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Short-term cardiac autonomic responses to primary immunization in hospitalized term and preterm infants: the prospective PVAD study

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Abstract

Background: Hospitalized preterm infants may experience transient apnea, bradycardia, or oxygen desaturation after routine immunization, but whether vaccination is accompanied by a broader alteration in cardiac autonomic regulation remains uncertain.

Methods: In this prospective single-center observational study, hospitalized term and preterm infants receiving primary hexavalent and 13-valent pneumococcal immunizations underwent continuous electrocardiographic recording from 24 h before to 48 h after vaccination. Linear and nonlinear heart rate variability indices were calculated over six consecutive 12-h windows. Overall temporal changes were assessed using Friedman tests. Prespecified paired

comparisons between each post-immunization window and the -12 to 0 h reference window used Wilcoxon signed-rank tests with Holm-Bonferroni correction.

Results: Twenty-five infants were enrolled and 21 had analyzable recordings; 18 were born before 32 weeks of gestation. No heart rate variability index showed a significant overall temporal change or a significant difference from the reference window after multiplicity correction. High-frequency power decreased from $85.05 \pm 100.39 \text{ ms}^2$ before immunization to $49.86 \pm 46.63 \text{ ms}^2$ at 0-12 h (adjusted $p=0.5271$) and $35.07 \pm 24.77 \text{ ms}^2$ at 12-24 h (adjusted $p=0.1643$).

Conclusions: Primary immunization was not associated with a detectable sustained short-term alteration in cardiac autonomic regulation. These findings are reassuring but do not exclude brief responses or effects confined to clinically vulnerable subgroups.

Keywords: autonomic nervous system; heart rate variability; immunization; infant; preterm infant; apnea; neonatal intensive care

Background

Preterm infants should generally receive routine vaccines according to chronological rather than corrected age, because delayed immunization prolongs vulnerability to vaccine-preventable disease. Contemporary reviews indicate that penta- and hexavalent vaccines are immunogenic and generally well tolerated in preterm infants, while also acknowledging a higher frequency of transient apnea, bradycardia, and oxygen desaturation after immunization than in term infants [1, 2]. This distinction is clinically important: vaccination remains strongly indicated, yet the optimal selection and duration of cardiorespiratory monitoring for infants who remain hospitalized at the time of their first doses are still debated.

Recent evidence has sharpened this question. In a multicenter randomized clinical trial of hospitalized infants born before 33 weeks, apnea within 48 h occurred more often after 2-month vaccination than during an unvaccinated observation period. However, the number and

1 duration of episodes did not differ, and no serious adverse events occurred [3]. Observational
2 studies similarly identify lower gestational age, ongoing respiratory support,
3 bronchopulmonary dysplasia, baseline cardiorespiratory instability, and caffeine exposure as
4 markers of higher post-immunization risk [4-6]. These findings support vaccination on
5 schedule while arguing against a uniform interpretation of post-vaccination risk across all
6 preterm infants.

7 The mechanisms underlying these transient events remain incompletely defined. Prematurity
8 is associated with delayed maturation of autonomic cardiac control, reduced complexity of
9 heart-rate dynamics, and altered cardiorespiratory coupling [7-9]. Vaccination produces a
10 controlled innate immune response, and the autonomic nervous system interacts
11 bidirectionally with inflammation through afferent immune sensing and efferent cholinergic
12 and adrenergic pathways [10]. Across inflammatory and septic states, reduced global heart
13 rate variability (HRV) and signal complexity are frequently observed. However, these
14 associations are not specific to vagal indices and are influenced by heart rate, respiration,
15 sleep state, treatment, and illness severity [11, 12]. In adults, short-lived HRV changes have
16 been reported after vaccination, but these observations cannot be directly extrapolated to
17 neonates [13].

18 Clinical cardiorespiratory event counts capture overt instability but may miss subclinical
19 shifts in autonomic adaptation. Conversely, HRV may help distinguish a broad autonomic
20 response from isolated apnea related to respiratory immaturity. Data combining prolonged
21 pre- and post-immunization electrocardiographic recordings with linear and nonlinear HRV
22 analysis in infants are scarce. We therefore conducted the Post-Vaccination Acquired
23 Dysautonomia (PVAD) study to determine whether primary immunization is followed by
24 measurable changes in cardiac autonomic regulation in hospitalized term and preterm infants.

We hypothesized that a vaccine-related inflammatory response would transiently reduce global and vagally mediated HRV during the first 24 h after immunization.

Methods

Study design and setting

PVAD was a prospective, single-center, non-invasive observational study conducted in the neonatal unit and neonatal intensive care unit of Saint-Etienne University Hospital, France, from March 8 to September 9, 2023. The report follows the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) recommendations.

Participants and immunization

All hospitalized term or preterm infants scheduled to undergo primary immunization during the study period were eligible. Infants received one dose of a 13-valent pneumococcal conjugate vaccine (Prevenar 13) together with a hexavalent diphtheria-tetanus-acellular pertussis-inactivated poliovirus-Haemophilus influenzae type b-hepatitis B vaccine (Hexyon or Infanrix hexa). Infants were excluded in the presence of hypoxic-ischemic encephalopathy, clinical evidence of sepsis, or treatment known to interfere directly with autonomic regulation, including beta-blockers.

Sample-size rationale

No formal a priori sample-size calculation was performed because no directly comparable neonatal HRV data were available to support a reliable effect-size estimate. The study was exploratory, and its pragmatic sample size was determined by the number of eligible hospitalized infants undergoing primary immunization during the predefined six-month recruitment period and by the feasibility of obtaining complete 72-h recordings.

ECG acquisition and analysis windows

Electrocardiographic (ECG) signals were acquired continuously for 72 h, beginning 24 h before and ending 48 h after immunization, using an IntelliVue MX550 monitor (Philips, the Netherlands) and three thoracic electrodes. Signals were transferred through the central

monitoring system to a dedicated acquisition computer and backed up every 24 h. Recordings were exported in .rf format with Zymed software (Philips) and analyzed offline using HRVanalysis software developed in the research laboratory in MATLAB R2016a (MathWorks, Natick, MA, USA).

The recording was divided into six consecutive 12-h windows: -24 to -12 h, -12 to 0 h, 0 to +12 h, +12 to +24 h, +24 to +36 h, and +36 to +48 h relative to vaccination. The prespecified reference window was -12 to 0 h. The preceding -24 to -12 h window was retained as an additional pre-immunization description and internal assessment of temporal variability.

Heart rate variability outcomes

After beat detection and artifact handling, HRV was characterized across complementary domains. Time-domain outcomes were heart rate, the standard deviation of normal-to-normal intervals (SDNN), the root mean square of successive differences (rMSSD), and the percentage of successive normal-to-normal intervals differing by >20 ms (pNN20). Frequency-domain outcomes were total power, low-frequency (LF) power, high-frequency (HF) power, and the LF/HF ratio. Because neonatal respiratory frequencies extend above adult HF limits, the HF band was defined as 0.15-2.0 Hz and LF as 0.04-0.15 Hz. Poincaré-plot indices comprised SD1, SD2, and SD1/SD2. Nonlinear scaling was assessed using short-term and long-term detrended fluctuation analysis exponents (α_1 -DFA and α_2 -DFA) and the Hurst-related DFA index (H-DFA). Interpretations of LF and LF/HF were deliberately cautious because neither is a pure measure of sympathetic activity, particularly in neonates and during non-stationary recordings [14].

Clinical variables

Recorded variables included sex, gestational age, birth weight, birth-weight percentile, small-for-gestational-age status, postnatal age at immunization, respiratory support, caffeine therapy, bronchopulmonary dysplasia severity, and cerebral hemorrhage.

Ethics approval and consent to participate

The protocol was reviewed by the Ethics Committee of Saint-Etienne University Hospital at its plenary meeting on March 8, 2023, and received a favorable opinion on April 5, 2023 (IRBN472023/CHUSTE; Institutional Review Board IORG0007394). The study involved human participants and human physiological data and was conducted in accordance with the Declaration of Helsinki and with all applicable guidelines and regulations. As the study involved minors, written informed consent to participate was systematically obtained before inclusion from the parents or legal guardians of each participant by signature of the participant information sheet.

Statistical analysis

Categorical variables were summarized as counts and percentages. Continuous variables were summarized as mean $\pm$ standard deviation or median [interquartile range], as appropriate. Distributional normality was assessed using the Shapiro-Wilk test. Because several HRV variables were not normally distributed and repeated measurements were obtained from the same infants, overall changes across the six consecutive 12-h periods were assessed using Friedman tests. Prespecified within-infant comparisons between each of the four post-immunization periods and the -12 to 0 h reference period were performed using paired Wilcoxon signed-rank tests. For each HRV variable, the four comparison p values were adjusted using the sequential Holm-Bonferroni procedure to control the family-wise error rate. Multiplicity adjustment was applied within each HRV variable across time-point comparisons but not across the different HRV variables because the analyses were exploratory and no single primary HRV endpoint had been prespecified. All tests were two-sided, and adjusted p values <0.05 were considered statistically significant. Statistical analyses were performed in MATLAB R2016a (MathWorks, Natick, MA, USA).

Results

Study population

Twenty-five infants were enrolled. Four ECG recordings were excluded because of connection failures, leaving 21 infants with analyzable recordings. Twelve (57%) were male. Nine infants (43%) were born extremely preterm, nine (43%) very preterm, two (10%) moderate-to-late preterm, and one (5%) at term. Median gestational age was 28 weeks + 1 day, and median birth weight was 1080 g. At immunization, median postnatal age was 60 days. Ten infants (48%) were receiving non-invasive respiratory support and caffeine; none was mechanically ventilated. Eighteen (86%) had bronchopulmonary dysplasia, and 10 (48%) had cerebral hemorrhage (Table 1).

Heart rate variability

None of the HRV indices showed a statistically significant overall change across the six recording periods, and no prespecified comparison between the reference period and a post-immunization period was significant after Holm-Bonferroni correction (Table 2). Heart rate increased numerically during the first 24 h and subsequently decreased, but comparisons with the reference window did not reach significance. Global HRV, represented by total power and SDNN, showed no consistent post-immunization reduction. Vagally weighted short-term indices decreased numerically after vaccination. HF power was $85.05 \pm 100.39 \text{ ms}^2$ during the reference window, $49.86 \pm 46.63 \text{ ms}^2$ at 0-12 h (adjusted $p=0.5271$), and $35.07 \pm 24.77 \text{ ms}^2$ at 12-24 h (adjusted $p=0.1643$). Corresponding changes in rMSSD, pNN20, and SD1 were not significant. LF power and the LF/HF ratio were also unchanged relative to baseline. Poincaré-plot and DFA indices provided no evidence of an acute alteration in autonomic complexity. Trajectories of heart rate, total power, and HF power across the full 72-h recording are shown in Figure 1.

Discussion

Principal findings

In this prospective study of continuous ECG recordings surrounding primary immunization, we found no statistically significant change in time-domain, frequency-domain, Poincaré, or fractal HRV indices during the 48 h after vaccination. The numerical fall in HF power and related short-term indices during the first 24 h was not statistically significant and recovered only partially thereafter. Given the small sample and high between-infant dispersion, these data are compatible with both no meaningful autonomic effect and a modest, transient response that the study was underpowered to detect.

The study addresses a different endpoint from post-immunization apnea trials. The recent randomized trial by Greenberg et al. demonstrated increased odds of at least one apnea event within 48 h among vaccinated hospitalized preterm infants, but no excess in event duration, positive-pressure ventilation, or serious adverse events [3]. Our absence of a detectable group-level HRV shift does not contradict that result. Apnea may arise in a susceptible subset, whereas HRV averaged across 12-h windows and across a heterogeneous cohort may dilute brief or subject-specific autonomic perturbations. Moreover, respiratory control immaturity, airway mechanics, sleep state, and treatment may influence apnea without producing a sustained change in the selected cardiac indices.

Relationship to inflammation and autonomic regulation

The biological rationale for PVAD was the bidirectional interaction between innate immunity and autonomic regulation. Systemic inflammation and sepsis are generally associated with reduced total HRV and reduced complexity, but the pattern is broad rather than selectively parasympathetic [11]. Experimental neonatal data also suggest that an inflammation-associated reduction in HRV may be substantially mediated by the accompanying rise in heart rate [12]. In contrast, routine vaccination evokes a smaller and self-limited inflammatory response. A prior randomized study in preterm infants reported increased C-reactive protein

1 levels and cardiorespiratory events after vaccination, but HRV findings were insufficient to
2 establish a causal autonomic pathway [15]. Our results therefore suggest that any
3 inflammatory-autonomic effect of routine primary vaccination is smaller than the
4 dysregulation observed during clinically significant infection, at least when assessed over 12-
5 h windows.

6 The numerical reduction in HF power deserves cautious interpretation. HF power, rMSSD,
7 pNN20, and SD1 emphasize short-term beat-to-beat variability and are influenced by vagal
8 modulation, but they also depend on respiratory pattern, heart rate, sleep state, and signal
9 stationarity. Almost half of the cohort received non-invasive respiratory support and caffeine,
10 both of which can modify cardiorespiratory dynamics [16]. The first post-vaccination night
11 also overlapped with circadian and sleep-stage changes that can alter HRV [17]. Without
12 synchronized data on respiratory status, sleep state, temperature, and inflammatory
13 biomarkers, the observed trajectories cannot be specifically attributed to vaccine-induced
14 inflammation.

15 **Clinical implications**

16 The results are reassuring regarding sustained cardiac autonomic dysregulation after primary
17 immunization. They support timely vaccination of hospitalized preterm infants and provide no
18 evidence that routine immunization results in a sepsis-like loss of HRV or complexity.
19 However, they should not be used to conclude that post-vaccination monitoring is universally
20 unnecessary. Recent randomized and observational evidence supports a transient increase in
21 apnea or other cardiorespiratory events among some hospitalized preterm infants, particularly
22 those with ongoing respiratory support or baseline instability [3-6]. Monitoring decisions
23 should therefore remain based on gestational maturity, postmenstrual age, current respiratory
24 status, recent apnea, and local guidance rather than on the present HRV findings alone.

Strengths and limitations

The principal strength was prolonged within-infant ECG acquisition beginning 24 h before vaccination and continuing for 48 h afterward, enabling each infant to serve as their own reference. The combined use of conventional and nonlinear indices provided a broader assessment than heart rate or event counts alone. The cohort also reflects the clinically relevant population of very preterm infants who remain hospitalized at the time of routine immunization.

Several limitations constrain interpretation. First, the pragmatic sample comprised only 21 analyzable infants from a single center and was insufficient for adjusted or subgroup analyses. Second, the cohort was heterogeneous with respect to gestational age, bronchopulmonary dysplasia, respiratory support, and caffeine exposure. Third, no unvaccinated concurrent control group was available; secular changes in sleep, care, respiratory support, and maturation could therefore mimic or obscure vaccine-related effects. Fourth, 12-h averaging may mask brief autonomic changes around fever, apnea, or desaturation. Fifth, inflammatory biomarkers, temperature trajectories, respiratory signals, sleep stage, and prospectively adjudicated cardiorespiratory events were not integrated into HRV analyses. Sixth, the large number of HRV indices increased the overall risk of multiplicity beyond the within-index Holm-Bonferroni correction. Finally, although distribution-appropriate tests were used, mean $\pm$ standard deviation summaries may not optimally describe right-skewed spectral measures and were retained to remain consistent with the prespecified results dataset.

Future research

A multicenter study should combine beat-level ECG with respiratory inductance, oxygen saturation, sleep state, temperature, and standardized apnea definitions and obtain inflammatory biomarkers at biologically relevant time points. Mixed-effects models could quantify within-infant trajectories while accounting for heart rate, gestational and postmenstrual age, respiratory support, caffeine, and baseline instability. Event-triggered

HRV analyses around apnea and desaturation may be more sensitive than fixed 12-h averages. Such work could test whether an autonomic signature identifies the small subset of infants who benefit most from extended observation, without delaying vaccination or unnecessarily prolonging hospitalization.

Conclusions

In this small prospective cohort, primary hexavalent and pneumococcal immunizations were not associated with a detectable sustained short-term change in cardiac autonomic regulation, as assessed by linear and nonlinear HRV. The findings argue against a sustained sepsis-like autonomic disturbance but do not exclude brief or subgroup-specific responses. They complement, rather than replace, clinical evidence on transient post-immunization apnea in hospitalized preterm infants.

Declarations

Abbreviations

BPD: bronchopulmonary dysplasia; DFA: detrended fluctuation analysis; ECG: electrocardiogram/electrocardiographic; H-DFA: Hurst-related detrended fluctuation analysis index; HF: high frequency; HRV: heart rate variability; LF: low frequency; NICU: neonatal intensive care unit; pNN20: percentage of successive normal-to-normal intervals differing by >20 ms; PVAD: Post-Vaccination Acquired Dysautonomia; rMSSD: root mean square of successive differences; SDNN: standard deviation of normal-to-normal intervals.

Ethics approval and consent to participate

The protocol was reviewed by the Ethics Committee of Saint-Etienne University Hospital at its plenary meeting on March 8, 2023, and received a favorable opinion on April 5, 2023 (IRBN472023/CHUSTE; Institutional Review Board IORG0007394). The study involved human participants and human physiological data and was conducted in accordance with the Declaration of Helsinki and with all applicable guidelines and regulations. As the study

involved minors, written informed consent to participate was systematically obtained before inclusion from the parents or legal guardians of each participant by signature of the participant information sheet.

Consent for publication

Not applicable. The manuscript does not contain identifiable individual participant data.

Availability of data and materials

The pseudonymized datasets generated and/or analyzed during the current study are not publicly available because they contain clinical data from a small cohort of hospitalized infants and public release was not covered by the ethics approval. They may be available from the corresponding author on reasonable request, subject to approval by the research team and applicable ethical, regulatory, and contractual requirements.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

FS contributed to investigation and preparation of the original draft. VP contributed to methodology, physiological analysis, statistical analysis, and review and editing of the manuscript. HP contributed to conceptualization, methodology, investigation, statistical analysis, supervision, and review and editing of the manuscript. All authors read and approved the final manuscript.

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Figure legend

Figure 1. Heart rate and heart rate variability trajectories around primary immunization. Trajectories of heart rate, total power, and high-frequency power across the 72-h recording. Points and error bars represent mean $\pm$ standard deviation for each consecutive 12-h interval. The vertical dashed line denotes vaccination at time 0.

Table 1. Participant characteristics

Characteristic	Total (N=21)
Male sex	12 (57%)
Extremely preterm (<28 weeks)	9 (43%)
Very preterm (28 to <32 weeks)	9 (43%)
Moderate-to-late preterm (32 to <37 weeks)	2 (10%)
Term ($\geq$ 37 weeks)	1 (5%)
Gestational age at birth, mean [median]	28 weeks + 3 days [28 weeks + 1 day]
Birth weight, g, mean [median]	1193 [1080]
Birth-weight percentile, mean [median]	45 [48]
Small for gestational age (<10th percentile)	5 (24%)
Postnatal age at immunization, days, mean [median]	58 [60]
Invasive mechanical ventilation	0 (0%)
Non-invasive respiratory support	10 (48%)
Caffeine therapy	10 (48%)
Bronchopulmonary dysplasia	18 (86%)
Mild	7 (33%)
Moderate	4 (19%)
Severe	7 (33%)
Cerebral hemorrhage	10 (48%)

Values are n (%) unless otherwise indicated.

1 **Table 2. Heart rate variability before and after primary immunization**

Index	-24/-12 h	-12/0 h	0/+12 h	+12/+24 h	+24/+36 h	+36/+48 h	Adj. p 0/+12 h	Adj. p 0/+24 h	Adj. p 0/+36 h	Adj. p 0/+48 h
Heart rate (bpm)	152.8±10.7	150.7±10.2	155.8±10.5	155.8±7.5	149.5±8.8	146.7±7.3	0.0778	0.0755	0.9863	0.2809
Total power (ms ²)	652.7±347.5	906.7±451.5	787.5±337.2	691.9±297.4	958.7±572.0	836.6±319.4	0.9548	0.6393	0.9990	0.9958
LF power (ms ²)	133.48±76.35	199.35±141.71	184.56±108.30	136.35±63.41	180.16±113.74	194.71±95.44	0.9983	0.5003	0.9943	1.0000
HF power (ms ²)	42.19±47.91	85.05±100.39	49.86±46.63	35.07±24.77	47.09±37.69	48.74±35.43	0.5271	0.1643	0.4430	0.4926
LF/HF	5.16±1.90	4.90±2.18	5.69±2.73	4.90±2.06	4.82±1.71	5.15±1.82	0.5557	1.0000	1.0000	0.9949
SDNN (ms)	27.31±6.51	31.08±6.18	27.89±6.25	27.29±6.82	31.42±9.00	30.42±5.52	0.5643	0.3754	1.0000	0.9993
rMSSD (ms)	10.97±3.96	14.85±6.65	12.41±4.88	12.28±6.49	12.46±3.97	13.30±4.80	0.8027	0.7664	0.8164	0.9653
pNN20 (%)	6.54±5.24	11.79±8.69	7.63±5.11	8.84±9.49	9.85±8.44	10.63±9.57	0.7263	0.9173	0.9859	0.9987
SD1 (ms)	7.33±2.13	9.83±3.86	8.04±2.54	8.36±3.94	8.44±2.57	8.90±3.01	0.6045	0.7807	0.8150	0.9615
SD2 (ms)	35.15±7.01	37.22±6.08	35.28±8.54	35.80±8.69	40.34±10.70	38.88±6.67	0.9439	0.9854	0.6964	0.9711
SD1/SD2	0.22±0.05	0.28±0.12	0.24±0.08	0.25±0.11	0.23±0.12	0.25±0.11	0.8182	0.9066	0.6511	0.8609
alpha1-DFA	1.20±0.20	1.13±0.25	1.16±0.22	1.16±0.27	1.19±0.30	1.20±0.32	0.9878	0.9910	0.7703	0.6566
alpha2-DFA	1.16±0.05	1.12±0.09	1.14±0.06	1.15±0.08	1.14±0.05	1.14±0.04	0.9702	0.8612	0.9409	0.9832
H-DFA	1.03±0.08	0.99±0.08	1.00±0.07	1.01±0.07	1.02±0.05	0.97±0.06	0.9956	0.9669	0.7961	0.9635

2 Values are mean ± standard deviation. The -12 to 0 h interval was the prespecified reference window. Overall temporal changes were assessed using
3 Friedman tests. Adjusted p values compare the reference window with each post-immunization interval using paired Wilcoxon signed-rank tests with
4 Holm-Bonferroni correction across the four comparisons performed for each HRV variable. DFA, detrended fluctuation analysis; HF, high frequency;
5 LF, low frequency; pNN20, percentage of successive normal-to-normal intervals differing by >20 ms; rMSSD, root mean square of successive
6 differences; SDNN, standard deviation of normal-to-normal intervals.

