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# "The Value of Clinical Examination in Preterm Newborns after Neonatal Sepsis: A Cross-sectional Observational Study."

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**ABSTRACT**

**Background**: Neonatal sepsis is an important risk factor for lesions in the brain of preterm newborns (PTNB) and the most effective strategies to minimize its deleterious effects are early detection and intervention.

**Aim**: To investigate the presence of neurological abnormalities in PTNBs after neonatal sepsis.

**Methods**: This was a prospective cross-sectional study with 100 PTNBs selected at random, 50 of the study group (sepsis) and 50 of the control group (non-sepsis). The neurological evaluation protocol adopted was the Hammersmith Neonatal Neurological Examination (HNNE).

**Results**: The PTNBs of the sepsis group had total HNNE scores lower than expected for normality in 86% of the cases, and the non-sepsis group in 26% ($p < .001$). Higher prevalence levels of altered scores in tone category ($p < .001$), tone patterns ($p = .026$), reflexes ($p = .002$), movements ($p < .001$), abnormal signs ($p < .001$) and behavior ($p < .001$).

**Conclusion**: The neurological dysfunctions after neonatal sepsis could be identified by clinical neonatal neurological evaluation.

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**KEYWORDS**
Neonatal sepsis; neurological examination; infant premature; developmental disabilities; early diagnosis

## Introduction

Neonatal sepsis can be defined as a clinical syndrome resulting from the invasion of microorganisms in the bloodstream during the first month of life. Its incidence is high in preterm newborns (PTNB), with up to 30 per 1,000 live births.[1]

Scientific evidence suggests a notable contribution to the mechanism of neurological dysfunction in PTNBs.[2,3] Since the systemic inflammatory response is a significant risk factor for white matter lesions characterized by cystic periventricular leukomalacia (PVL).[4,5] Several clinical studies have established through neuroimaging the association between neonatal infections and brain axonal lesions in PTNBs.[2,6–9]

This neuronal damage may result in neurodevelopmental impairment in these children. Two meta-analyses have shown that children born prematurely with sepsis records have worse rates of intellectual and motor development, incidence of Cerebral Palsy (CP), and visual and auditory dysfunctions.[10,11]

However, existing literature indicates that the earlier the intervention is started in these children, the higher the ability to minimize the neurodevelopmental impairment, due to neuronal plasticity.[12] In this context, the importance of detecting neonates with neurological abnormalities is evident. Clinical evaluation remains the primary choice among several tools for this purpose.[13]

Among several methods of neonatal neurological evaluation, we highlight that of Dubowitz, later also known as the Hammersmith Neonatal Neurological Examination (HNNE).[14] This method was validated for PTNBs[14] and continues to be used in scientific research and clinical practise.[15]

The scientific literature emphasizes its predictive power of development in PTNBs, as in two Finnish studies, one with 216 children, in which the HNNE results were significantly associated with sensorimotor performance at the age of two years,[12] and another with 98 participants, in which abnormalities in the early evaluation were predictors of neuromotor dysfunctions at the age of 11 Years.[16]

Thus, the relationship between neonatal sepsis and lesions in the cerebral white matter of PTNBs, with resulting damages to neurodevelopment, has already been established, as well as the relevance of early identification of those who require follow-up and the validity of neonatal neurological evaluation for this purpose. However, no studies were found to analyze the neurological behavior of PTNBs with neonatal sepsis.

Thus, this study aimed to investigate the presence of neurological abnormalities in preterm infants after sepsis by the neonatal clinical evaluation.

## Methods

This was a prospective cross-sectional study. The sample of 100 participants was obtained consecutively.

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LIST OF ABBREVIATIONSCP: Cerebral Palsy;ELBW: extremely low birthweight;FMABC: The ABC Faculty of Medicine;GA: gestational age;HNNE: Hammersmith Neonatal Neurological Examination;LM: last menstrual period;LBW: low birthweight;LT: late preterm;NMRI: neuromagnetic resonance imaging;PIVH: periventricular/intraventricular hemorrhage;PTNB: preterm newborns;PVL: periventricular leukomalacia;VLBW: very low birthweight;WBC: white blood cells.

The population of the study group (sepsis group) consisted of 50 PTNBs after the sepsis diagnosis. The control group (non-sepsis group) consisted of 50 low-risk PTNBs assessed for neurological dysfunctions, according to inclusion criteria and with no records of sepsis in medical records.

Inclusion criteria for the non-sepsis group consisted of gestational age at birth up to 36 weeks, age equivalent to term birth at the time of assessment (between 37 and 42 weeks), absence of records of sepsis or neonatal asphyxia, Apgar score of the 5$^{th}$ minute ≥ 7 and normal cranial ultrasound or showing only minimal abnormalities. The same criteria were adopted for the composition of the sepsis group, except for the need for cranial ultrasound without significant abnormalities, and the clinical-laboratory diagnosis of neonatal sepsis recorded in the chart was determinant, because the sample was obtained by consulting medical records.[17,18]

The exclusion criteria for both groups were in use at the time of evaluation of mechanical ventilation, sedation or in the period of septic shock, the presence of congenital malformations, congenital infections of the Nervous System or genetic syndromes.

The participants were hospitalized in the Neonatal Intensive Care Unit (ICU) and in the nursery or monitored at the preterm follow-up outpatient clinic at the University Municipal Hospital of São Bernardo do Campo, São Paulo, Brazil, a teaching hospital of the ABC Faculty of Medicine (FMABC). The project was approved by the Research Ethics Committee of the FMABC under opinion nº 1.357.613/2015. Parents were approached personally, they agreed to participate in the survey and signed the Informed Consent Form before the evaluation.

The PTNBs' birth weight was classified as low birthweight (LBW) (<2500 g), very low birthweight (VLBW) (<1500 g) and extremely low birthweight (ELBW) (<1000 g). The expected weight for gestational age (GA) was classified by Intergrowth 21.[19] Gestational age at birth was classified by neonatologists as per one of the criteria: date of last menstrual period (LMP) in three cases, gestational ultrasound in three cases, and objective evaluation calculated by the New Ballard method in 94 cases.

The diagnosis of sepsis was given by the medical staff of the hospital in question. Neonatal sepsis was considered in the presence of a positive blood culture and/or clinical and laboratory signs suggestive of infection.[17] Clinical signs included worsening of respiratory distress: tachypnea, sternal and/or subcostal retraction, groaning and cyanosis, apnea, body temperature instability, hyper- or hypoglycemia, poor peripheral perfusion, food intolerance, arterial hypotension, and underactive infants.[17]

Laboratory parameters included: complete blood count with three or more altered parameters according to Rodwell et al[18] and/or C-reactive protein > 0.5 mg/L; negative or not performed blood culture; no evidence of infection at another site; and established and maintained antimicrobial therapy. Rodwell et al[18] considered the following hematological parameters: leukocytosis (white blood cells [WBC] ≥ 25,000 at birth, or ≥ 30,000 between 12 to 24 hours, or > 21,000 at over 48 hours of life), leukopenia (WBC ≤ 5,000); neutrophilia or neutropenia; increased number of immature neutrophils; increased neutrophilic index; ratio of immature over segmented neutrophils ≥ 0.3; neutrophils with toxic granulation and vacuolization; and thrombocytopenia Neonatal sepsis: risk factor for development 295 (< 150,000 platelets).

The classification of sepsis into early and late followed the Brazilian national criteria for diagnosis in neonatology. Early sepsis was one recorded within the first 48 hours of life, and late, if it was recorded after that period.[18]

The neurological evaluation protocol adopted was HNNE,[14] since it is validated for preterm infants, it does not require formal certification or training, its application is brief and has a self-explanatory guide to the achievement and scoring of each item.[13] The HNNE is also validated for its predictive power of neurodevelopment and for its sensitivity in identifying clinical neurological abnormalities in preterm infants with brain lesions on imaging exams.[12,16]

In addition, it is possible to conduct online HNNE training for evaluators on the official website. It also makes available the forms to register the evaluation, free of charge.[20]

The HNNE consists of 34 items grouped into six categories: tone (ten items), tone patterns (five items), reflexes (six items), movements (three items), abnormal signs (three items) and behavior (seven items). It consists of items that can be scored, and each item has three to five options that consider the expected responses to normality for each of the gestational ages between 37 and 42 gestational weeks at the time of evaluation. The composition of the scores is calculated by adding all the items within each category. The protocol has raw scores which are converted to optimality scores in the following way by using data from healthy term infants. An optimality score of 1 is given to raw scores that are seen in 10–90th percentile, score of 0.5 is given for findings seen in 5–10th percentile and 90–95th percentile. And score of 0 is given to findings see in <5th or >95th percentile. Optimality scores ≥ 30.5 out of 34 are considered normal. The following scores are considered optimal for each category: tone (9–10), tone patterns (5), reflexes (4–6), movements (3), abnormal signs (3) and behavior (6–7).[14]

The neurological evaluations were performed on the PTNBs when they reached corrected gestational age, between 37 and 42 weeks, as validated, by the same evaluator.

At the time of the evaluation, among the sepsis group PTNB, 47 remained hospitalized and three under follow-up at the preterm follow-up outpatient clinic. While in the group no-sepsis, only four remained hospitalized. The hospitalized preterm infants were clinically stable, did not use respiratory support, participated in the Kangaroo Method and were in the preparation phase for hospital discharge.

### Statistical Analysis

In the analysis of the results, the qualitative variables were shown by absolute and relative frequency and the quantitative ones by the median, 25$^{th}$ and 75$^{th}$ percentile values and respective 95% confidence intervals, according to the Shapiro-Wilk normality test, with $p < .05$.

The Chi-square test was used to compare and associate the classifications of the total scores, and in each category of the assessment obtained by the sepsis and non-sepsis groups, as well as to compare the scores classifications obtained by PTNBs

with early and late sepsis, clinical and verified sepsis and those developing septic shock or not. The Bonferroni correction was performed to verify the applicability conditions. The Mann-Whitney test was used to compare the values of the scores in the categories and totals.

The Chi-square test (qualitative variables) and the Mann-Whitney test (quantitative variables) were used to identify the association between the other factors and the total score in the sepsis group.

The Enter method logistic binary regression was used to evaluate the effect of neonatal sepsis on the clinical neurological alterations of PTNBs. All analyses were performed in the statistical software Data Analysis and Statistical Software for Professionals (Stata) version 11.0 and *p*-values below 0,05 were considered as statistically significant.

No studies were found to reference the sample calculation. Thus, a convenience study sample was used with 100 preterm infants, and the effect size was calculated later. For this, the Cohen's d test was used, with a value of 1.74 (95% CI: 1.28; 2.20). The power of the test was 1.00.

## Results

Table 1 shows the maternal characteristics and the studied PTNBs.

The prenatal examination was performed in 48 mothers (96%) of the sepsis group and 49 (98%) of the non-sepsis group ($p$ = .812). Premature rupture of membranes was found in 40% of the mothers in the sepsis group and 28% in the non-sepsis group ($p$ = .445). The median of its duration was 57.94 hours (0–576) in the former and 4.42 (0–99) in the latter ($p$ = .002).

Regarding the characteristics of PTNBs, the proportion of females in the sepsis group was 50% and 56% ($p$ = .548) in the non-sepsis group. Surfactants were administered to 62% and 38% of PTNBs in the sepsis and non-sepsis group, respectively ($p$ < .001). All participants in the sepsis group underwent antibiotic therapy, with a median duration of 18.6 days (7–84) and only one in the non-sepsis group followed this course for 13 days, due to respiratory infection ($p$ < .001).

Regarding the diagnosis of sepsis, 41 had clinical sepsis and 9 had proven sepsis. And regarding the classification, 27 developed early sepsis, 6 developed late sepsis, and 17 developed early and late sepsis.

The hemoculture examination was performed in 84% and was positive only 9%. The infectious agents detected were Gram-positive in 1, *Streptococcus b* in 1, *Bacillus* gram negative + *Klebsiella pneumoniae* in 1, *Escherichia coli* in 3, *Escherichia coli* + *Enterococcus faecalis* in 1 and *Escherichia coli* + *Bacillus* gram-negative in 1.

In the HNNE neurological evaluation, the sepsis group had a higher percentage of sub-optimal sores for all the categories evaluated (Table 2). The median total score was 22 (20–24) in the sepsis group and 32 (31.26–33) in the non-sepsis group ($p$ < .001) (Table 3). Table 4 shows that the sepsis group had significantly sub-optimal scores (below the 5th centile) in 26 of the 34 evaluation items.

Logistic binary regression was performed to evaluate the effect of sepsis, adjusted for maternal variables (schooling and antenatal corticosteroid use) and variables of PTNBs (birth weight, gestational age, neonatal respiratory diseases, and neurological complications), on the probability of sub-optimal total neurological evaluation score. PTNBs were 7.08 (95% CI 2.13–23.53, $p$ = .001) times more likely to have a sub-optimal inadequate neurological evaluation score, compared to those without this condition (Table 5).

When comparing the HNNE categories' score, considering the proven sepsis and clinical groups, there was no statistically significant difference for tone ($p$ = .384), tone patterns ($p$ = 0487), reflexes ($p$ = .580), movements ($p$ = .181), abnormal signs ($p$ = .066) and behavior ($p$ = .400) and neither for the total scores ($p$ = .783). Between groups early and late sepsis, a more significant percentage of inadequacy for "movements" ($p$ = .031) and "behavior" ($p$ = .011) was found in the late sepsis group. For the other variables, no significant differences were observed: tone ($p$ = .093), tone patterns ($p$ = .062), reflexes ($p$ = .089), abnormal signs ($p$ = .113) and totals ($p$ = .181).

No significant differences were found when comparing the performance in the evaluation of PTNBs with sepsis that progressed to septic shock or not. The following *p*-values were found in the following categories: tone ($p$ = .242), tone patterns ($p$ = .725), reflexes ($p$ = .503), movements ($p$ = .595), abnormal signs ($p$ = .899) and behavior ($p$ = .967) and in the total scores ($p$ = .595).

## Discussion

In this study, PTNBs who developed sepsis in the neonatal period had a higher prevalence of abnormalities in neurological behavior detected by HNNE. Among the 50 participants in the sepsis group, 43 (86%) underperformed for normality. This group showed significant proportions of changes in all categories of the evaluation, as well as in most of its items.

To date no studies describing the neurological performance of PTNBs with neonatal sepsis to enable comparative analysis are available. However, two national studies investigating possible risk factors for neurological lesions in the neonatal period found an association between the presence of sepsis and abnormalities in the HNNE assessment.[21,22] In the first one, consisting of 30 PTNBs, the only factor associated with abnormalities in the evaluation was the presence of sepsis.[21] In the second study, with 20 PTNBs, 83.3% of PTNBs with neonatal infections had below-normal scores.[22]

However, the total scores and in the different HNNE categories obtained in this study, by the non-sepsis group, are like to the normative data of 209 low-risk PTNBs for neurological dysfunctions in Australian study.[23] And the scores obtained by the sepsis group are like those obtained by PNTBs with structural brain injuries, detected by Neuromagnetic Resonance Imaging (NMRI) from two studies, one with 100 PNTBs from New Zealand and the other with 119 PNTBs from Australia.[24,25]

The findings of the publications mentioned above, contribute to reaffirm the reliability of the HNNE results found in the present study.

The frequent neurological abnormalities found in PTNBs after a septic episode are due to the systemic inflammatory response associated with the extreme vulnerability of the

**Table 1.** Comparison of maternal characteristics and stratified PTNB by presence or absence of neonatal sepsis.

| Characteristics | Sepsis (n = 50) | Non-sepsis (n = 50) | p* |
|---|---|---|---|
| | n (%) | | |
| **MATERNAL** | | | |
| **Habits** | | | |
| Smoking | 7 (14) | 11 (22) | 0.298 |
| Alcoholism | 4 (8) | 2 (4) | 0.400 |
| Illicit drugs | 2 (4) | 0 (0) | 0.153 |
| **Schooling** | | | 0.007 |
| ≤ 8 years | 5 (10) | 4 (8) | |
| 8–12 years | 15 (30) | 15 (30) | |
| = 12 years | 14 (28) | 27 (54) | |
| > 12 years | 7 (14) | 4 (8) | |
| Not informed | 9 (18) | 0 (0) | |
| **Use of medication** | 27 (54) | 20 (40) | 0.161 |
| Corticosteroid | 18 (36) | 6 (12) | 0.005 |
| Anti-hypertensive | 10 (20) | 9 (18) | 0.799 |
| Antibiotic | 16 (32) | 12 (24) | 0.373 |
| **Gestational diseases** | | | |
| Maternal infection | 27 (54) | 19 (38) | 0.108 |
| Maternal hypertension | 19 (38) | 13 (26) | 0.198 |
| Others | 2 (4) | 12 (24) | 0.004 |
| **Type of delivery** | | | 0.523 |
| Normal | 21 (42) | 18 (36) | |
| Cesarean | 29 (58) | 31 (62) | |
| Forceps | 0 (0) | 1 (2) | |
| **PTNB** | | | |
| **Median (IR) p**** | | | |
| **Gestational age at birth** | 31,22 (24–36) | 34,76 (30–36) | <0,001 |
| **Birthweight** | 1419 (550–2300) | 2007(1250–2470) | <0,001 |
| **Gestational age at assessment** | 38,90 (37,42,57) | 38,8 (37–42) | 0,004 |
| **Multiple births** | **n (%)** | | **p*** |
| Yes | 12 (24) | 11 (22) | 0.500 |
| **Weight classification** | | | <0.001 |
| LBW | 26 (52) | 43 (86) | |
| VLBW | 14 (28) | 7 (14) | |
| ELBW | 10 (20) | 0 (0) | |
| **Weight/Gestational Age Classification** | | | 0.517 |
| Adequate | 33 (66) | 36 (72) | |
| Small | 17 (34) | 14 (28) | |
| Large | 0 (0) | 0 (0) | |
| **Neonatal intercurrences** | 50 (100) | 35 (70) | |
| Jaundice | 24 (48) | 26 (52) | 0.532 |
| **Respiratory** | 45 (90) | 26 (52) | |
| Respiratory distress syndrome | 23 (46) | 6 (12) | <0.001 |
| Respiratory distress | 21 (42) | 20 (40) | 0.839 |
| Pulmonary hypertension | 4 (8) | 0 (0) | 0.041 |
| Pneumomediastinum | 0 (0) | 1 (2) | 0.315 |
| Pneumothorax | 4 (8) | 0 (0) | 0.041 |
| Laryngomalacia | 0 (0) | 1 (2) | 0.315 |
| Chronic lung disease | 14 (28) | 0 (0) | <0.001 |
| **Heart-related** | 30 (60) | 5 (10) | |
| Persistent oval foramen | 28 (56) | 3 (6) | <0.001 |
| Interventricular communication | 2 (4) | 0 (0) | 0.153 |
| **Neurological** | 6 (12) | 0 (0) | |
| Seizure | 5 (10) | 0 (0) | 0.022 |
| Meningitis | 1 (2) | 0 (0) | 0.315 |
| **Ultrasound Scanner** | 50 (100) | 14 (28) | <0.001 |
| Normal | 9 (18) | 42 (84) | <0.001 |
| PIVH I | 13 (26) | 5 (10) | |
| PIVH II | 20 (40) | 3 (6) | |
| PIVH III | 5 (10) | 0 (0) | |
| PVL | 3 (6) | 0 (0) | |
| **Respiratory support** | 45 (90) | 14 (28) | <0.001 |
| Invasive Ventilation | 37 (74) | 1 (2) | <0.001 |
| Noninvasive | 41 (82) | 5 (10) | <0.001 |
| Oxygen therapy | 31 (62) | 10 (20) | <0.001 |

**Captions: PIVH (peri-intraventricular hemorrhage). PVL (periventricular leukomalacia).**

*** Chi-square test's level of significance. ** Mann-Whitney test's level of significance.**

**Table 2.** Number and percentage of scores below the published optimal scores for each of the subscales and the overall total score of the HNNE neurological assessment, in the PTNB of the sepsis group and the non-sepsis group.

| | Published Optimal Scores | Sepsis (n = 50) | Non-sepsis (n = 50) | p* |
|---|---|---|---|---|
| | | n (%) | | |
| *Categories* | | | | |
| Tone | (9–10) | 39 (78) | 10 (20) | <0.001 |
| Tone patterns | (5) | 34 (68) | 23 (46) | 0.026 |
| Reflexes | (4–6) | 13 (26) | 2 (4) | 0.002 |
| Movements | (3) | 43 (86) | 16 (32) | <0.001 |
| Abnormal signs | (3) | 19 (38) | 3 (6) | <0.001 |
| Behavior | (6–7) | 27 (54) | 4 (8) | <0.001 |
| *Total Score* | >30,5 | 43 (86) | 13 (26) | <0.001 |

*** Chi-square test's level of significance.**

**Table 3.** Median (confidence interval) of the scores obtained in the categories of the HNNE neurological assessment and total, in the PTNB of the sepsis group and the non-sepsis group.

| | Sepsis (n = 50) | Non-sepsis (n = 50) | |
|---|---|---|---|
| | Median (CI 95%) | | p* |
| *Categories* | | | |
| Tonus | 6 (5–7) | 10 (9–10) | <0.001 |
| Tone patterns | 4 (4–4) | 5 (4–5) | 0.005 |
| Reflexes | 4 (4–5) | 6 (5.76–6) | <0.001 |
| Movements | 1 (1–2) | 3 (3–3) | <0.001 |
| Abnormal signs | 3 (2.26–3) | 3 (3–3) | <0.001 |
| Behavior | 5 (4–6) | 7 (7–7) | <0.001 |
| *Total Score* | 22(20–24) | 32 (31,26 − 33) | <0.001 |

**CI: Confidence Interval**

***Mann-Whitney test's significance level**

developing brain in the neonatal period.[5] The lesion mechanism begins with increased pro-inflammatory cytokines, which easily cross the blood-brain barrier, striking the glial cells, causing hypomyelination and possible cerebral white matter lesions, besides inducing neuronal apoptosis and impairing differentiation of neurons and cerebral blood flow, favoring the occurrence of ischemic and hemorrhagic events.[2,4]

Clinical studies with neuroimaging evidence brain lesions in PTNBs after neonatal sepsis. One British study with 117 participants[26] and another Australian study, with 192[6] individuals found a significant association of white matter lesions detected by NMRI and neonatal sepsis. Also, a multicenter study with 32 centers from several countries and a sample of 910 PTNBs reaffirms these findings, since it found an association between neonatal infections and brain lesions seen in the cranial ultrasound exam.[7]

Like the motivation of this study, many investigations in the last decades have been devoted to evaluating the presence of neurological abnormalities resulting from neonatal sepsis. However, neurodevelopmental disorders were noted. In these, children with a history of sepsis had significant proportions of neuromotor, cognitive, attention deficit and hyperactivity disorders.[27,28]

Here, no differences were found in the neurological performance of PTNBs with sepsis identified by clinical parameters and confirmed by positive hemoculture. In both cases, the losses were evident. This is in accordance with the results in other publications using neuroimaging, which found lesions in

**Table 4.** Percentage of PTNBs that scored zero on items of the HNNE neurological assessment.

| Items of the assessment | Sepsis (n = 50) | Non-sepsis (n = 50) | p* |
|---|---|---|---|
| | n (%) | | |
| Posture | 9 (18) | 6 (12) | 0.401 |
| Arm recoil | 20 (60) | 3 (6) | <0.001 |
| Arm traction | 27 (54) | 9 (18) | <0.001 |
| Leg recoil | 22 (44) | 9 (18) | 0.005 |
| Leg traction | 20 (40) | 4 (8) | <0.001 |
| Popliteal angle | 17 (34) | 4 (8) | 0.001 |
| Head control 1 | 22 (44) | 3 (6) | <0.001 |
| Head control 2 | 16 (32) | 1 (2) | <0.001 |
| Head lag | 21 (42) | 3 (6) | <0.001 |
| Ventral suspension | 22 (44) | 6 (12) | <0.001 |
| Flexor tone 1 | 3 (6) | 0 (0) | 0.079 |
| Flexor tone 2 | 2 (4) | 1 (2) | 0.558 |
| Leg extensor tone | 10 (20) | 14 (28) | 0.349 |
| Neck extensor tone | 22 (44) | 6 (12) | <0.001 |
| Increased extensor tone | 19 (38) | 8 (16) | 0.013 |
| Tendon reflex | 25 (50) | 1 (2) | <0.001 |
| Suck/gag | 15 (30) | 2 (4) | 0.001 |
| Palmar grasp | 15 (30) | 0 (0) | <0.001 |
| Plantar grasp | 17 (34) | 7 (14) | 0.019 |
| Moro reflex | 12 (24) | 7 (14) | 0.202 |
| Placing | 5 (10) | 7 (14) | 0.538 |
| Spontaneous movements 1 | 19 (38) | 3 (6) | <0.001 |
| Spontaneous movements 2 | 38 (76) | 6 (12) | <0.001 |
| Head raising prone | 31 (62) | 13 (26) | <0.001 |
| Abnormal hand or toe postures | 8 (16) | 2 (4) | 0.046 |
| Tremor | 12 (24) | 2 (4) | 0.004 |
| Startle | 5 (10) | 1 (2) | 0.092 |
| Eye movements | 9 (18) | 2 (4) | 0.025 |
| Auditory orientation | 9 (18) | 0 (0) | 0.002 |
| Visual orientation | 30 (60) | 12 (24) | <0.001 |
| Alertness | 20 (40) | 1 (2) | <0.001 |
| Irritability | 19 (38) | 3 (6) | <0.001 |
| Cry | 18 (36) | 6 (12) | 0.005 |
| Consolability | 4 (8) | 2 (4) | 0.400 |

***Chi-square test's level of significance.**

**Table 5.** Logistic binary regression evaluating the effect of neonatal sepsis on clinical neurological alterations of PTNBs.

| Variables | β | S.E. | Wald | Df | p | Odds ratio | (IC 95%) |
|---|---|---|---|---|---|---|---|
| Sepsis | 1.96 | 0.61 | 10.2 | 1 | 0.001 | 7.08 | (2.13; 23.53) |
| Schooling | −0.37 | 0.56 | 0.44 | 1 | 0.507 | 0.68 | (0.22; 2.07) |
| Corticosteroid | −0.53 | 0.75 | 0.50 | 1 | 0.477 | 0.58 | (0.13; 2.54) |
| Birth weight | −0.07 | 0.79 | 0.00 | 1 | 0.927 | 0.92 | (0.19; 4.44) |
| Gestational age | 1.17 | 0.78 | 2.20 | 1 | 0.137 | 3.23 | (0.68; 15.16) |
| Respiratory disease | 0.53 | 0.59 | 0.80 | 1 | 0.369 | 1.70 | (0.53; 5.45) |
| Neurological complications | 0.53 | 0.84 | 0.40 | 1 | 0.523 | 1.71 | (0.33; 8.87) |

* Dependent variable: scores <30,5.
* Independent variables: sepsis (yes), schooling (<12 years), use of antenatal corticosteroids (yes), birth weight (<1500 grams), gestational age (<32 weeks), neonatal respiratory diseases (yes), neurological complications (yes: meningitis, seizure or PIHV grade III and IV)

β: inclination,, DF: degree of freedom, S.E: standard error, 95% confidence interval.

the cerebral white matter in both sepsis conditions,[22] and these were also identified as the main isolated risk factors for minor neurological dysfunctions, between four and six years of age.[29] This is because axonal tracts are more affected by the systemic inflammatory process rather than infectious agents itself.[5]

However, the PTNBs with late sepsis had worse performances in the HNNE movements and behavior categories, with significant results and with a tendency to the significance of tone, tone patterns and reflexes. The prominent role of this type of sepsis in neurological dysfunctions was evidenced by a French developmental follow-up study, consisting of 139 PTNBs, which compared the frequency of CP at five years of age among those with early and late sepsis. The latter had a greater, significant association with this type of motor dysfunction. Possibly, its contribution to worse outcomes is related to the fact that this type increases the presence of associated morbidities (hypotension, disseminated intravascular coagulation, chronic lung disease, and severe PIVH).[30]

Studies have shown that changes in the categories of movements (assessment of the quantity and quality of spontaneous movements and the ability to raise head in prone) and of behavior (assessment of auditory and visual orientation, alertness, irritability, cry and consolability) are associated with abnormalities on magnetic resonance imaging at term and neuromotor outcome at the age of 11 years.[16,31] In the study by Brown et al (2009), changes in the movements and behavior categories showed greater associations with white matter abnormalities at term, respectively.[31] And in the one performed by Setänen et 297 al (2016), the category that best predicted neurological status at 11 years of age was behavior.[16]

In this study, gestational ages and birth weight were significantly lower in the sepsis group, possibly because they were risk factors for the development of sepsis in the neonatal period.[3,32] This fact could consist a bias, influencing the results. However, a study that aimed to establish the range of neurological findings in HNNE of PTNBs, born with gestational ages at birth between 25 and 34 weeks, at term age, showed that the total scores did not differ significantly between ages. All members of the study had normal neurodevelopment at 18 months of age. And their results reiterate that the neurological behavior of PTNBs is similar at term age, regardless of gestational age at birth.[33]

The non-sepsis group was composed, mostly of late-preterm (LP), an expected fact, since it represents 70% of the whole preterm population. In addition, two studies that evaluated LP, by HNNE, showed that the responses in the evaluation observed in this population were like those found in term-born and very preterm infants assessed at term age. So, suggesting that the same proforma can be used as a screening tool for LP.[34,35]

Furthermore, gestational ages at birth were not associated with the presence of neurological lesions in our study, since 26 out of 50 preterm newborns in the sepsis group had gestational ages greater than 31 weeks. This data that is in agreement with the results of studies showing that neurological sequelae are not present only in extreme but also in moderate and LP.[3,36]

The clinical evaluation of HNNE was effective in the detection of neurological alterations. Its predictive power for neurodevelopmental dysfunctions has been emphasized since the publication of its first version in 1981, even in more recent papers.[12,16]

All HNNE categories had a higher frequency of abnormalities in the sepsis group, but the categories of tone, movements, abnormal signs, and behavior were even more significant compared to the non-sepsis group. In a developmental follow-up

study, abnormalities in the tone patterns, tone and behavior categories correlated with sensorineural dysfunctions at the age of two Years.[12] Another study also found an association of changes in the behavior category with the presence of CP at 11 years of age. These data reinforce the long-term neurological impairment of neonatal sepsis.[16]

To apply HNNE, the PTNBs should reach corrected gestational age, that is, 37 weeks. During the data collection period of this study, most individuals in the non-sepsis group had already been discharged from the hospital and performed longitudinal follow-up at the outpatient clinic at the time of the evaluation, while most of the members of the sepsis group were still hospitalized in the ICU or medium-risk unit. This may be one of the limitations of this study since the literature indicates that the hospitalization environment may contribute to worse rates in the neurological evaluation.[15]

The other limitations of the present study are the differences found between the characteristics of the groups, especially regarding weight and gestational ages at birth, presence of respiratory complications, ultrasound abnormalities and low rate of positive bacterial cultures in the group with sepsis.

It was evidenced that children born prematurely and who developed a septic condition in the neonatal period require longitudinal monitoring of neurodevelopment since the probability of dysfunction is high, and only early intervention can minimize its deleterious effects.[32]

Finally, it can be inferred that it is possible to detect neurological abnormalities due to sepsis from the neonatal period. Even if such occurrences are transient and the milder manifestations can progress to resolution by the second year of life,[13] follow-up studies showed that children with long-term dysfunctions already had alterations in HNNE.[12,36,38]

## Conclusion

PTNBs with neonatal sepsis evidenced unfavorable clinical changes in neurological evaluations by HNNE. As no factors associated with these abnormalities were detected, the clinical neurological evaluation could suggest that the presence of sepsis in PTNBs may played a prominent role in the etiology of neurological dysfunctions.

Thus, we can conclude that neurological dysfunctions in preterm newborns after neonatal sepsis could be identified by clinical neonatal neurological evaluation.

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## Declaration Of Conflicting Interests

The Authors declares that there is no conflict of interest'.

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