Pointwise Hypothesis Testing of Biomedical Near-Infrared Spectroscopy Signals

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Abstract. This study uses a pointwise statistical approach to analyze Near-Infrared Spectroscopy (NIRS) signals in preterm infants with and without Patent Ductus Arteriosus (PDA). Three oxygenation signals—SpO_2, rSO_2-1 (cerebral), and rSO_2-2 (renal)—were analyzed across no-PDA, PDA, and hsPDA groups. Smoothed signals were tested using pointwise ANOVA and Tukey HSD to detect significant group differences. Results showed distinct patterns in rSO_2-1 and rSO_2-2, with the hsPDA group standing out in rSO_2-1 and the no-PDA group in rSO_2-2, demonstrating the value of this method in biomedical signal analysis.

Keywords: Biomedical Near-Infrared Spectroscopy Signals, Pointwise Data Analysis, Functional Data Analysis, Pointwise Hypothesis Testing, Pointwise ANOVA, Pointwise post-hoc Tukey's HSD Test

1 Introduction

Analyzing biomedical signals like Near-Infrared Spectroscopy (NIRS) is challenging due to their continuous nature, high dimensionality, and clinical noise. NIRS, a non-invasive method for monitoring tissue oxygenation [1], is particularly relevant in the context of Patent Ductus Arteriosus (PDA)—a condition where a fetal blood vessel fails to close after birth, potentially leading to serious complications [2]. Since PDA affects tissue oxygenation, NIRS data can offer valuable diagnostic insights.

However, most existing studies simplify NIRS signals to discrete averages, often missing time-based patterns essential for clinical interpretation. This study aims to address that gap by smoothing noisy NIRS signals and applying pointwise hypothesis testing to detect statistically significant differences in oxygenation patterns between preterm infants with different PDA statuses.

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2 Related Works

Previous studies on NIRS in neonatology have mainly compared average oxygenation values between PDA groups. Van der Laan et al. used non-parametric tests and found no significant differences [3], while others, including Schwarz and Navikienė, applied ANOVA-based methods and identified some group-level differences, particularly involving the hsPDA group [4, 5]. However, these approaches often rely on summary statistics, missing time-specific patterns in the signals.

Functional Data Analysis (FDA) has been proposed as a more suitable framework for continuous biomedical data, with applications like functional PCA and canonical correlation offering richer temporal insights [6]. Still, its use in clinical research remains limited. This study builds on that gap by using pointwise testing on smoothed NIRS signals to capture interpretable, time-localized differences between groups.

3 Dataset and Methodology

Dataset

Study was carried out in the tertiary-level neonatal intensive care unit of the Neonatology Center, Vilnius University Hospital Santaros Klinikos, from 2017 November to 2020 June. The study was approved by the Vilnius Regional Biomedical Research Ethics Committee (No.158200-17-940-446, issued on 2017 September 12th). And registered at clinicaltrials.com (reg. No NCT04295395). Informed parental consent was obtained before enrolment.

Infants included in the study were very-low-birth-weight (<1500 g), born at <32 weeks gestation, and at least 72 hours old. NIRS measurements were taken using the NONIN SenSmart X-100 system with neonatal sensors (8004CB-NA, EQUANOX™). Sensors were placed on the forehead for cerebral and on the lower back for renal oxygenation. Recordings were continuous over 12 hours, with brief repositioning every 3 hours to prevent skin irritation.

Using NIRS technology, 3 signals had been measured:

- Cerebral oxygenation levels (rSO 2-1);
- Renal oxygenation levels (rSO 2-2);
- Blood oxygenation (SpO 2).

Table 1. Newborn groups description

Group	Description	Number of patients
no-PDA	Newborns with closed ductus arteriosus therefore without PDA	63
PDA	Newborns with heamodynamically insignificant patent ductus arteriosus thus without treatment	41
hsPDA	Newborns with heamodynamically significant patent ductus arteriosus thus with pharmacological treatment	20

Data Preparation

Firstly data had to be prepared. The dataset consisted of continuously collected medical data from preterm newborns, which inevitably included missing values (NAs) due to factors such as signal interruptions or technical issues. To address these missing values, a two-step approach was applied:

- signals with 30% or more NA values for a specific patient were excluded
- signals with less than 30% missing data were imputed using interpolation – for NAs within the signal linear interpolation was used, for NAs at the beginning of the signal the first observed value was carried backward to fill in missing values, for NAs at the end of the signal the last observed value was carried forward to fill in missing values.

Moreover, any oxygenation values lower than 20 were replaced with 20, ensuring that the data aligned with the physiological expectations.

Functional Data Representation and Smoothing

Since NIRS signals were collected every 1 minute over a 12-hour period, Functional Data Analysis (FDA) framework was adopted to transform these high-frequence, discrete observations into smooth, continuous functions. This approach enables more robust statistical analysis by preserving temporal dependencies and reducing noise. For this, B-spline basis functions were used to represent each discrete signal as continuous curve. B-spline basis functions are particularly suited for modeling non-periodic physiological data due to its flexibility and computational efficiency [7]. Each discrete signal is represented using B-spline basis functions:

$$x(t) \approx \sum_{m=1}^{M} c_k \phi_k(t), \tag{1}$$

where $\phi_m(t)$ are the B-spline basis functions, c_m are the corresponding coefficients, and M is the number of basis functions. The choice of M controls the smoothness of the function: higher values provide flexibility, while lower enforce smoother trends.

To determine the optimal number of basis functions and the degrees of smoothness, Generalized Cross-Validation (GCV) was used:

$$GCV = \frac{n \cdot SSE}{(n - df)^2}, \tag{2}$$

where *SSE* is the sum of squared errors, *n* is the number of observations, and *df* is the effective degrees of freedom. Then the elbow method together with optim.basis function from fda.usc library [8] were used to determine the optimal number of basis functions.

Hypothesis testing

Pointwise ANOVA

To detect localized differences in NIRS signals across groups, pointwise Analysis of Variance (ANOVA) was applied. Although it does not analyze curves as a whole, it provides valuable insights into the differences between data groups and the statistical significance of those differences [9]. This method checks the null hypothesis that all groups have the same mean value at each time *t* against the alternative hypothesis that at least one pair of data groups has different mean values at time *t*. Null and alternative hypothesis can be expressed as:

$$\begin{aligned} &H_0 : \mu_2(t) = \ldots = \mu_k(t) \\ &H_1 : \exists i, j \; such \; that \; \mu_i(t) \neq \mu_j(t) \end{aligned} \tag{3}$$

To test hypothesis at each time, the total sum of squares (*SST*) is calculated by summing between group sum of squares (*SSB*) and the within-group sum of squares (*SSW*), where:

$$SSB(t) = \sum_{j=1}^{k} n_j \left(\overline{Y}_j(t) - \overline{Y}(t)\right)^2, \tag{4}$$

where $\overline{Y_j}(t) = \frac{1}{n_j}\sum_{i=1}^{n_j} Y_{ij}(t)$ is the mean for group j at the time t and $SSW(t) = \sum_{j=1}^{k}\sum_{i=1}^{n_j}\left(Y_{ij}(t) - \overline{Y_j}(t)\right)^2$.

The F-statistic is then calculated at every time t as:

$$F(t) = \frac{MSB(t)}{MSW(t)}, \tag{5}$$

where $MSB(t) = SSB(t)/(k - 1)$ is the mean square between groups, $MSW(t) = SSW(t)/(N - k)$ is the mean square within groups.

This means that the F-statistic compares the ratio of variance explained by group differences to the variance due to random error. By comparing $F(t)$ to the F-distribution, the p-value and significance of the difference at each time t are determined.

Pointwise Post Hoc Analysis – Tukey HSD Test

To investigate pairwise differences between groups, Tukey's Honest Significant Difference (HSD) test is used as a post hoc analysis. This pointwise test evaluates whether the mean values of two groups differ significantly at each time point t. The difference is defined as:

$$\Delta_{k_1,k_2}(t) = \left|\overline{Y_{k_1}}(t) - \overline{Y_{k_2}}(t)\right|, \tag{6}$$

Where $\overline{Y_{k_1}}(t)$ and $\overline{Y_{k_2}}(t)$ are the mean values of groups k_1 and k_2 at the time t, respectively.

Tukey HSD test calculates the critical value for pairwise comparisons using the studentized range distribution. This calculation incorporates the number of groups, the total number of observations, and the mean square within groups (obtained from the ANOVA calculation). The test identifies statistically significant differences between group pairs at each time point, providing a more granular view of group-level differences over time.

4 Results and Discussion

NIRS signals were smoothed using B-spline basis functions to reduce noise and prepare the data for statistical analysis. The optimal number of basis functions was selected for each signal and group using Generalized Cross-Validation (GCV) and validated with the Elbow method.

The number of basis functions varied depending on the signal complexity: hsPDA groups required fewer functions (12–16), while no-PDA groups showed more variability, requiring up to 57. This reflects differences in signal structure across groups.

Figures 1–3 show raw (colored) and smoothed (black) curves for each group and signal: SpO_2, rSO_2-2, and rSO_2-1. The smoothing clearly reduced measurement noise while preserving key physiological trends.

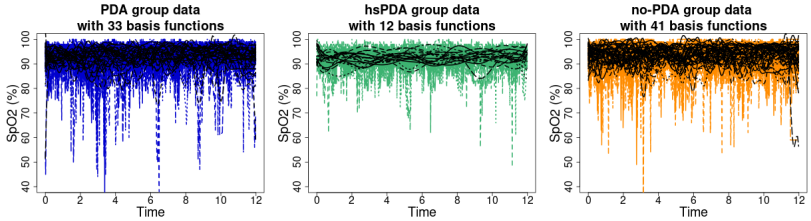

Fig. 1. Raw SpO_2 (blue, green and orange) signals and smoothed curves (black) in newborn groups (PDA, hsPDA, no-PDA): X-axis represents time (hours), and Y-axis represents blood oxygenation levels

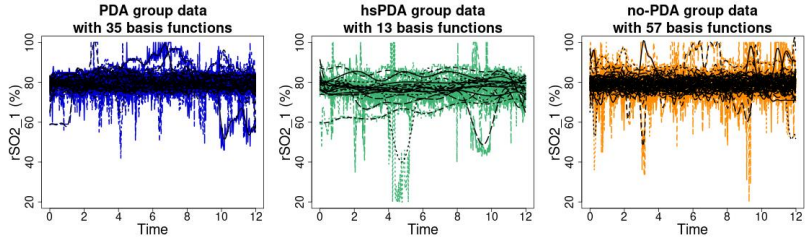

Fig. 2. Raw rSO_2-1 (blue, green and orange) signals and smoothed curves (black) in newborn groups (PDA, hsPDA, no-PDA): X-axis represents time (hours), and Y-axis represents blood oxygenation levels

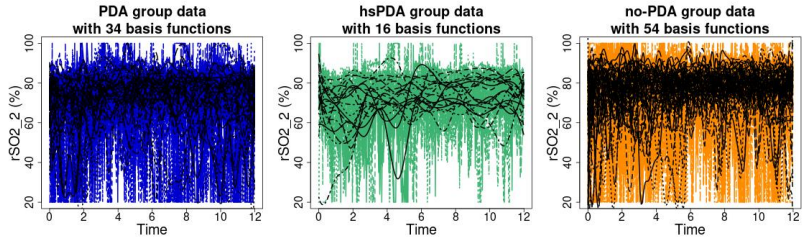

Fig. 3. Raw rSO_2-2 (blue, green and orange) signals and smoothed curves (black) in newborn groups (PDA, hsPDA, no-PDA): X-axis represents time (hours), and Y-axis represents blood oxygenation levels

To identify statistically significant differences in oxygenation signals between newborn groups, pointwise ANOVA and Tukey HSD post hoc tests were applied to the smoothed signals.

As shown in Figure 4, pointwise ANOVA for SpO_2 (blood oxygenation) revealed mostly non-significant differences across groups, with *p*-values rarely falling below the 0.05 threshold. The Tukey HSD plots (Fig. 5) confirm this finding, with no sustained significant pairwise differences. This suggests that arterial oxygen saturation remains relatively stable across PDA statuses.

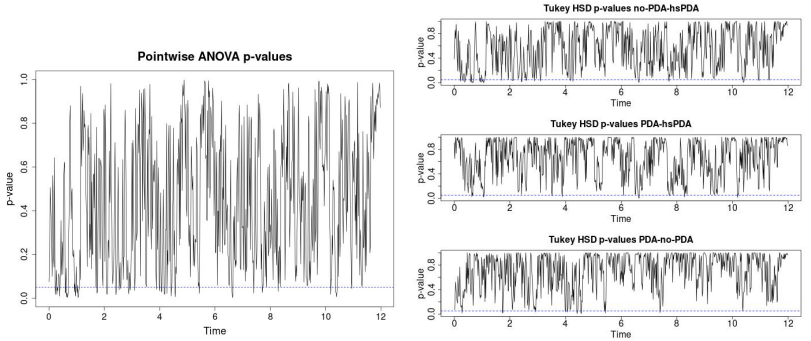

Fig. 4. Pointwise ANOVA p-values for rSO_2-1 and pointwise Tukey HSD p-values for SpO_2 signals across time

Figure 5 presents pointwise ANOVA results for rSO_2-1 (cerebral oxygenation), with more time periods with significant differences compared to SpO_2 signal. The time period with the most significant differences is between 2 and 6 hours, with additional peaks before 2 hour and more frequent peaks between 6 and 10 hours, while the only period without significant *p*-values is from 10 to 12 hours. The post-hoc test revealed that PDA and no-PDA groups show only a few isolated peaks with statistically significant differences, while hsPDA with no-PDA and hsPDA with PDA pairs show statistically significant differences for longer and continuous time periods (Fig. 5). It shows that the hsPDA group, when compared to the other two groups, has the most significant differences over time.

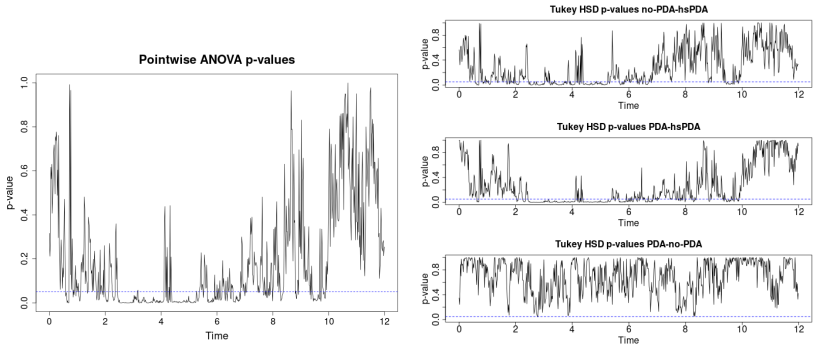

Fig. 5. Pointwise ANOVA p-values for rSO_2-1 and pointwise Tukey HSD p-values for rSO_2-1 signals across time

Figure 6 shows that pointwise ANOVA identified extended time regions with significant group differences in rSO_2-2 (renal oxygenation), particularly between hours 1 and 3, around 6th hour and from 8 to 12 hours, with additional peaks at other times. The Tukey HSD post-hoc test shows that the PDA and no-PDA pair shows the most significant differences, the hsPDA and no-PDA group also exhibit many periods with significant differences, while in contrast, the hsPDA and PDA pair shows the fewest significant differences (Fig. 6). From Tukey HSD tests, it is evident that the no-PDA group differs the most from the other two groups, as it has continuous periods of significant differences with both PDA and hsPDA groups.

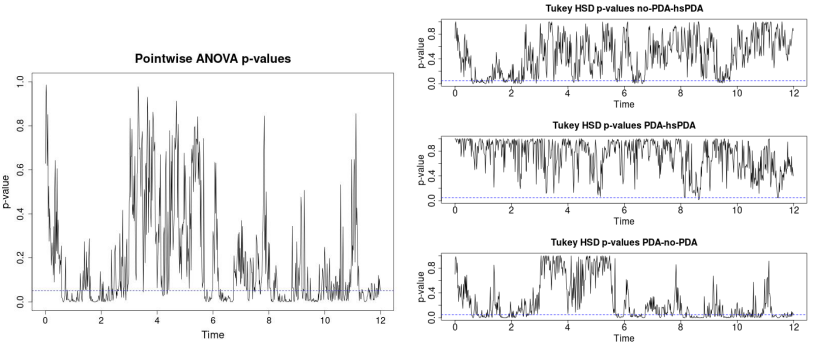

Fig. 6. Pointwise ANOVA p-values for rSO_2-2 and pointwise Tukey HSD p-values for rSO_2-2 signals across time

5 Conclusions

This study applied a pointwise statistical approach to smoothed NIRS signals to investigate group differences in oxygenation patterns among preterm infants with and without PDA. The results showed that while SpO_2 remained largely similar across groups, significant differences were observed in cerebral and renal oxygenation. The hsPDA group showed distinct patterns in rSO_2-1, while the no-PDA group stood out in rSO_2-2.

These findings demonstrate that pointwise analysis can reveal time-specific physiological differences that are often missed by traditional summary-based methods. This approach offers a simple yet powerful framework for analyzing biomedical signals and may support more nuanced interpretations in clinical research.

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