

# Vaginal Delivery Is Associated with Neurochemical Evidence of Increased Neuroaxonal Remodelling in Infants from the KUNO-Kids Health Study: Cross-Sectional Analysis

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## Keywords

Biomarker · Birth mode · Central nervous system · Neuron · Serum

## Abstract

**Aim:** Little is known about neonatal brain plasticity or the impact of birth mode on neurointegrity. As a reflection of neuroaxonal damage, the neuronal structural protein neurofilament light chain (NfL) has emerged as a highly specific biomarker. Our purpose was to test the hypothesis that vaginal delivery is associated with increased NfL in neonates.

**Methods:** NfL concentrations were measured using single-molecule array immunoassay in umbilical cord serum from healthy term neonates enrolled in the prospective KUNO-Kids Health Study. NfL values were investigated for independent influencing factors using linear and logistic models, followed by post hoc propensity score-matching. **Results:** Of 665 neonates,  $n = 470$  (70.7%) were delivered vaginally and  $n = 195$  (29.3%) by cesarean section. Median serum NfL was significantly higher after vaginal delivery 14.4 pg/mL (11.6–

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18.5) compared to primary 7.5 pg/mL (6.1–8.9) and secondary cesarean delivery 9.3 pg/mL (7.5–12.0). Multivariable logistic regression models showed delivery mode and gestational age to be independently associated with NfL. Propensity score-matching analysis confirmed that assisted vaginal delivery generated higher NfL compared to vaginal (non-assisted), while lowest levels were associated with cesarean section. **Interpretation:** Our data confirm the significant impact of birth mode on neonatal NfL levels. The persistence of these differences and their potential long-term impact have yet to be investigated.

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## Introduction

Cesarean section (CS) rates continue to rise worldwide, fueling the interminable debate on the impact of delivery mode on the long-term health of our next generation [1]. Recent World Health Organization data show wide-ranging CS rates, from about 2% in Madagascar and Ethiopia to over 50% in Brazil and Egypt [2], propelled by factors not only medical but also cultural, social, and economic [3]. Effects on child development, whether cardiorespiratory [4, 5], metabolic [1, 6], or immunologic [7], have been widely discussed. However, we know relatively little about the impact of parturition on neonatal neurointegrity [3]. Yet, early neuronal damage can lead to brain volume loss and the development of neuronal disorders throughout life [8]. Hence, identifying a diagnostic and prognostic biomarkers of recent, ongoing, and future neurologic damage will enable both early-stage detection and optimized care [9]. The difficulty of noninvasive access to the central nervous system (CNS) explains why the quest for neurologic biomarkers has been ongoing for years.

There are a couple of promising neurologic biomarkers for neurological brain injury all present within cells of the CNS, including S100B in glial cells, neuron-specific enolase in neurons, and neurofilaments (Nf) expressed in axons [10]. As members of the intermediate filament family, Nf are highly specific neuronal scaffolding proteins comprising of four subunits: the Nf chain triplet – light (NfL), medium (NfM), and heavy (NfH) – plus alpha-internexin in the CNS or peripherin in the peripheral nervous system [10]. Upon neuroaxonal damage, Nf are released into cerebrospinal fluid and blood compartment [10], prompting the recent development of a highly sensitive single-molecule array (Simoa) immunoassay detecting even low concentrations of NfL in peripheral

blood [11]. Subsequent studies have validated NfL as a biomarker for neuroaxonal injury [12].

Our group recently proposed NfL as a blood biomarker for neonatal injury and showed for the first time that cord blood NfL levels are higher after vaginal delivery (VD) than after primary (elective) CS (PCS). We also found higher NfL levels in the first week of life in brain-damaged infants [8]. Further studies have shown that serum NfL dynamics in early life predict neurodevelopmental outcome in preterm infants with and without intraventricular hemorrhage [13, 14]. We conducted this study to test the hypothesis that cord blood NfL levels reflect the mode of delivery, to explore additional potential predictors of NfL at birth, and to establish a large database of NfL values in healthy neonates for future studies with sick neonates of all kinds, including, e.g., perinatal hypoxic-ischemic encephalopathy, perinatal stroke, and congenital infections.

## Materials and Methods

This study was part of the ongoing prospective birth cohort KUNO-Kids Health Study based in the Perinatal Center at the University Children's Hospital Regensburg, Germany [15]. The study was approved by the Ethics Committee of the University of Regensburg (file number: 14-101-0347 and 19-1646-101). Participating parents provided written informed consent. All cord blood samples collected in the KUNO-Kids Health Study from the start of 2015 through the end of 2019 were available for the study. Of the total  $n = 717$  newborns with umbilical cord blood samples, we excluded the following: (a)  $n = 20$  withdrawals of consent; (b)  $n = 5$  incomplete data; (c)  $n = 12$  insufficient serum volume remaining; (d)  $n = 13$  multiples; and (e)  $n = 2$  transfers to neonatology care, leaving a total of 665 serum samples from healthy singleton term newborns remaining with their mothers after birth who were eligible for analysis.

Maternal and infant's characteristics are summarized in Table 1, and definitions were used as previously published [16]. Details on any complications prior to pregnancy (including asthma bronchiale, diabetes, hypothyreosis, depression, thrombophilia, inflammatory bowel disease, obesity), any prenatal complications occurring during pregnancy (preeclampsia, gestational diabetes, complications in previous pregnancy), or any delivery complications (including medical induction of labor, premature rupture of membranes, non-reassuring CTG, fetus outside weight centiles 3–97, and signs of maternal or fetal infection) were collected from the charts. Signs of maternal or fetal infection were defined as the presence of at least one of the following parameters at delivery: maternal fever  $>38^{\circ}\text{C}$ , increased CRP or leukocyte counts, stained fluid, fetal tachycardia.

All umbilical cord blood samples were collected in serum tubes according to a standard operating procedure, followed by transfer to the central laboratory service, centrifugation, distribution into aliquots, and storage at  $-80^{\circ}\text{C}$  until batchwise analysis. Technicians were blinded to neonate's clinical information. NfL was measured using the highly sensitive Simoa enzyme-linked immuno-

**Table 1.** Baseline characteristics stratified by delivery mode

Outcome	Total (n = 665)	VD (n = 470)	Primary C-section (n = 97)	Secondary C-section (n = 98)	p value
<i>Maternal characteristics</i>					
Maternal age, years	32 (29, 35)	32 (28, 34)	33 (31, 36)	33 (30, 35)	<0.001
Gravidity, 2 or more, n (%)	330 (49.6)	220 (46.8)	65 (67.0)	45 (45.9)	0.001
Parity, 2 or more, n (%)	261 (39.2)	174 (37.0)	58 (59.8)	29 (29.6)	<0.001
GA, weeks	39.7 (38.9, 40.6)	40.0 (39.3, 40.6)	38.7 (38.1, 39.1)	39.7 (40.0, 40.7)	<0.001
Any complications prior to pregnancy, n (%)	245 (36.8)	156 (33.2)	51 (52.6)	38 (38.8)	0.001
Any prenatal complications, n (%)	318 (47.8)	185 (39.4)	76 (78.4)	57 (58.2)	<0.001
Any delivery complications, n (%)	404 (60.8)	269 (57.2)	44 (45.4)	91 (92.9)	<0.001
Signs of infection, n (%)	63 (9.5)	41 (8.7)	2 (2.1)	20 (20.4)	<0.001
CTG non-reassuring, n (%)	91 (13.7)	54 (11.5)	2 (2.1)	35 (35.7)	<0.001
Preterm rupture of membranes, n (%)	194 (29.2)	146 (31.1)	–	48 (49.0)	–
Vaginal assistance, n (%)		56 (11.9)	–	–	–
<i>Infant's characteristics</i>					
Sex, n (%)	323 (48.6)	236 (50.2)	46 (47.4)	41 (41.8)	0.31
Birth weight, g	3,430 (3,125, 3,746)	3,140 (3,150, 3,740)	3,470 (2,950, 3,726)	3,473 (3,073, 3,820)	0.54
Birth length, cm	52 (50, 53)	52 (50, 53)	52 (50, 53)	52 (50, 54)	0.58
Head circumference, cm	35 (34, 36)	35 (34, 36)	35 (34, 36)	35 (34, 36)	0.05
Apgar 1 min, n (%)					0.004
<8	38 (5.7)	22 (4.7)	4 (4.1)	12 (12.2)	
8	48 (7.2)	38 (8.1)	2 (2.1)	8 (2.1)	
9	506 (76.1)	359 (76.4)	75 (77.3)	72 (73.5)	
10	73 (11.0)	51 (10.9)	16 (16.5)	6 (6.1)	
Apgar 5 min, n (%)					<0.001
<8	6 (0.9)	0 (0.0)	2 (2.1)	4 (4.1)	
8	16 (2.4)	10 (2.1)	1 (1.0)	5 (5.1)	
9	59 (8.9)	42 (8.9)	5 (5.2)	12 (12.2)	
10	584 (87.8)	418 (88.9)	89 (91.8)	77 (78.6)	
Apgar 10 min, n (%)					<0.001
8	1 (0.2)	1 (0.2)	0 (0.0)	0 (0.0)	
9	29 (4.4)	13 (2.8)	3 (3.1)	13 (13.3)	
10	635 (95.5)	456 (97.0)	94 (96.9)	85 (86.7)	
pH in arterial umbilical cord blood	7.26 (7.20, 7.31)	7.23 (7.19, 7.29)	7.31 (7.28, 7.34)	7.30 (7.27, 7.32)	<0.001
Acidosis (cases with pH <7.15), n (%)	139 (20.9)	132 (28.1)	3 (3.1)	4 (4.1)	<0.001
BE in arterial umbilical cord blood	–4 (–7, –2)	–5 (–7, –3)	–2 (–3, 0)	–3 (–5, –1)	<0.001
NfL, pg/mL	13.0 (9.3, 17.1)	14.4 (11.6, 18.5)	7.5 (6.1, 8.9)	9.3 (7.5, 12.0)	<0.001

Data are median (IQR) or counts (percentages) when appropriate. For definitions of any complications prior to pregnancy, any prenatal complications occurring during pregnancy, any delivery complications or signs of infection please refer to the Materials and Methods section.

sorbent assay (NF-LIGHT; Quanterix Corporation, Billerica, MA, USA). Intra- and interassay variability were both <10%. Repeated measures were performed for the few samples with intra-assay coefficients of variation >20% [8, 16].

#### Statistical Analysis

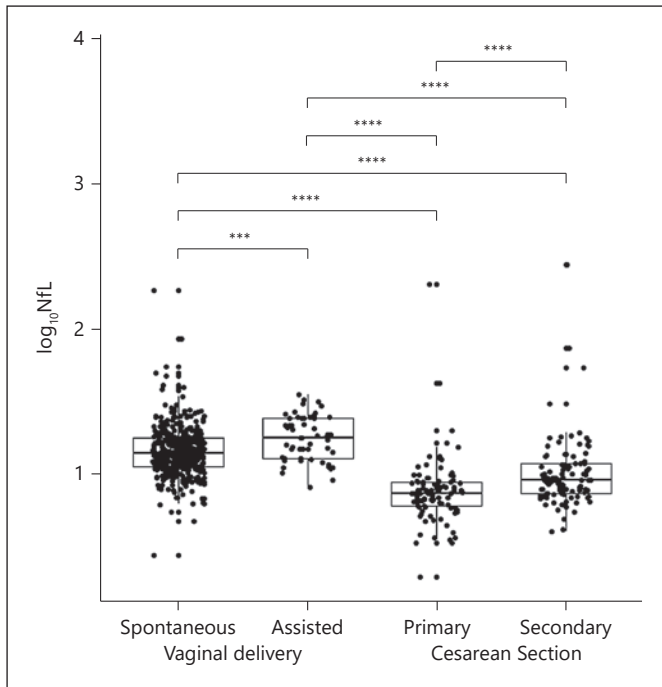
The study population was grouped based on the delivery mode. Spontaneous and assisted (vacuum extraction) VD was compared to PCS and secondary CS (SCS). Differences between groups were summarized using descriptive statistics with the Kruskal-Wallis test for continuous variables and the  $\chi^2$  test (or variants thereof) for categorical variables. Quantitative data are shown as median (IQR) and qualitative data as counts (percentages). Univariable-

and multivariable-adjusted linear models were fitted with  $\log_{10}$  NfL as the dependent variable and the baseline characteristics listed in Table 1 as independent variables. The independent variables selected for multiple linear regression were those statistically significant at the 10% level in univariable analyses. In the event of collinearity in the multivariable analysis, the least statistically significant factor was excluded in multivariable analysis. The multivariable analysis used forward then backward selection with  $p < 0.1$  as the inclusion criteria. VD was used as the reference group throughout. A post hoc 1:1 matched propensity score analysis was performed to confirm the results of the original multiple linear regression. All analyses were performed in R version 3.6.1 (or later) with  $p < 0.05$  considered statistically significant.

**Table 2.** Estimates of factors affecting NfL levels

Outcome log <sub>10</sub> NfL, pg/mL	Univariable		Multivariable	
	estimate (95% CI)	<i>p</i> value	estimate (95% CI)	<i>p</i> value
Delivery mode				
Vaginal	Reference		Reference	
Primary C-section	-0.29 (-0.33, -0.25)	<0.001	-0.24 (-0.28, -0.19)	<0.001
Secondary C-section	-0.17 (-0.21, -0.12)	<0.001	-0.17 (-0.21, -0.12)	<0.001
Gravidity, 2 or more	-0.03 (-0.07, 0.001)	0.05	-	NS
Parity, 2 or more	-0.04 (-0.08, -0.01)	0.01	-	NS
GA (10 days steps)	0.06 (0.05, 0.08)	<0.001	0.05 (0.04, 0.07)	<0.001
Any complications prior to pregnancy	-0.04 (-0.08, -0.01)	0.01	-	NS
Any prenatal complications	-0.05 (-0.08, -0.02)	0.005	-	NS
Head circumference, cm	-0.01 (-0.03, -0.002)	0.02	-0.02 (-0.03, -0.01)	<0.001
Apgar 1 min (per category)	-0.02 (-0.04, -0.00)	0.05	-0.03 (-0.05, -0.01)	<0.001

All independent variables from Table 1 were included in the model, except those collinear. No effect was found for maternal age, any delivery complications, infant's sex, birth weight, birth length, and Apgar 5 and 10 min.



**Fig. 1.** Box plot of NfL levels stratified by delivery mode; pairwise tests adjusted for multiple testing (4 subgroups) with \*\*\**p* < 0.001, \*\*\*\**p* < 0.0001.

## Results

Most of the 665 infants analyzed in this study were born by VD (*n* = 470, 70.7%), split between spontaneous (*n* = 414, 62.3%) and assisted (*n* = 56, 8.4%). The remaining 195 neonates (29.3%) were delivered by CS, *n* = 97 (14.6%) by

PCS, and *n* = 98 (14.7%) by SCS. Population baseline characteristics were stratified by birth mode (Table 1).

In the overall VD population, irrespective of assistance, median serum NfL was 14.4 pg/mL (IQR 11.6–18.5). Those born by assisted VD had significantly higher NfL levels (18.0 pg/mL [IQR 13.1–24.7]) than those born spontaneously (14.1 pg/mL [IQR 11.5–18.1]; *p* < 0.001). In contrast, levels in the CS population were significantly lower compared to overall VD with PCS (7.5 pg/mL [IQR 6.1–8.9]) and SCS (9.3 pg/mL [IQR 7.5–12.0]; *p* < 0.001) (Fig. 1).

In the linear model with log<sub>10</sub> NfL as the dependent variable, both delivery mode (VD as reference) and gestational age (GA) at birth had significant independent influence on NfL (*p* < 0.001), whereas parity and complications, both prior to gestation and during pregnancy, were significant in the univariable but not in the multivariable model (Table 2). Since GA was collinear with other independent variables such as birth weight and length, these variables were excluded from the multivariable analysis (Table 2). Apgar 1 min was an independent predictor in the MV analysis, but pH and BE were not independent predictors of NfL after adjusting for delivery mode and GA.

Another linear model was fitted, focusing on the birth modalities with the highest NfL values (VD and SCS, results not shown). As in the preceding analysis, SCS and GA at birth had significant independent influence on NfL, as did premature rupture of membranes. Due to inherent imbalance between the VD and SCS at baseline, we performed a 1:1 matched propensity score analysis including data from 97 babies, matched on the characteristics in Table 1, which confirmed the independent influ-

ence of SCS (estimate [95% confidence interval]:  $-0.173$  [ $-0.235, -0.112$ ];  $p < 0.001$ ) and GA ( $0.054$  [ $0.019, 0.088$ ];  $p = 0.003$ ) on NfL values.

Lastly, we analyzed the effects of VD in the spontaneous and assisted subgroups using  $\log_{10}$  NfL as the dependent variable. We observed peak NfL levels after adjusting for assisted VD ( $0.067$  [ $0.014, 0.120$ ];  $p = 0.015$ ) and GA ( $0.041$  [ $0.026, 0.055$ ];  $p < 0.001$ ). In contrast, in the CS subgroup analysis, adjustment for PCS ( $-0.246$  [ $-0.289, -0.203$ ];  $p < 0.001$ ), SCS ( $-0.160$  [ $-0.202, -0.118$ ];  $p < 0.001$ ), and Apgar 1 min ( $-0.024$  [ $-0.041, -0.008$ ];  $p = 0.005$ ) was in each case associated with low NfL levels.

## Discussion

Our large cross-sectional study on healthy term neonates showed that serum levels of the neuroaxonal injury marker NfL are twice as high in vaginal-born neonates than in their cesarean-born counterparts. Within each group, NfL levels are higher after assisted than after spontaneous VD, and higher after emergency than after elective CS. Infant stress during birth is commonly recorded by clinical parameters such as the Apgar score and biochemical indicators such as umbilical blood pH and base excess, and these findings were confirmed in our univariable analyses, with both higher pH and Apgar score being correlated with lower NfL levels. Multivariable logistic regression models revealed delivery mode as the most important predictor of serum NfL at birth.

The process of vaginal birth facilitates cardiovascular transition from placental to lung breathing [4]. There is evidence that CS-born infants have different hormonal, physical, bacterial, and medical exposures, any and all of which can subtly alter neonatal physiology, impacting short- and long-term outcomes [17, 18]. However, the effects of CS on the cognitive outcome have only recently attracted the attention they deserve. Although some studies have described lower cognitive performance in CS-born children [19, 20], a recent systematic review found the evidence for such an association to be inconclusive and suggested that future studies better distinguish between elective and emergency CS [21]. In any case, neurointegrity is the basis for future neurodevelopment, while serum NfL is now established as a promising biomarker of neuronal damage, with increased cerebrospinal fluid and serum levels predicting an adverse neurological outcome in both adults and preterm newborns [10, 13].

The evidence we have presented here of increased plasma NfL values in vaginal-born infants compared to

their cesarean-born counterparts is novel and has not been published before. It confirms our recently published observation [8]. Vaginal birth is preceded by uterine contractions that are the natural driving force for the fetal release of stress hormones inextricably linked to neonatal transition [22]. Infant stress during birth is commonly recorded by biochemical indicators such as umbilical blood pH and base excess and clinically by the Apgar score. Multivariable regression analysis revealed that delivery mode accounts strongest for serum NfL values at birth. Delivery mode also remained the dominant predictor of NfL in a propensity score analysis performed to correct for confounders.

The main source of NfL in peripheral blood is the CNS, though not in our context but in general, peripheral nerves also contribute, as demonstrated in adult peripheral neuropathy [23]. NfL is not without precedent: we have known for years that compression of the fetal cranium largely explains the increased serum levels of the CNS biomarker S100B after vaginal, as opposed, to cesarean birth [24, 25]. It has also been shown that even mild traumatic brain injury in rugby players causes a significant increase in plasma NfL 1 hour after the event [26]. Cranial imaging in asymptomatic term neonates reveals intracranial hemorrhage as a common incidental finding in up to one quarter of uneventful vaginal, but not cesarean-born, deliveries [27].

The age-dependent evolution of serum NfL levels is a well-known phenomenon [28]. Normal aging likely entails morphological brain changes, which are strongly related to NfL levels. The rather high levels in newborns have been reported to decrease by late childhood, apparently reflecting the substantial brain growth into adolescence. In adulthood, levels increase linearly until middle age, when they start to rise in a nonlinear manner, reflecting NfL accumulation due to neuronal damage [8, 29]. On the one hand, an accelerated loss of brain mass correlates directly with an increase in NfL levels. On the other, baseline NfL levels appear as strongly independent determinant of future brain volume loss [29]. We therefore need to determine whether the birth mode difference in NfL levels persists beyond the first days of life. A recent study in preterm infants with severe brain damage, namely, intraventricular hemorrhage and periventricular hemorrhagic infarction, investigated the significance of serum NfL dynamics in serial samples taken over the first months of life. NfL levels correlated with maturity, birth weight, postnatal age at measurement, and brain damage severity. NfL independently predicted motor but not cognitive outcome up to 2 years of age [13]. However, as the

cohort was relatively small, an influence on cognitive outcome can by no means be ruled out [8]. In fact, it appears that the key to clinically predictive relevance lies in the individual dynamics of NfL rather than in absolute values [14].

Increased Nf levels imply neuroaxonal damage and point toward neuronal loss [15]. However, in the context of a natural uncomplicated process, namely, spontaneous VD, it appears inappropriate to judge increased NfL when compared to an artificial process, namely, CS, as something negative or futile. Perhaps it is just a matter of insignificant collateral damage in the long term that nature accepts, e.g., the death of neurons already on an irreversible path to apoptosis. But it is also conceivable that increased NfL after spontaneous VD reflects the initiation of a profound developmental process of the brain, also known as synaptic pruning, which is part of natural brain maturation [30].

### *Strengths and Limitations*

Major strengths of our study include the sample size, the prospective design of the KUNO-Kids health study, and the homogeneous population profile, comprising healthy singleton term neonates only, thereby reinforcing the validity of our insights. However, the study also has limitations. First, it lacks serial blood samples enabling us to explore the postnatal dynamics of NfL. Second, we had no complementary cerebral imaging data allowing us to test for an association between NfL level and intracranial hemorrhage. And finally, perhaps most intriguingly, our study lacks information on cognitive outcome.

### **Conclusion**

Our results support the hypothesis that birth mode per se impacts newborn neurointegrity, at least in the short term. We have shown that compared to CS, VD is associated with significantly increased NfL levels in peripheral blood, independently of the biochemical and clinical markers of birth stress, suggesting that mechanical force during VD may play a role. The clinical relevance of these differences have yet to be investigated.

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#### *KUNO-Kids: Members of the Study Group*

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### **Statement of Ethics**

This study was approved by the Ethics Committee of the University of Regensburg (file number: 14-101-0347 and 19-1646-101) and conducted in accordance with the Declaration of Helsinki. Written informed consent was obtained from parents.

### **Conflict of Interest Statement**

Angelika Berger has advised and/or received speaker fees and research and travel support from Abbvie, Chiesi, Pfizer, Schülke, Milupa, Nestle, MCA Scientific Events, and the Anniversary Fund of the Österreichische Nationalbank. Jens Kuhle has advised and/or received speaker fees and research and travel support from EC-TRIMS, the Swiss MS Society, University of Basel, Bayer, Biogen,

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## Author Contributions

Katja Kürner: conceptualization, data curation, investigation, project administration, and writing – original draft and review and editing. Katharina Goeral: data curation, investigation, and writing – original draft and review and editing. Andrew Atkinson: data curation, formal analysis, investigation, methodology, software, validation, visualization, and writing – original draft and review and editing. Susanne Brandstetter: conceptualization, data curation, investigation, methodology, project administration, soft-

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## Data Availability Statement

All data generated or analyzed during this study are included in this article. Further inquiries can be directed to the corresponding author.

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