total ventilatory support (d)	42 ± 24	41 ± 30
Total invasive ventilatory support (d)	22 ± 22	22 ± 19
Patent ductus arteriosus	18	16
Pharmacological treatment	11	13
Oxygen requirement at 36 weeks post conceptional age	20	17
Necrotizing enterocolitis (grade ≥ II) ‡	2	3
Intraventricular hemorrhage (grade I and II)	5	3
Retinopathy of prematurity †	6	11
Pregnancy characteristics:		
Maternal hypertension *	6	6
Gestational diabetes	2	3
Chorioamnionitis	2	7
Betamethasone	24	24
Cesarean delivery	18	19
Singleton pregnancy	22	18
Infant status 24h prior to immunization:		
Chronological age (d)	66 ± 7	67 ± 7.7
Post menstrual age (wk)	36.4 ± 2	37.4 ± 2
Weight (g)	2565 ± 533	2865 ± 487
Caffeine (n infants)	6	2
Cardiorespiratory events (CRE)/ patient	8.6±11.1	6.7±7.7

‡ There were no surgical cases of necrotizing enterocolitis.

† Any grade of retinopathy of prematurity considering most did not have completed retinal

vasculature.

* Including chronic hypertension, gestational hypertension and preeclampsia.

Values are numbers or means ± standard deviation. No statistically significant differences between groups.

Table 2: Effect of immunization within the Placebo group (n=28)

	Before immunization	After immunization	P value
Infant characteristics			
Temperature (°C)	36.8 ± 0.2	37.1 ± 0.5	0.009
Heart rate (bpm)	153 ± 10	155 ± 10	NS
Saturation (%)	95 ± 3	95 ± 4	NS
Total CRE / patient / 24h	8.6 ± 11.1	14 ± 12.8	0.08
CRP (mg/l)	0.68 ± 1.0	20.1 ± 12.7	0.000001
PgE2 (pg/ml)	384 ± 235	396 ± 270	NS
Polysomnography analyses			
Mean (msec)	401 ± 28	393 ± 32	NS
SD (msec)	9 ± 5	9 ± 6	NS
rMSSD (msec)	5 ± 2	4 ± 2	NS
LF (103 msec2)	24 ± 26	33 ± 47	NS
HF (103 msec2)	4.9 ± 6.5	4.5 ± 4.8	NS
LF/HF	5.9 ± 3.7	6.2 ± 4.7	NS
SD1 (msec)	3.2 ± 1.6	3.1 ± 1.5	NS
SD2 (msec)	12.1 ± 6.9	12.8 ± 8.7	NS
Alpha 1	1.29 ± 0.17	1.29 ± 0.15	NS
Alpha 2	0.71 ± 0.18	0.72 ± 0.27	NS
ApEn	1.17 ± 0.23	1.13 ± 0.17	NS

bpm: beats per minute, CRE: Cardio-respiratory events, CRP: C-reactive protein, PgE2: Prostaglandin E2.

Heart rate variability analysis (See Methods): Mean: mean cardiac cycle length, SD: magnitude of global heart rate variability, rMSSD: square root of the mean squared differences of successive RR intervals, LF: low frequency, HF: high frequency, LF/HF: sympathetic/parasympathetic balance, SD1: short-term variability, SD2: short-term and long-term variability, ApEn: Approximate entropy. Values are means ± standard deviation.

Table 3: Effect of immunization within the Ibuprofen group (n=28)

	Before immunization	After immunization	P value
Infant characteristics			
Temperature (°C)	36.7 ± 0.3	37.1 ± 0.3	0.000001
Heart rate (bpm)	150 ± 12	155 ± 10	NS
Saturation (%)	96 ± 3	96 ± 3	NS
Total CRE / patient / 24h	6.7 ± 7.7	6.8 ± 9.7	NS
CRP (mg/l)	1.1 ± 3.0	16.6 ± 9.7	0.000001
PGE2 (pg/ml)	473 ± 285	355 ± 272	NS
Polysomnography analyses			
Mean (msec)	397 ± 30	384 ± 27	0,10
SD (msec)	9.9 ± 5.8	8.1 ± 4.7	NS
rMSSD (msec)	4.8 ± 1.9	4.5 ± 2.1	NS
LF (103 msec2)	32 ± 40	23 ± 43	NS
HF (103 msec2)	4.4 ± 4.0	3.8 ± 4.0	NS
LF/HF	6.5 ± 5.5	5.6 ± 3.7	NS
SD1 (msec)	3.4 ± 1.3	3.2 ± 1.5	NS
SD2 (msec)	13.6 ± 8.2	11.0 ± 6.6	NS
Alpha 1	1.30 ± 0.13	1.30 ± 0.13	NS
Alpha 2	0.75 ± 0.25	0.65 ± 0.20	NS
ApEn	1.18 ± 0.17	1.25 ± 0.12	0.03

bpm: beats per minute, CRE: Cardio-respiratory events, CRP: C-reactive protein, PGE2: Prostaglandin E2.

Heart rate variability analysis (See Methods): Mean: mean cardiac cycle length, SD: magnitude of global heart rate variability, rMSSD: square root of the mean squared differences of successive RR intervals, LF: low frequency, HF: high frequency, LF/HF: sympathetic/parasympathetic balance, SD1: short-term variability, SD2: short-term and long-term variability, ApEn: Approximate entropy. Values are means ± standard deviation.

Table 4: Comparison of changes observed after immunization in infants who received Placebo or Ibuprofen

	Δ Pre/post immunization		
	Placebo	Ibuprofen	P value
Infant characteristics			
Δ Temperature (°C)	0.3 ± 0.4**	0.3 ± 0.4**	NS
Δ Heart rate (bpm)	3 ± 12	5 ± 9	NS
Δ Saturation (%)	0 ± 2	0 ± 3	NS
Δ Total CRE / patient / 24h	5.4 ± 10.0	0.1 ± 8.0	0.03
Δ CRP (mg/l)	19 ± 13**	16 ± 9**	NS
Δ PgE2 (pg/ml)	12 ± 304	-118 ± 355	NS
Polysomnography analyses			
Δ Mean (msec)	-8 ± 28	-10 ± 36	NS
Δ SD (msec)	1 ± 7	-1 ± 7	NS
Δ rMSSD (msec)	0 ± 2	1 ± 7	NS
Δ LF (10^3 $msec^2$)	8 ± 47	-4 ± 56	NS
Δ HF (10^3 $msec^2$)	0 ± 4	-1 ± 4	NS
Δ LF/HF	0.2 ± 5	-0.9 ± 4.9	NS
Δ SD1 (msec)	-0.1 ± 1.3	0.9 ± 5.5	NS
Δ SD2 (msec)	0.7 ± 9.1	-1.9 ± 9.2	NS
Δ Alpha 1	0 ± 0.15	-0.03 ± 0.19	NS
Δ Alpha 2	0 ± 0.28	-0.11 ± 0.33	NS
ΔApEn	-0.03 ±0.3	0.08 ± 0.16*	0.08

Significant increase within the group: * $p<0.05$; ** $p<0.001$

bpm: beats per minute, CRE: Cardio-respiratory events, CRP: C-reactive protein, PgE2: Prostaglandin E2.

Heart rate variability analysis (See Methods): Mean: mean cardiac cycle length, SD: magnitude of global heart rate variability, rMSSD: square root of the mean squared differences of successive RR intervals, LF: low frequency, HF: high frequency, LF/HF: sympathetic/parasympathetic balance, SD1: short-term variability, SD2: short-term and long-

term variability, ApEn: Approximate entropy.

Values are means ± standard deviation.

Figure 1

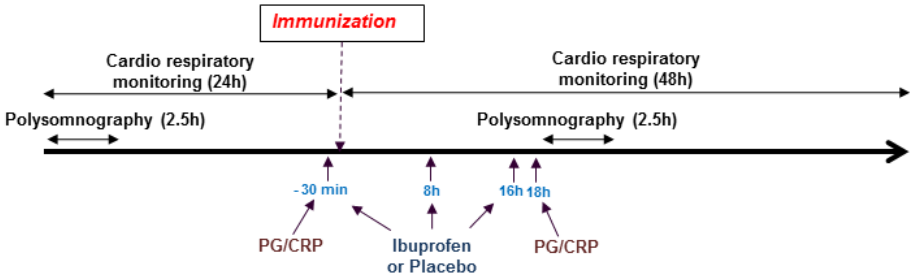
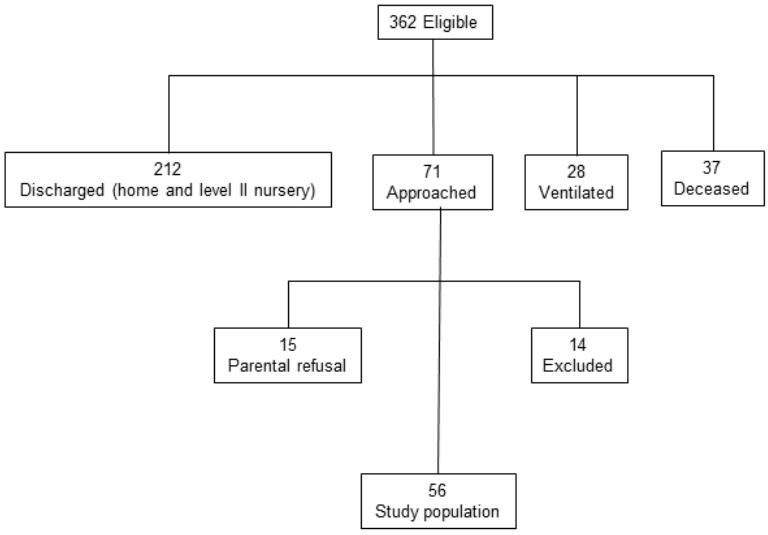

Immunization
Cardio respiratory monitoring (24h)
Cardio respiratory monitoring (48h)
Polysomnography (2.5h)
Polysomnography (2.5h)
-30 min
8h
16h
18h
PG/CRP
Ibuprofen or Placebo
PG/CRP

Figure 2


362 Eligible
212
Discharged (home and level II nursery)
71
Approached
28
Ventilated
37
Deceased
15
Parental refusal
14
Excluded
56
Study population