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Serum neurotrophins at birth correlate with respiratory and neurodevelopmental outcomes of premature infants

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Abstract

Objective: Preterm birth is a significant cause of infant morbidity and mortality, which are primarily the result of respiratory and neurodevelopmental complications. However, no objective biomarker is currently available to predict at birth the risk and severity of such complications. Thus, we sought to determine whether serum neurotrophins concentration measured at birth correlate with risk for later development of bronchopulmonary dysplasia (BPD) and long-term neurodevelopmental outcomes.

Methods: This study prospectively included 223 newborns admitted to neonatal intensive care units (NICU) and divided into three groups: (i) preterm infants who developed BPD; (ii) preterm infants who did not develop BPD; (iii) term infants. An exploratory cohort was enrolled in West Virginia, followed by a validation cohort recruited in four NICUs in Ohio. Specimens for serum and tracheal neurotrophins concentrations were collected within 48 h of admission. Infants requiring a fraction of inspired oxygen >0.21 for at least 28 days were diagnosed with BPD. Neurodevelopmental outcomes were extrapolated from Bayley Scales of Infant Development—Third Edition (BSID-III) administered at the 24-month follow-up visit.

Results: Serum brain-derived neurotrophic factor (BDNF) concentration at birth had significant negative correlation with later diagnosis of BPD ($P=0.011$) and with duration of invasive ventilation and oxygen supplementation ($P=0.009$ and 0.015 , respectively). Serum nerve growth factor (NGF) concentration at birth had significant positive correlation with BSID-III cognitive and language composite scores at 24 months ($P<0.001$ and 0.010 , respectively).

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CONFLICT OF INTEREST

None.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Conclusions: These data suggest that serum neurotrophins concentrations measured at birth provide prognostic information on subsequent respiratory and neurodevelopmental outcomes.

Keywords

biomarkers; bronchopulmonary dysplasia (BPD); developmental biology; neonatal pulmonary medicine

1 INTRODUCTION

Preterm birth is a significant cause of morbidity and mortality worldwide.^{1,2}

Bronchopulmonary dysplasia (BPD) is a form of chronic lung disease and one of the most common morbidities of prematurity,³ with infants born extremely preterm being most at risk and requiring prolonged hospitalizations in the neonatal intensive care unit (NICU).⁴

Presently, neonatologists cannot provide parents of premature infants with precise predictions regarding their child's prognosis, set definite expectations for the neonatal period, or consider early use of more aggressive management. In fact, most biomarkers studied over the years have low predictive accuracy and none is currently used in routine clinical care or shown to be useful for predicting longer-term respiratory outcomes.

Preterm birth—especially when complicated by BPD—is also associated with high risk for long-term neurodevelopmental deficits,⁵ which is perhaps the most important clinical outcome for preterm infants and their families but for which there are neither behavioral nor neurological tests available to identify those patients who would benefit most from early intervention. Indeed, with the recent advances in perinatal care, the survival rate of premature infants has increased significantly, but the infants who survive often experience disabilities extending into school age and adolescence. Yet, the first formal objective assessment of neurodevelopmental impairment in premature-born infants is usually obtained only after completion of the first or second year of life using the Bayley Scales of Infant Development—Third Edition (BSID-III).

During embryogenesis, the development of the respiratory tract is closely associated with the formation of peripheral neuronal networks, which is orchestrated by coordinated expression of neurotrophic factors and their cognate receptors.⁶ In addition, neurotrophins are expressed in the airways by non-neuronal resident cells like epithelium, smooth muscle, fibroblasts, and vascular endothelium, and neurotrophin receptors have been localized on migrating immuno-inflammatory cells like mast cells, eosinophils, and T lymphocytes. The same neurotrophins play critical anti-apoptotic and neuroprotective roles during development of the central nervous system (CNS), thereby affecting axon growth, neurotransmitter expression, morphologic differentiation, and higher neuronal functions.

Thus, the physiological expression of neurotrophins like the nerve growth factor (NGF) and brain-derived growth factor (BDNF), as well as their high-affinity tropomyosin receptor kinase A and B (TrkA and TrkB) receptors, is essential for normal development and function of both respiratory and nervous systems. In this study, we tested in two independent cohorts the hypothesis that serum neurotrophins concentrations measured at birth correlate with the risk of BPD diagnosis and its clinical course. As a secondary endpoint, we measured

neurodevelopmental outcomes in a subgroup of infants from the validation cohort tested with BSID-III at 24 months of age. To our knowledge, this is the first report of a putative biomarker for early assessment of premature-born infants that is physiologically relevant to multiple organ systems and is easily and reproducibly measurable through minimally invasive technique.

2 | METHODS

2.1 | Study design

The initial exploratory analysis involved a prospective cohort study of newborns admitted to the NICU at West Virginia University (WVU) Children's Hospital in Morgantown, WV between January 2011 and September 2012. To determine the extent to which the observed trends applied to new subjects who were not part of the initial analysis, data from the exploratory cohort were used to power a new independent trial—"Neurotrophin Expression in Infants as a Predictor of Respiratory and Neurodevelopmental Outcomes" ([ClinicalTrials.gov](https://clinicaltrials.gov/ct2/show/study?term=14-1270) Unique Protocol Identifier: 14-1270)—aimed at testing the association between serum BDNF and NGF concentrations and clinical outcomes in a prospective validation cohort of full-term and preterm born newborns admitted to four separate NICUs located in Northeast Ohio, at the Cleveland Clinic Main Campus, Hillcrest Hospital, Fairview Hospital, and MetroHealth Hospital between February 2014 and January 2016.

We compared three groups: (i) premature infants who developed BPD; (ii) premature infants who did not develop BPD; and (iii) term infants. Prematurity was defined as gestational age (GA) <37 complete weeks. Infants who had undergone transfusion at any time prior to sample collection were excluded from this study because of concerns that the transfused blood could alter serum neurotrophins concentrations. The Institutional Review Boards of the West Virginia University, Cleveland Clinic Health System, and MetroHealth System approved all protocols followed in this study and written parental informed consent was obtained from all participants.

A subgroup of preterm-born infants enrolled in the validation cohort and receiving follow-up care at the Cleveland Clinic underwent neurodevelopmental testing administered by specialized pediatricians and nurse specialists using BSID-III at 24 months of age. Outcome measures included the cognitive composite score (CCS), language composite score (LCS), and motor composite score (MCS).

2.2 | Supporting information

All definitions regarding maternal and neonatal characteristics and outcomes are summarized in appendix S1, whereas all information about sample processing and data collection can be found in appendix S2.

2.3 | Statistical analysis

Data were analyzed in collaboration with the Cleveland Clinic's Department of Quantitative Health Sciences using the Statistical Analysis System (SAS) software version 9.4 (SAS Institute, Cary, NC). All analyses were performed on a complete-case basis and all tests

were two-tailed and performed at an overall significance level of 0.05. Data from the exploratory study conducted at WVU (log-scale mean and standard deviation for each study group) were used to perform sample size calculations for the validation study conducted in Ohio. We computed sample sizes required to compare each pair of groups using a Wilcoxon rank sum test with an alpha level of 0.05 for each pairwise comparison and power of 80%. Calculations were performed using the POWER procedure in the SAS 9.4 software.

For the validation study, subject sibling groups were identifiable and analyses account for the correlation between siblings using generalized linear models with Generalized Estimating Equations (GEE). To assess the association between serum neurotrophins in sets of siblings, two siblings from each family were randomly selected and Pearson correlation coefficients with 95% confidence intervals from the Fisher's Z transformation were computed on their data. Study groups were compared on continuous and categorical patient and maternal characteristics using linear and logistic regression models with GEE. An overall significance criterion of 0.05 was used for the effect of group with the Tukey's Multiple Comparison procedure for pairwise group comparisons. Fisher's Exact and Wilcoxon Rank-Sum tests were used to compare groups on categorical characteristics where sample size did not allow the use of these regression models.

Alternating logistic regression was used to predict the development of BPD by serum neurotrophins in the subgroup consisting of preterm infants only, considering multiple births. The alternating logistic model is a special type of logistic model with GEE, modeling within-sibling correlated as a log odds ratio. Similarly, linear regression models with GEE were used to predict GA as a function of serum BDNF and multiple births. Because of the large proportions of subjects with no days of oxygen or ventilation therapy, zero-inflated negative binomial models for ventilator days and oxygen days were created to assess the association between serum neurotrophin levels and these clinical outcomes after adjusting for GA, GA, NGF or BDNF, and their interaction were entered into both the negative binomial and logistic regression parts of the model and were included in the final model if significant at the 0.05 level. GA and serum neurotrophins were assessed for inclusion in the models as continuous variables (normal scale and log-transformed) or classified in quartiles. Models with the smallest Akaike Information Criteria that showed non-significant over dispersion via a Chi-square test were selected as the final models. The analysis of clinical outcomes was performed using one randomly chosen subject per family due to the strong correlation between both serum neurotrophin levels and clinical outcomes among siblings.

For the analysis of neurodevelopmental data, comparisons of continuous variables were performed by ANOVA or Kruskal Wallis test depending on the distribution of the variable. Chi-Square test or Fisher's Exact test were used to compare binary and nominal categorical variables, while Kruskal Wallis test was used with ordinal variables. Associations between serum neurotrophins concentrations at birth and BSID-III scores were calculated using the Spearman Correlation Coefficient (Rho).

3. | RESULTS

3.1 | Exploratory cohort

Fifty-four intubated and mechanically ventilated newborns admitted to the WVU NICU were included in the exploratory study. Among these, 10 were born at full term, 21 were preterm born infants who did not develop BPD, and 23 were preterm born infants who developed BPD. Full-term newborns required intubation for conditions including congenital heart disease, congenital diaphragmatic hernia, respiratory distress, and persistent pulmonary hypertension. In this pilot study (Table S3; see Supporting Information), the median concentration of NGF in the TAF was lowest in full-term infants, doubled in preterm infants who did not develop BPD, and almost tripled in preterm infants who developed BPD. Also, median BDNF concentration in the TAF of full-term newborns was much lower than in preterm newborns independently from later development of BPD. A different trend was observed with the blood assay. Median serum concentrations of NGF and BDNF in the full-term group tended to be similar to the preterm without BPD group, but much higher than the preterm with BPD group.

Due to the limited sample size, differences in neurotrophins concentration among the three groups did not reach statistical significance, but these data allowed to perform sample size calculations to power the independent multicenter validation study described below. Also, BDNF and NGF were not significantly correlated with each other in the serum, and the concentration of each neurotrophin in serum was not significantly correlated with the concentration of the same neurotrophin in the TAF. As expected (Table S4; see Supporting Information), the three groups differed significantly for gestational age, birth weight, antenatal steroid exposure, number of surfactant doses, number of days of invasive ventilation, duration of oxygen supplementation, length of stay (LOS) in the hospital, and severe intraventricular hemorrhage (IVH).

3.2 | Validation cohort

Of the 647 newborns screened, 223 were consented and 171 were included in the final analysis. Among these, 47 were born at term, 92 were preterm born infants who did not develop BPD, and 32 were preterm born infants who developed BPD (Figure 1). As in the exploration cohort, the study groups included in the validation cohort differed significantly for GA and birth weight ($P < 0.001$), but the distribution of gender, race, and ethnicity did not differ significantly among the groups (Table 1). Also, the three groups differed significantly for multiple births, siblings in the study, birth by vaginal delivery, 1- and 5-min Apgar scores, antenatal exposure to steroids, number of surfactant doses, antenatal antibiotic exposure, number of days with invasive ventilation or supplemental oxygen, discharge with supplemental oxygen, and number of co-morbidities including retinopathy of prematurity (ROP) and IVH.

Notably, serum BDNF concentration at birth differed significantly among the three groups in that preterm infants with BPD had significantly lower serum BDNF than preterm infants without BPD and term infants (Figure 2; $P < 0.001$). NGF serum concentration at birth also differed significantly among groups ($P = 0.010$), as preterm infants with BPD had

significantly lower serum NGF) than preterm infants without BPD and term infants. Serum BDNF concentration at birth was not associated with race (white vs others), ethnicity (Hispanic vs non-Hispanic), or maternal smoking ($P = 0.27, 0.80, 0.19$, respectively). However, in univariable analysis, BDNF was significantly lower in male versus female infants ($P = 0.001$) and remained lower in males after adjusting for multiple birth and GA ($P = 0.003$; data not shown). Also, this study cohort contained 21 sets of siblings, including 18 pairs of twins and 3 sets of triplets. Serum neurotrophin levels were strongly correlated within sets of siblings in the study cohort, and the Pearson correlation coefficient (95% confidence interval) for sibling pairs (two chosen at random from sets of triplets) was 0.69 (0.35–0.86) for BDNF and 0.94 (0.86–0.98) for NGF (Figure S5).

There was a moderately strong positive association between serum BDNF concentration and GA (Spearman Correlation Coefficient = 0.51), and a linear regression model for GA predicted by a function of serum BDNF and multiple birth suggested serum BDNF levels rise during the third trimester and level off when GA reaches full term. Furthermore, twins and triplets had significantly lower predicted GA (mean 3.0 weeks; 95% confidence interval 1.6–4.3 weeks) at a given BDNF than singleton newborns (Figure S6). In an alternating logistic regression model for BPD in preterm infants ($n = 124$) predicted by GA and serum BDNF, we found lower levels of serum BDNF were significantly associated with higher risk of BPD ($P = 0.011$) after adjusting for GA (Figure 3). In contrast, serum NGF did not correlate with BPD risk in preterm infants after adjusting for GA ($P = 0.64$).

Of the 171 newborns, 117 (68%) had no days on the ventilator, and 106 (62%) had no days on oxygen. The associations between serum BDNF, serum NGF, and the number of days of invasive ventilation and supplemental oxygen were assessed in separate zero-inflated models for each outcome and neurotrophin using one randomly selected subject per family ($n = 147$). In these models, high levels of serum BDNF were associated with a low probability of any ventilator or oxygen use ($P = 0.009$ and $P = 0.015$, respectively), and high levels of serum NGF (analyzed on the log scale) were associated with fewer days of ventilator or oxygen use ($P = 0.001$ and $P = 0.003$, respectively). GA was analyzed in quartiles in the NGF-ventilator model and as a continuous variable in all other models. There was no evidence that the associations between clinical outcomes and serum neurotrophin levels differed by GA (all interactions $P > 0.05$), although newborns with low GA were at the highest risk for ventilator and oxygen use and required the highest number of days of use (Figure 4).

Forty-three premature infants enrolled in the verification cohort with a median GA of 29.9 [interquartile range, IQR 26.9, 31.6] weeks received follow-up care at the Cleveland Clinic, and 39 (91%; 27 infants without BPD and 12 with BPD) of them completed the BSID-III at median chronological age of 24.1 [23.2, 26.1] months and 22.1 [21.1, 24.1] months corrected age (Table 2). Median CCS was 100.0 [90.0, 105.0]; median LCS was 94.0 [83.0, 100.0]; and median MCS was 100.0 [91.0, 107.0]. Serum BDNF levels measured at birth showed no significant correlation with neurodevelopmental outcomes, whereas serum NGF levels measured at birth had highly significant positive correlation with LCS ($P < 0.001$) and significant positive correlation with CCS ($P = 0.010$), but no significant correlation with MCS ($P = 0.61$).

4 | DISCUSSION

Advances in neonatal care have increased the survival of extremely premature infants, but the prevalence of BPD has not decreased,^{7,8} and children with BPD are twice as likely to require re-hospitalization in the first 2 years of life than premature children without BPD.⁹ Although all infants born preterm have higher rates of respiratory problems than infants born at full term,^{9–11} many infants with BPD experience sequelae even into adolescence and adulthood,¹² and long-term follow-up of these infants has documented airway hyperreactivity,^{13,14} hyperinflation,¹⁴ higher rates of airway obstruction,^{9,14,15} and impairment in gas transfer.¹⁵

4.1 | Primary outcomes

In this study, we show a highly significant negative correlation between serum BDNF concentration measured at birth and the risk of later diagnosis of BPD based on the definition published by Jobe and Bancalari in 2001 on behalf of an NICHD/NHLBI consensus panel. In more practical terms, using the alternating logistic regression model shown in Figure 3, we can predict the probability of the development of BPD with great accuracy just by knowing the infant's GA and serum BDNF level. For instance, an infant born at 28 weeks GA with a serum BDNF level 10 000 pg/mL will have 40% probability of being diagnosed with BPD 1 month later, whereas an infant born with the same GA will have 60% chance of BPD if serum BDNF level is 5000 pg/mL or only 10% if serum BDNF level is 20 000 pg/mL. Also, if serum BDNF is 10 000 pg/mL at birth, the probability of BPD diagnosis is 90% if GA is 25 weeks, but only 40% if GA is 28 weeks.

Furthermore, we have shown that lower serum BDNF and NGF levels at birth are significantly associated with worse clinical outcomes during the neonatal period, for example, a longer requirement for invasive mechanical ventilation and oxygen supplementation, even after adjusting for GA. Therefore, this simple, inexpensive, and minimally invasive test, either alone or in combination with GA, can improve our ability to prognosticate earlier the development of chronic lung disease and adopt a more precise management strategy for premature newborns. Indeed, several strategies to reduce the onset of BPD—including corticosteroids, vitamin A, and caffeine—have been tested in randomized trials, but only a few have shown significant results, and their use remains controversial.¹⁶ One of the possible explanations might be the inability to identify “at risk” subgroups shortly after premature birth, thereby failing to include those patients who will benefit most from treatment.

Importantly, the strong correlation among siblings with regards to serum neurotrophins concentration suggests that their expression is primarily driven by genetic determinants and the prenatal environmental milieu. Accordingly, the trends observed in the serum data from the exploratory cohort were fully confirmed in the validation cohort despite the two sample populations were completely independent, managed by different medical teams, and living in different geographical areas, that is, rural West Virginia versus urban Northeast Ohio.

In the exploratory cohort, we also measured local airway neurotrophins concentration in samples obtained by suctioning the newborns' trachea within the first 48 h of life.

Surprisingly, we observed a trend opposite to the systemic concentration, that is, higher NGF and BDNF concentration in premature infants that eventually developed BPD compared to preterm without BPD and term newborns. This finding is consistent with previous studies showing localized NGF overexpression in the bronchoalveolar lavage of patients with airway inflammatory conditions, including bronchiolitis, asthma, and allergic rhinitis.^{17–19} Although BPD is known to have multifactorial etiology, inflammatory mechanisms operating within the respiratory tract play a major role in the pathophysiology of this condition,^{20,21} and because signaling through the NGF/TrkA axis exerts profound and highly compartmentalized effects on neurons, resident airway cells, and immuno-inflammatory pathways,⁶ it is likely to also play a role in the pathogenesis of BPD.

On the other hand, the detection of lower BDNF serum concentration in the infants with BPD included in the exploratory cohort is consistent with previous observations that physiologic expression of the high-affinity BDNF receptor (TrkB) is necessary for the normal development of the lung. Indeed, selective mutation of this gene results not only in altered airway innervation and deficits in control of breathing but also in structural abnormalities of the lung parenchyma similar to those observed in BPD.²² Nonetheless, the small sample size and the number of patients for whom samples could not be obtained limited this preliminary study. Therefore, we elected to independently confirm our findings in a larger multicenter cohort. This validation study focused on serum measurements because it was done after widespread implementation of the intubation-surfactant-extubation (INSURE) strategy, which promoted immediate reinstitution of Nasal Continuous Positive Airway Pressure (NCPAP) after surfactant administration²³ and rapidly decreased the number of intubated patients from whom TAF samples could be obtained.

For the analysis of all data reported throughout this manuscript, we used only the current NICHD/NHLBI definition of BPD. However, for a complete assessment, we also verified whether our results would have been different if alternative definitions of BPD were considered. Under the 28-day definition, infants were diagnosed as having BPD if they required $\text{FiO}_2 > 21\%$ at any point by day of life 28. Under the 36-week postmenstrual age definition, infants were diagnosed as having BPD if they required $\text{FiO}_2 > 21\%$ at 36 weeks post-menstrual age. Along with the NICHD/NHLBI definition, these definitions were grouped to create a composite definition of BPD. Results using the composite definition of BPD were similar to the data presented here with minimal changes to subject classification.

Our data also show a significant positive association between serum BDNF concentration at birth and GA and build upon a previous analysis of cord blood samples demonstrating that serum BDNF levels are highest in infants born at 36 weeks GA and lowest in infants born at 24–28 weeks.²⁴ This is important because GA is not always precisely known due to menstrual irregularities, uncertain date of conception, or lack of adequate prenatal care, and even the popular Ballard Maturational Assessment is highly subjective and biased by inter-operator variability.²⁵ Indeed, the estimated GA of a neonate using the complete Ballard scale could differ by more than 7 weeks if the same neonate were rated by another physician, while the neurologic and physical parts of the scale are even more inaccurate with differences up to 9–10 weeks.²⁶

4.2 | Secondary outcomes

In addition to the respiratory complications, strong epidemiologic evidence suggests that children with BPD are also at increased risk for neurodevelopmental sequelae compared to premature children without BPD, which surprisingly cannot be predicted by the severity of their respiratory impairment.²⁷ In our study, serum NGF concentration measured at birth in premature newborns was strongly associated with their language outcomes at 24 months, and a significant correlation was also found for cognitive outcomes. In contrast, NGF was not predictive of motor development, and no relationship was found between BDNF and any neurodevelopmental variable, which confutes the conclusions of a previous smaller study using cord blood samples and proposing BDNF as a better marker of brain maturity.²⁴ The association of different neurotrophins with different clinical outcomes reflects different patterns of biological activity targeting central and peripheral neuronal subpopulations, as well as non-neuronal tissues.

The results of the present study are consistent with our previous work, in which we found that children with severe head trauma develop within 2 h a remarkable increase in the cerebrospinal fluid (CSF) concentration of NGF, with a further increase at 48 h post-trauma.²⁸ More importantly, higher NGF levels after head trauma not only correlated with the severity of neurologic compromise but also predicted better neurological outcomes confirming the neuroprotective role exerted by this factor. In the same study, BDNF exhibited only modest change, and its concentration did not correlate with the severity of brain injury or with neurologic outcomes, suggesting that different neurotrophic factors act on different target cells and neural pathways and serve different functions after CNS injury. Our findings are also consistent with the timing of neurotrophic factors expression in animal models of experimental brain lesions, indicating that this is an early event upstream from the complex cascade of local inflammatory host response after CNS injury.²⁹

The overexpression of NGF at birth is likely to represent a critical defensive mechanism of the premature brain in response to neuronal death following brain injury. In addition, NGF up-regulation may also play a key role in the ensuing inflammatory response and have beneficial impact on the regenerative capacity of the injured brain by arresting the ongoing degeneration of cholinergic projections to the cerebral cortex and upregulating central nicotinic receptors, which results in improved cortical blood flow and counteracts cerebral ischemia and neuronal death.³⁰ Because of the immaturity of the blood-brain barrier at this stage, serum neurotrophins concentrations reflect concentrations of the same proteins in the CNS,³¹ making them useful for the prognosis of long-term neurodevelopmental outcomes. Even more importantly, it has been reported that intraventricular NGF administration improves neuronal survival in infants suffering from brain injury, and that neurotrophic factors promote striatal neurogenesis and cellular proliferation after brain injury, making the therapeutic administration of exogenous NGF an intriguing therapeutic option for the management of neurodevelopmental sequelae of prematurity.³²

Among the limitations of this study, neurodevelopmental outcomes were a secondary endpoint analyzed post hoc and for which the study was not powered. Therefore, further analysis is needed as more data are collected. Nevertheless, the findings of the present study are novel, provide preliminary data for a later larger study, and suggest that serum

neurotrophins meet most of the criteria defining an “ideal” biomarker. Indeed, they are relatively easy to obtain, quickly and inexpensively measured, physiologically relevant to the disease condition, and accurately predict relevant and actionable clinical outcomes across a variety of treatments and populations.³³

Although other strategies have been proposed for the prediction of BPD and its complications,^{34–36} our model performed better than most other published biomarker with a ROC AUC 0.960 and was also significantly better than other popular respiratory biomarkers like fractional exhaled NO (FeNO) or periostin (both with ROC AUC of approximately 0.7).³⁷ Other strengths of this study compared to previous publications include the relatively large size of the sample used as independent confirmation cohort and its stringent adherence to the NICHD/NHLBI consensus definition of BPD. Furthermore, we find remarkable that, despite substantial differences between the two cohorts with respect to macro-environmental conditions (eg, rural vs urban), the confirmation of the biomarker validity emphasized the robustness of the results. Nonetheless, we do recognize that the relatively small number of infants diagnosed with BPD is an important limitation of our study.

In summary, we have shown that measurements of serum neurotrophic factors concentration in premature newborns can improve our ability to prognosticate at birth their risk of being diagnosed with BPD and their clinical outcomes. BDNF serum concentration at birth correlates with duration of invasive mechanical ventilation and supplemental oxygenation. NGF serum concentration at birth is associated with long-term neurodevelopmental outcomes. Serum BDNF and NGF are physiologically relevant to lung and brain development, easily measurable with minimal costs, and accurately predict relevant clinical outcomes across a variety of treatments and populations. In clinical practice, these biomarkers may aid neonatologists to make early management decisions, as well as set expectations for the family during and after the NICU admission. On the research side, neurotrophins can help understand better the pathophysiology of the disease, provide early indications on the disease process, help develop clinical guidelines, determine the population that will respond best to a particular treatment, perhaps even open the path to future replacement therapy.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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FIGURE 1. Serum neurotrophins at birth correlate with respiratory and neurodevelopmental outcomes of premature infants

Summary of recruitment (validation cohort). A total of 647 newborns were screened, and 171 were included in the final analysis. Among these, 47 were term newborns, 92 preterm newborns who did not develop BPD, and 32 preterm newborns who developed BPD.

FIGURE 2. Serum neurotrophins at birth correlate with respiratory and neurodevelopmental outcomes of premature infants

Serum neurotrophins measurements. (A) Boxplot showing serum brain-derived neurotrophic factor (BDNF) protein levels by study group; (B) scatter plot showing serum BDNF protein levels by gestational age; (C) boxplot showing serum nerve growth factor (NGF) protein levels by study group; (D) scatter plot showing serum NGF protein levels by gestational age. Significant differences in serum levels of both BDNF ($P < 0.001$) and NGF ($P = 0.010$) were found at birth among the three study groups.

FIGURE 3. Serum neurotrophins at birth correlate with respiratory and neurodevelopmental outcomes of premature infants

Prediction of BPD Risk. Plots show an alternating logistic regression model for BPD in preterm infants ($n = 124$) predicted by gestational age and serum BDNF with adjustment for correlation among siblings. Curves of GA <25 weeks were not shown due to small sample size; curves of GA >33 weeks were not shown because the probabilities were small for all serum BDNF levels.

FIGURE 4. Serum neurotrophins at birth correlate with respiratory and neurodevelopmental outcomes of premature infants

Prediction of number of days of ventilation and oxygen supplementation. Gestational age was analyzed in quartiles in the NGF-ventilator model and as a continuous variable in all other models, with gestational ages of 27 (solid light gray line), 32 (dashed light gray line), 36 (solid black line), and 40 (dashed black line) weeks used for prediction.

TABLE 1

Clinical and demographic comparisons among term, preterm without BPD, and preterm with BPD infants in the validation cohort

Variable	Full term (n = 47)	Preterm without BPD (n = 92)	Preterm with BPD (n = 32)	P-value
Gestational age (weeks), median [Q1, Q3]	39.7 [38.6, 40.3] ^{2,3}	33.9 [31.4, 35.1] ^{1,3}	26.0 [25.6, 27.9] ^{1,2}	<0.001 ^a
Birth weight (grams), median [Q1, Q3]	3590.0 [3115.0, 3850.0] ^{2,3}	1930.0 [1430.0, 2330.0] ^{1,3}	808.5 [718.5, 962.5] ^{1,2}	<0.001 ^a
Gender (female), n (%)	24 (51)	48 (52)	13 (41)	0.56 ^b
Race, n (%) [*]				0.53 ^d
Black or African American	8 (17)	19 (21) ³	10 (31) ²	
White	33 (70)	62 (67)	17 (53)	
Other	6 (13)	11 (12)	5 (16)	
Ethnicity, n (%) [*]				0.99 ^d
Hispanic/Latino	2 (4)	3 (3)	1 (3)	
Non-Hispanic/Latino	38 (81)	67 (73)	26 (81)	
Unknown/not reported	7 (15)	22 (24)	5 (16)	
Multiple births, n (%)	0 (0) ^{2,3}	37 (40) ¹	12 (38) ¹	<0.001 ^d
Maternal age (years), n [Q1, Q3]	29.0 [25.0, 33.0]	30.0 [26.0, 33.0]	29.0 [26.0, 33.5]	0.41 ^a
History of smoking, n (%)	20 (43)	32 (35)	15 (47)	0.52 ^b
Reported drug use, n (%)	8 (17)	8 (9)	2 (6)	0.34 ^b
Preeclampsia, n (%)	4 (9)	17 (18)	7 (22)	0.20 ^b
Chorioamnionitis, n (%)	19 (40)	20 (22)	12 (38)	0.061 ^b
Gestational diabetes, n (%)	5 (11)	13 (14)	1 (3)	0.26 ^d
Antenatal steroids, n (%)	1 (2) ^{2,3}	56 (61) ¹	28 (88) ¹	<0.001 ^d
Antenatal antibiotics, n (%)	37 (79) ²	88 (96) ¹	30 (94)	0.038 ^c
Method of delivery, n (%)				0.004 ^b
Vaginal delivery	30 (64) ³	46 (50)	7 (22) ¹	
Cesarean section	17 (36)	46 (50)	25 (78)	
One minute apgar, median [Q1, Q3]	8.0 [7.0, 8.0] ³	7.0 [6.0, 8.0] ³	3.5 [1.2, 7.0] ^{1,2}	<0.001 ^a
Five minute apgar, median [Q1, Q3]	9.0 [8.0, 9.0] ^{2,3}	8.0 [7.0, 9.0] ^{1,3}	6.0 [5.0, 8.0] ^{1,2}	<0.001 ^a
Ten minute apgar (if applicable) [*] , median [Q1, Q3]	7.0 [6.5, 8.0]	8.0 [7.0, 8.0]	6.0 [6.0, 7.0]	0.11 ^a
Doses of surfactant, n (%)				<0.001 ^d
0	45 (96) ^{2,3}	70 (76) ^{1,3}	1 (3) ^{1,2}	
1	1 (2)	17 (18)	13 (41)	
2	0 (0)	3 (3)	12 (38)	

Variable	Full term (<i>n</i> = 47)	Preterm without BPD (<i>n</i> = 92)	Preterm with BPD (<i>n</i> = 32)	<i>P</i> -value
3	1 (2)	2 (2)	6 (19)	
Invasive ventilation (days), median [Q1, Q3]	0.0 [0.0, 0.0] ³	0.0 [0.0, 0.0] ³	14.5 [3.0, 24.0] ^{1,2}	<0.001 ^c
Supplemental oxygen (days), median [Q1, Q3]	0.0 [0.0, 0.0] ^{2,3}	0.0 [0.0, 2.0] ^{1,3}	76.0 [40.0, 109.0] ^{1,2}	<0.001 ^c
Home oxygen, <i>n</i> (%)	0 (0) ³	0 (0) ³	8 (26) ²	0.001 ^d
Length of hospital stay (days), median [Q1, Q3]	5.0 [3.0, 6.0] ^{2,3}	20.0 [11.0, 45.0] ^{1,3}	105 [77.0, 120.0] ^{1,2}	<0.001 ^a
Retinopathy of prematurity, <i>n</i> (%)	0 (0) ³	15 (16) ³	22 (69) ^{1,2}	<0.001 ^d
Intraventricular hemorrhage, <i>n</i> (%)	0 (0) ³	4 (4) ³	12 (38) ^{1,2}	<0.001 ^d
I	0 (0)	3 (100)	4 (36)	
II	0 (0)	0 (0)	2 (18)	
III	0 (0)	0 (0)	1 (9)	
IV	0 (0)	0 (0)	4 (36)	
Culture positive sepsis, <i>n</i> (%)	1 (2)	1 (1)	3 (9)	0.074 ^d

* Data not available for all subjects. Missing values: Ten-min Apgar = 143; Home oxygen = 1. *p*-values:

^alinear regression with GEE;

^blogistic regression with GEE;

^clogistic regression with GEE with outcome analyzed as any versus none;

^dFisher's Exact test with Bonferroni correction for multiple comparisons;

^eWilcoxon rank sum test with Steel-Dwass multiple comparison.

Tukey's multiple comparison procedure was used for pairwise ad-hoc comparisons unless otherwise stated:

¹Significantly different from full term;

²Significantly different from preterm without BPD;

³Significantly different from preterm with BPD.

TABLE 2

Correlation between serum neurotrophins (NT) concentrations at birth and BSID-III scores at 24 months of age

NT	Variable	<i>n</i>	Rho	95% CI	<i>P</i> -value
BDNF	Cognitive composite score	39	0.20	(−0.13, 0.53)	0.22
	Overall language composite score	39	0.04	(−0.29, 0.38)	0.79
	Overall motor composite score	39	0.13	(−0.20, 0.46)	0.43
NGF	Cognitive composite score	39	0.41	(0.11, 0.71)	<i>0.010</i>
	Overall language composite score	39	0.57	(0.30, 0.84)	<i><0.001</i>
	Overall motor composite score	39	0.08	(−0.25, 0.42)	0.61

Bold and italics express statistically significant values.