frontiers | Research Topics

Developmental origins of health and disease: Impact of preterm birth

Edited by
Lynette Kay Rogers, Krithika Lingappan and Jonathan L. Slaughter

Published in
Frontiers in Pediatrics

ISSN 1664-8714
ISBN 978-2-83251-336-1
DOI 10.3389/978-2-83251-336-1

About Frontiers

Frontiers is more than just an open access publisher of scholarly articles: it is a pioneering approach to the world of academia, radically improving the way scholarly research is managed. The grand vision of Frontiers is a world where all people have an equal opportunity to seek, share and generate knowledge. Frontiers provides immediate and permanent online open access to all its publications, but this alone is not enough to realize our grand goals.

Frontiers journal series

The Frontiers journal series is a multi-tier and interdisciplinary set of open-access, online journals, promising a paradigm shift from the current review, selection and dissemination processes in academic publishing. All Frontiers journals are driven by researchers for researchers; therefore, they constitute a service to the scholarly community. At the same time, the *Frontiers journal series* operates on a revolutionary invention, the tiered publishing system, initially addressing specific communities of scholars, and gradually climbing up to broader public understanding, thus serving the interests of the lay society, too.

Dedication to quality

Each Frontiers article is a landmark of the highest quality, thanks to genuinely collaborative interactions between authors and review editors, who include some of the world's best academicians. Research must be certified by peers before entering a stream of knowledge that may eventually reach the public - and shape society; therefore, Frontiers only applies the most rigorous and unbiased reviews. Frontiers revolutionizes research publishing by freely delivering the most outstanding research, evaluated with no bias from both the academic and social point of view. By applying the most advanced information technologies, Frontiers is catapulting scholarly publishing into a new generation.

What are Frontiers Research Topics?

Frontiers Research Topics are very popular trademarks of the *Frontiers journals series*: they are collections of at least ten articles, all centered on a particular subject. With their unique mix of varied contributions from Original Research to Review Articles, Frontiers Research Topics unify the most influential researchers, the latest key findings and historical advances in a hot research area.

Find out more on how to host your own Frontiers Research Topic or contribute to one as an author by contacting the Frontiers editorial office: frontiersin.org/about/contact

Developmental origins of health and disease: Impact of preterm birth

Topic editors

Lynette Kay Rogers — The Research Institute at Nationwide Children's Hospital, United States

Krithika Lingappan — Baylor College of Medicine, United States

Jonathan L. Slaughter — The Research Institute at Nationwide Children's Hospital, United States

Citation

Rogers, L. K., Lingappan, K., Slaughter, J. L., eds. (2023). *Developmental origins of health and disease: Impact of preterm birth*. Lausanne: Frontiers Media SA.

doi: 10.3389/978-2-83251-336-1

Table of contents

frontiers | Frontiers in Pediatrics

TYPE Editorial
PUBLISHED 04 January 2023
DOI 10.3389/fped.2022.1120208

OPEN ACCESS

EDITED AND REVIEWED BY
Eugene Dempsey,
University College Cork, Ireland

*CORRESPONDENCE
Lynette Kay Rogers
✉ lynette.rogers@nationwidechildrens.org

SPECIALTY SECTION
This article was submitted to Neonatology, a section of the journal Frontiers in Pediatrics

RECEIVED 09 December 2022
ACCEPTED 12 December 2022
PUBLISHED 04 January 2023

CITATION
Rogers LK and Slaughter JL (2023) Editorial: Developmental origins of health and disease: Impact of preterm birth.
Front. Pediatr. 10:1120208.
doi: 10.3389/fped.2022.1120208

Editorial: Developmental origins of health and disease: Impact of preterm birth

Lynette Kay Rogers[1,2]* and Jonathan L. Slaughter[1,2]

[1]Department of Pediatrics, The Ohio State University, Columbus, OH, United States, [2]Department of Pediatrics, Abigail Wexner Research Institute, Nationwide Children's Hospital, Columbus, OH, United States

KEYWORDS

preterm birth, maternal environment, intrauterine growth restriction, adult disease, prematurity

Editorial on the Research Topic
Developmental origins of health and disease: Impact of preterm birth

The pivotal concept of developmental origins of disease was first identified in the 1980s and has been the topic of numerous investigations related to adult-onset diseases such as obesity, diabetes, and coronary artery disease (1). Maternal-fetal interactions effect the fetus directly in the context of maternal illness or nutritional deprivation or can establish epigenetic changes that effect basic metabolic function such as obesity, diabetes, and the HPA axis (2, 3). While preterm birth itself is not a direct maternal influence, it is often the result of an adverse *in utero* environment that precipitates the early birth. Further, preterm birth has been associated with impaired structural and function development of organ systems in the offspring, thus making preterm birth a unique predisposing factor for adult disease (4). The manuscripts published within this special issue address factors associated with maternal health, growth of the infant, and morbidities associated with early birth and poor growth.

Mautner et al. assessed maternal physical and mental health between women with early preterm birth vs. late preterm birth. Early preterm birth was associated with depressive symptoms and lower health-related quality of life (HRQoL) scores, but late preterm birth mothers had even higher depressive symptoms; both of which may affect the care provided to their respective infants." Both Motte-Signoret et al. and Gonzales-Garcia et al. studied small for gestational age or intrauterine growth restricted (IUGR) infants. Motte-Signoret measured growth factor (GF), insulin growth factor 1 (IGF1), and insulin resistance in small for gestational age (SGA) infants. SGA infants demonstrated resistance to GF and IGF1 with insulin resistance. These findings could explain initial defects in early catch-up growth, risks for later catch-up growth, and higher prevalence of metabolic syndrome in later life. Gonzales-Garcia compared the Fenton 2013 growth charts to those from the International Fetal and Newborn Growth Consortium for the 21st Century (Intergrowth 21st) Project (IW-21) for assessing to intra-(IUGR) and extrauterine (EUGR) growth restriction in very low birthweight infants. There was concordance between the charts for IUGR

growth trajectories but not for EUGR infants. The dynamic IW-21 was more restrictive but better predicted morbidities in EUGR infants.

Reports on the negative effects of preterm birth included identifying predictors of severe necrotizing enterocolitis (NEC) and impaired neurodevelopmental outcomes and indicated that early identification of risk factors might ultimately lead to improved outcomes. Lin et al. sought to identify predictive indicators of necrotizing enterocolitis (NEC) by comparing biomarkers between NEC with portal venous gas (PVG) and NEC without PVG. C-reactive protein (CRP), fibrinogen degradation product, and blood glucose demonstrated predictive value for NEC-PVG. Current outcomes of the Neuroprem 2 cohort study were reported by Lugli et al. Out of 502 very preterm infants 9.6% had severe disability and 5.4% had cerebral palsy. Gestational age and periventricular hemorrhage were most highly associated with severe disability. Along these same lines, Wibowo et al. measured bone mineral content in newborns and found it lower in underdeveloped countries, higher in males, and negatively correlated with maternal cigarette usage. Hole et al. utilized a preterm piglet model to study early motor development. Preterm piglets took shorter steps than term piglets in early stages of walking but rapidly adapted with no differences within 3 days. Overall conclusions from the combined studies were that early interventions are needed to prevent later delays.

Khasawneh et al. retrospectively analyzed determinates of late hospital discharge. The majority of all preterm births analyzed were late preterm infants. While several parameters such as gestational age and maternal and neonatal morbidities were correlated with length of stay (early, ≤3 days vs. late >3 days), there was no correlation between length of stay after birth and later readmission.

In summary, growth restriction and small birth weight can have permanent consequences on the metabolic health of the infant. Furthermore, the morbidities associated with preterm birth such as NEC, and motor and neurodevelopmental disabilities are likely to contribute to deficits in the quality of life once these infants reach adulthood. Prematurity and SGA should be considered overall risk factors for adult health and quality of life.

Author contributions

Both authors have contributed to the editorial. All authors contributed to the article and approved the submitted version.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

References

1. Barker DJ. Fetal origins of coronary heart disease. *Br Med J.* (1995) 311:171–4. doi: 10.1136/bmj.311.6998.171

2. Nobile S, Di Sipio Morgia C, Vento G. Perinatal origins of adult disease and opportunities for health promotion: a narrative review. *J Pers Med.* (2022) 12 (2):157. doi: 10.3390/jpm12020157

3. Roseboom T, de Rooij S, Painter R. The Dutch famine and its long-term consequences for adult health. *Early Hum Dev.* (2006) 82:485–91. doi: 10.1016/j.earlhumdev.2006.07.001

4. Mericq V, Martinez-Aguayo A, Uauy R, Iniguez G, Van der Steen M, Hokken-Koelega A. Long-term metabolic risk among children born premature or small for gestational age. *Nat Rev Endocrinol.* (2017) 13:50–62. doi: 10.1038/nrendo.2016.127

frontiers in Pediatrics

ORIGINAL RESEARCH
published: 25 May 2021
doi: 10.3389/fped.2021.680869

Maternal and Neonatal Factors Affecting Bone Mineral Content of Indonesian Term Newborns

Tunjung Wibowo[1], Neti Nurani[1], Janatin Hastuti[2], Alifah Anggraini[1], Rina Susilowati[3], Mohammad Hakimi[4], Madarina Julia[1] and Mirjam Van Weissenbruch[5]*

[1] Department of Child Health, Faculty of Medicine, Public Health, and Nursing, Universitas Gadjah Mada/Dr. Sardjito General Hospital, Yogyakarta, Indonesia, [2] Department of Health Nutrition, Faculty of Medicine, Public Health and Nursing, Universitas Gadjah Mada, Yogyakarta, Indonesia, [3] Department of Histology and Molecular Biology, Faculty of Medicine, Public Health and Nursing, Universitas Gadjah Mada, Yogyakarta, Indonesia, [4] Department of Obstetrics and Gynaecology, Faculty of Medicine, Public Health and Nursing, Universitas Gadjah Mada, Yogyakarta, Indonesia, [5] Department of Neonatology, Amsterdam University Medical Center Location VU University Medical Center, Emma Children's Hospital, VU University Amsterdam, Amsterdam, Netherlands

OPEN ACCESS

Edited by:
Lynette Kay Rogers, The Research Institute at Nationwide Children's Hospital, United States

Reviewed by:
Ishmael Miguel Inocencio, Hudson Institute of Medical Research, Australia
Pei Yuen Ng, National University of Malaysia, Malaysia

****Correspondence:***
Tunjung Wibowo
tunjungwibowo@ugm.ac.id

Specialty section:
This article was submitted to Neonatology, a section of the journal Frontiers in Pediatrics

Received: *15 March 2021*
Accepted: *04 May 2021*
Published: *25 May 2021*

Citation:
Wibowo T, Nurani N, Hastuti J, Anggraini A, Susilowati R, Hakimi M, Julia M and Van Weissenbruch M (2021) Maternal and Neonatal Factors Affecting Bone Mineral Content of Indonesian Term Newborns. Front. Pediatr. 9:680869. doi: 10.3389/fped.2021.680869

Background: Interactions between the genome and intrauterine environment can affect bone mineralization in newborns and even in adult life. Several studies show that intrauterine fetal bone mineralization or early postnatal bone condition influences the risk of osteoporosis in later life.

Objectives: To determine whole body bone mineral content (WB BMC) and factors that influence neonatal WB BMC in Indonesian term newborns.

Subjects/Methods: A cross-sectional study was conducted in Dr. Sardjito General Hospital, Yogyakarta, Indonesia. A total of 45 term, appropriate for gestational age (AGA) newborns were included in this study. BMC was assessed by dual-energy x-ray absorptiometry (DXA) in the first week of life. Weight (g), length (cm) and head circumference (cm) were measured at birth. Data on maternal characteristics were obtained from the maternal health records or reported by the mothers.

Results: WB BMC measured in the present study (mean ± SD: 33.2 ± 9.3 g) was lower than WB BMC of similar populations in developed countries. Multiple linear regression showed that birth weight, birth length, and gestational age had a positive association with WB BMC ($p = 0.048$, 0.017, and <0.001, respectively), while maternal cigarette exposure had a negative association with WB BMC ($p = 0.012$). Male infants had significantly higher of WB BMC than female ($p = 0.025$). These determinants contribute to 55% variability of WB BMC.

Conclusions: WB BMC in Indonesian term newborns is lower than populations in developed countries. Birth weight, length, gestational age, sex, and maternal cigarette exposure during pregnancy are significantly associated with WB BMC observed in Indonesian newborns.

Keywords: neonatal bone mineral content, birth weight, gestational age, sex, maternal smoking

INTRODUCTION

Osteoporosis has a significant impact on a person's quality of life as well as being a massive economic burden for the country. In 1990, the worldwide annual direct and indirect burden of hip fracture was estimated at US $ 34.8 billion, rising to $ 131.5 billion by 2050 (at a rate of $ 21,000 per patient) (1). There is growing evidence of an interaction between the genome and the environment in the expression of several chronic diseases including osteoporosis (2). Data showed that intrauterine fetal bone mineralization or bone condition at birth will influence the risk of osteoporosis later in life (3).

Minton et al. (4) observed an association between gestational age and birth weight with the newborns' WB BMC in a Caucasian population in the United States (4). A study in Korea showed the influence of season of birth, cord serum 25-hydroxyvitamin D, maternal diabetes, alcohol consumption and maternal smoking during pregnancy on fetal bone mineralization (5).

A systematic review of the AGA-term infant population with predominantly Caucasian ethnicity found that the WB BMC value measured by Hologic DXA was 66.2 g (95% CI 65.4–67.05), while with Lunar DXA it was 78.9 g (95% CI 78.4–79.4) (2). It has been widely known that bone mass is influenced by race and ethnicity (6, 7). A study in a multiethnic population of prepubertal children using DXA showed ethnic differences in total body WB BMC (7). Research in seven Asian countries that aimed to assess bone health using heel ultrasound in adults aged 46–85 years indicated that Indonesian men and women had the poorest bone health (8). In addition, it was obvious that most pregnant women in Indonesia were Vitamin D deficient even though they live in a tropical country where sun exposure was abundant (9, 10).

With respect of cigarette smoking, Indonesia has one of the highest prevalence of active smokers in the world, with 55.8 % of men aged 10 years and over, compared to just 1.9 % of women of the same age (11). In view of these information, we aimed to assess the Indonesian term newborns' WB BMC and its associated factors.

METHODS

Study Population

We enrolled 45 term infants hospitalized in the newborn nursery of Dr. Sardjito General Hospital from March 2018 to March 2019. Inclusion criteria were gestational age $\geq$ 37 weeks, appropriate for gestational age (AGA), and medically stable. Infants with major congenital abnormalities, bone disorders, gastrointestinal, or renal diseases were excluded.

Maternal Characteristics

Pre-coded questionnaires were used to collect data on maternal characteristics consisting of maternal age (y), parity, education level, and cigarette exposure during pregnancy. Information on pre-pregnancy weight and maternal height were taken from the maternal health records or reported by the mothers. Pre-pregnancy maternal body mass index (BMI) was calculated as pre-pregnancy weight (kg) divided by height (m) squared. The Institute of Medicine (IOM) classification system was used to categorize BMI (kg/m^2): underweight, <18.5; normal, 18.5 to <24.99; overweight, 25 to <29.99; and obese, $\geq$30 (12). Maternal hemoglobin level (mmol/l) before delivery was taken from medical records. Hemoglobin levels were measured using Sysmex XN-1000 (Sysmex, Kobe, Japan) with flow cytometry method (13). Anemia in pregnancy was defined as hemoglobin level < 6.83 mmol/l (= 11 g/dl) (14).

Newborn Characteristics

Newborn characteristics consisted of gestational age, gender, and the anthropometric measurements, that is, weight, length and head circumference, were obtained within 24 h after delivery. Gestational age was defined using the first trimester ultrasonogram or Dubowitz score, when the ultrasonogram information was not available. Weight in grams (g) was measured using calibrated electronic digital scale (Seca 727, Hamburg, Germany) to the nearest 0.1 g. Length was measured using a standard length-measuring board (Seca GmbH & Co. Hamburg, Germany) by two trained nutritionists to the nearest 0.1 centimeter (cm). Head circumference was measured with a non-stretch measuring tape to the nearest 0.1 cm. Infants were classified as AGA if their birth weight were $\geq$ 10th and < 90th percentile for gestational age, using PediTools Fenton 2013 (15).

WB BMC, WB BMC except head, trunk BMC and legs BMC were measured using dual-energy X-ray absorptiometry (DXA; Prodigy: GE-Lunar Corp., Madison, WI, USA) as describe elsewhere (16). Assessment of BMC in newborns using DXA is an accurate method with less radiation exposure and fast scan time (2, 17). Calibration was performed using a calibration block supplied by the manufacturer consisting of tissue-equivalent material with three bone-simulating chambers. For quality control, the baby's body weight measured by the DXA were re-checked using a digital scale (SECA 727). The difference of body weight measurement by DXA and digital scale in this present study was 3.5%.

The BMC was assessed within the first week of life. The mother was asked to breastfeed the baby before being transported to the scanning room to help keep the baby calm and minimize movements during scanning. Babies were placed in a portable carriage and brought to the scanning room. Most newborns became drowsy after swaddling, which facilitated their standardized positioning, thus minimizing movement artifacts in the scanning. During scanning, infants were in supine position on the scan table. The scan began at the top of the head and moved in a rectilinear pattern down the body to the feet. The scan was stopped and repeated if there was a significant movement. BMC was measured in grams (g).

Statistical Analysis

Statistical software SPSS for Windows, version 18.0 (IBM Corp., Chicago, IL, USA) was used to perform data analyses. Data were expressed as means $\pm$ standard deviation (SD) or ratio. The associations between WB BMC at birth and the determinants (sex, gestational age, birth weight, birth length, maternal anemia, maternal smoking exposure, and pre-pregnancy BMI) were analyzed using linear regression. The mean differences between

TABLE 1 | Baseline characteristics of the study subjects ($N = 45$).

Characteristics	Mean ± SD or N (%)
Maternal	
Age (years)	32.1 ± 6.1
Parity[a]	
Primipara	18 (40.0%)
Multipara	27 (60.0%)
Height (m)	1.56 ± 0.05
Pre-gestational BMI (kg/m^2)	23.8±4.1
Pre-gestational Nutritional Status (*N*)	
Underweight (BMI < 18.5)	3 (6.7%)
Normal (18.5 < BMI < 24.99)	27 (60.0%)
Overweight (25 < BMI < 29.99)	11 (24.4%)
Obese (BMI ≥ 30)	4 (8.9%)
Anemia (*N*)	
Yes (hemoglobin <6.83 mmol/l)	20 (44.4%)
No (hemoglobin ≥ 6.83 mmol/l)	25 (55.6%)
Education (*N*)	
University	24 (53.3%)
High school and lower	21 (46.7%)
Cigarette exposure	
Yes	8 (17.8%)
No	37 (82.2%)
Neonatal	
Gender (*N*)	
Male	23 (51.1%)
Female	22 (48.9%)
Birth weight (g)	3134.1 ± 281.1
Birth length (cm)	49.4 ± 1.8
Head circumference (cm)	34.1 ± 1.4
Gestational age (weeks)	38.7 ± 1.0
Age of DXA measurement (days)	3.6 ± 2.7

[a]*Parity: the number of times that the mother has given birth; primipara: mother has given birth once; multipara: mother given birth more than once.*
BMI, body mass index.

subgroups were identified using ANOVA and *post hoc* Tukey's test. Independent *t*-test was also used to detect mean difference between two groups. Significance was set at $p < 0.05$. Because there are no data of newborn BMC available in Indonesia, we decided not to calculate sample size. We start with observational study to evaluate BMC in Indonesian newborns.

The study was approved by the Medical and Health Research Ethics Committee of the Faculty of Medicine, Public Health, and Nursing, Universitas Gadjah Mada, Yogyakarta. Written parental informed consent was obtained from both parents of the study subjects in accordance with the appropriate Ethics Committee requirements.

RESULTS

A total of 45 term AGA newborns consisting of 23 male and 22 female newborns were included in this study. The subjects' characteristics are shown in **Table 1**. The mean ± SD of the gestational age was 38.7 ± 1.0 weeks (range of 37.0–40.9 weeks), while the mean ± SD of birth weight was 3,134.1 ± 281.1 g (range 2,486 −3,745 g). Most mothers had multiparous pregnancy (60%), normal pre-gestational nutritional status (60%), and no cigarette exposure (82.2%). All mothers with cigarette exposure were passive smokers. Most mothers had no anemia (55.6%). The results of BMC scans were showed in **Table 2**.

TABLE 2 | Mean ± SD BMC of whole body, whole body except head, trunk and legs.

Site	Mean ± SD
WB BMC (g)	33.2 ± 9.3
WB BMC except head (g)	13.7 ± 5.0
Trunk BMC (g)	6.2 ± 2.7
Legs BMC (g)	6.7 ± 7.0

BMC, bone mineral content; WB BMC, whole body BMC.

TABLE 3 | Mean of bone mineral content according to determinant variables.

Characteristics	WB BMC (mean ± SD)	*P*-value
Sex		
Male (23)	36.2 ± 9.3	0.028[a]
Female (22)	30.2 ± 8.3	
Maternal cigarette exposure		
Yes (8)	27.3 ± 9.1	0.046[a]
No (37)	34.5 ± 8.9	
Pre-gestational nutritional status		
Underweight (BMI < 18.5) (3)	27.5 ± 5.7	0.336[b]
Normal (18.5 < BMI < 24.99) (27)	34.9 ± 10.0	
Overweight (25 < BMI < 29.99) (11)	29.9 ± 8.7	
Obese (BMI ≥ 30) (4)	34.5 ± 2.4	

[a]*p-value for independent sample t-test.*
[b]*p-value for one-way Anova.*
BMI, body mass index; WB BMC, whole body bone mineral content.

WB BMC was significantly higher in male infants compared with female infants ($p = 0.028$). Infants born to mothers exposed to cigarette smoke had a significantly lower WB BMC than those not exposed ($p = 0.046$) (**Table 3**).

Linear regression analysis showed that sex, gestational age, birth weight, birth length and maternal cigarette exposure were significantly associated with WB BMC at birth (**Table 4**).

Multiple linear regression analysis was also performed to assess which variables were independently associated with WB BMC. Variables with $p < 0.25$ in the simple regression analysis were included in the multiple regression model (18). The results of the multivariate linear regression analysis showed that neonatal factors, that is, birth weight, birth length, and gestational age were positively and independently associated with WB BMC ($p = 0.048$, 0.017, and <0.001, respectively). Male infants had significantly higher of WB BMC than female ($p = 0.025$). On the other hand, only maternal smoking exposure was negatively associated the newborns' WB BMC ($p = 0.012$). Those determinants contribute to almost 50% of the variance of WB BMC (adjusted $R^2 = 0.55$).

TABLE 4 | Determinants of WB BMC at birth by linear regression analysis.

Independent variable	Simple linear regression analysis				Multiple linear regression analysis			
	β	95% CI*	*p*	R^2	β	95% CI*	*p*	Adjusted R^2
Sex (0 = female, 1 = male)	5.93	0.56–11.31	0.031	0.10	4.52	0.61–8.44	0.025	0.55
Gestational age (weeks)	4.36	1.96–4.78	0.001	0.24	4.24	2.32–6.16	<0.001	
Birth weight (g)	0.02	0.01–0.03	<0.001	0.25	0.01	0.00–0.02	0.048	
Birth length (cm)	2.27	0.33–4.21	0.023	0.12	1.78	0.34–3.22	0.017	
Maternal hemoglobin level (g/dl)	−1.07	−2.62–0.48	0.270	0.02				
Maternal cigarette exposure (0 = no, 1 = yes)	−7.13	−14.22–(-0.04)	0.049	0.09	−6.48	−11.47–(−1.48)	0.012	
Maternal pre-gestational BMI (kg/m^2)	−0.22	−0.92–0.47	0.523	0.01				
Maternal height (m)	18.55	−37.77–74.88	0.510	0.01				
Maternal education (0 = University,1 = Senior high school and lower)	−1.34	−7.01–4.33	0.636	0.01				

CI, confidence interval for β; BMI, body mass index.

DISCUSSION

The present study investigated the association between maternal and neonatal conditions and BMC assessed with DXA in term AGA Indonesian newborns at birth. The results of our study showed that birth weight, -length, gestational age, sex, and maternal smoking exposure had significant associations with BMC.

The International Society for Clinical Densitometry (ISCD) advises that spine and whole body scans be used to diagnose bone disorders in children. However, there are currently no clear guidelines for the infant. In infants, measuring the whole body except head may be preferred because of inaccuracies in the algorithm for determining the BMC of the skull. Spine vertebrae especially the lumbar is recommended because of their speed and precision of measurement, easily identified bony landmarks, and less subjected to movement artifact. However, because of its excellent precision and the fact that it measures overall bone density, the total body is still a preferred site (19). Moreover, data from an earlier study conducted by Gallo and colleagues showed that the WB BMC measurement data up to 6 months were robust and consistent with other studies (20).

The WB BMC observed in this study was much lower than the total body BMC observed in several studies conducted in developed countries. A systematic review of term infants, mostly from developed countries, found a mean WB BMC of 68.8 g and 65.9 g for male and female infants, respectively (2). This systematic review also reported that the highest WB BMC was seen in newborns from North America followed by Europe and Africa while Asian newborns had the lowest WB BMC (2).

It is well-known that vitamin D is essential for mineral-bone homeostasis. Several studies have reported that maternal serum 25-hydroxyvitamin D (25 [OH] D) concentrations are associated with offspring bone mass (21–23). Low levels of vitamin D in pregnant women may be one of the causes of low WB BMC in neonates in Indonesia. A study conducted in the same setting as our present study (Yogyakarta) detected vitamin D deficiency in 90% of cord blood samples (24). Two others studies conducted in two different places in Indonesia found that the prevalence of vitamin D deficiency in early pregnancy was very high, namely in Minangkabau: 82.8% and in Jakarta: 99.6% (9, 10). A study in Korea observed that in winter, maternal low vitamin D status resulted in a marked reduction in WB BMC of the newborn (25). However, a more recent randomized clinical trial reported that vitamin D supplementation during pregnancy did not increase offspring WB BMC compared with placebo (26). Unfortunately, maternal 25 (OH) D levels were not measured in our study.

The supply of calcium from maternal sources plays an important role in the normal development of fetal bones. Maternal hypocalcemia may stimulate intrauterine parathyroid hyperplasia causing poor mineralization of the fetal skeleton (27). Previous research conducted in two different places in Indonesia, that is, Java and Sumatra, shows that the calcium intake of pregnant women in both areas was below the recommended daily allowance (28, 29).

As observed in previous research, the findings of the present study found an increase in WB BMC that was consistent with rising birth weight and length (5, 30). This makes sense because WB BMC is dependent on the size and density of the bone, and WB BMC differences can represent bone size and density differences (31).

Our findings are consistent with the results of a previous study indicating that birth weight and birth length were strongly, positively associated with neonatal bone mass even after adjusting for sex and gestational age (32). In the last trimester of pregnancy, a dramatic increase in calcium transfer across the placenta to fetal circulation will be followed by increased fetal bone mineralization. In a healthy term human fetus, two-thirds of total body calcium is transported during the last trimester. The peak accretion is between 36 and 38 weeks of gestation (33, 34). Hence, under normal uteroplacental circumstances, the more mature the fetus, the better the bone mineralization. Data also showed that birth weight also predicts later bone mass (35).

Our results indicate that there is a significant difference in WB BMC between male and female term infants, in which male infants have a higher WB BMC than female infants. These findings are consistent with previous studies of total body composition which showed that males had relatively more lean and less fat mass than females (36) and bone is a lean body component. The difference in body composition between male and female fetuses is most likely influenced by sex steroid hormones. *In utero*, the testes produce testosterone, which is believed to increase lean body mass in fetal life. In the first week of life, male infant testosterone levels increase rapidly and nearly match the concentrations achieved in adult males, whereas the ovaries are relatively silent regarding estrogen production, and do not release significant amounts of testosterone during perinatal development (36).

The differences in WB BMC between male and female term infants at birth are, however, still conflicting. Several studies have shown that males have a significantly higher BMC at birth than female infants (6, 37–39). Meanwhile, several other studies at birth observed no difference in male and female newborns WB BMC (5, 16, 35). Data in adults showed that the incidence of osteoporosis and fractures in women is higher than in men, and this is because the bone mass of women is lower than that of men. Data also suggested that these differences might already occur early in life (40). Namgung and colleagues reported that in term infants there were differences in WB BMC between males and females at the beginning of life but not at birth (5). This phenomenon was also supported by other research which found that male infants aged 1–18 months had higher total body bone mass than female infants, even after controlling for weight, length and race (6).

Another important finding in this study is the effect of smoking exposure on bone mineralization. In this study, all respondents who reported exposure to cigarette smoke were not active smokers. This finding is in line with national data, which showed that prevalence of smoking among women in Indonesia was much lower than that of men (11). The results of our study indicated that exposure to cigarette smoke during pregnancy had a detrimental effect on neonatal WB BMC even though these pregnant women were not active smokers. A cohort study conducted in Finland showed the harmful effects of passive smoking in children on their bone health in adulthood (41).

The negative effects of smoking during pregnancy on neonatal bone mineralization had been demonstrated by several previous studies (35, 42). Evidence from an animal study on 4-week-old mice whose mothers were exposed to cigarette smoke observed that cigarette exposure during pregnancy had a negative influence on bone microarchitecture of the offspring (42).

Several possible mechanisms have been proposed to explain the relationship between maternal smoking and infant bone mass. Maternal smoking has detrimental effect on placental function, causing impairment of uteroplacental blood flow and leading to reduce fetal oxygen carrying capacity. Disruption of the placenta will also cause disruption of mineral transport from mother to fetus. Since fetal bone mineralization is determined by the transfer of placental minerals, fetal bone mineral accretion rate and the rate at which fetal bone is resorbed, impaired mineral transport will cause disruption of fetal bone mineralization (5, 43). Another possible mechanism is due to the high concentration of cadmium as a tobacco smoke contaminant which has specific effects on osteoblast function and on trophoblast calcium transport. This condition may interfere with fetal bone development (16, 35). Exposure to cigarette smoke in pregnant women is also suspected to decrease calcium absorption and lead to bone demineralization. Indirectly, this can affect fetal bone mineralization (30).

However, some results from studies investigating the relationship between maternal smoking with offspring's bone mineralization are inconclusive (44). A study published in 1991 found that in full-term infants, bone mineralization was not significantly altered by maternal cigarette smoking. However, this study showed the limitation that the bone mineralization was examined at one third of the distal of the radius (30). This site is not recommended by the ISCD, as there are few normative data for pediatric forearm studies, and the values obtained may only be useful when compared with subsequent studies (19, 45). A recent systematic review aimed at exploring the effect of maternal smoking during pregnancy on the bone mineral density (BMD) of children or neonates concluded that smoking during pregnancy has no direct effect on BMD of the offspring. Other factors such as placental weight, birth weight, and present body size of children may confound the relation between BMD and smoking during pregnancy (46).

One of the weaknesses in research on maternal smoking or exposure to cigarette smoke during pregnancy is that the data on smoking exposure were obtained from subjective measurements such as a self-reporting questionnaire (46). This is also the case in our study.

STRENGTHS AND LIMITATIONS

To our knowledge our study is the first attempt to use DXA in an Indonesian population to measure bone mass in term newborns. The availability of instruments to assess BMC is one of the barriers to conducting research of bone mass in newborns in developing countries. Furthermore, bone mass measurement has often been an area of debate, with many researchers using various methods to report bone mass. However, DXA has been considered to be the gold standard for bone mass measurement and is used in both clinical and academic studies. DXA is also an ideal method because radiation exposure is low and scan time is fast (2).

The small sample size of the research will limit the generalizability of the findings of this study. This disadvantage can, however, be accounted for since all of the study participants were of Javanese origins, which is Indonesia's largest ethnicity. Another limitation of this study is its cross-sectional study design which makes it difficult to assess if our findings are only a temporal effect.

CONCLUSIONS

The WB BMC in our study population was lower than other populations in developed countries. Birth weight, -length,

gestational age, sex, and maternal cigarette exposure during pregnancy were independently and significantly associated with WB BMC.

DATA AVAILABILITY STATEMENT

The datasets presented in this article are not readily available because the dataset is part of neonatal body composition research and will be analyzed for other publications. Requests to access the datasets should be directed to tunjungwibowo@ugm.ac.id.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Medical and Health Research Ethics Committee (MHREC) Faculty of Medicine Gadjah Mada University-Dr. Sardjito General Hospital. Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin.

AUTHOR CONTRIBUTIONS

TW performed the conception and investigation of the study, performed the statistical analysis, prepared the tables, and wrote the final version of the manuscript. NN identified the statistical methods and validated the statistical analysis results. JH reviewed and edited the final version of the manuscript and did the verification of the reproducibility results. AA helped the investigation of the study and maintained the research data. MH, MJ, and MV obtained funding for the study, advised the study design, supervised the statistical analysis, interpretation of the results, and reviewed the final version of the manuscript. All authors contributed to the article and approved the submitted version.

FUNDING

The work presented in this paper was funded by Faculty of Medicine, Public Health and Nursing, Universitas Gadjah Mada, the General Director of Higher Education and Dr. Sardjito General Hospital.

ACKNOWLEDGMENTS

We are extremely grateful to all of the families who took part in this study. Many thanks for doctors, nurses for their help in recruiting the participants and also all team members of this study including the interviewers, radiologist, nurses and receptionist.

REFERENCES

1. Harvey N, Dennison E, Cooper C. Osteoporosis: impact on health and economics. *Nat Rev Rheumatol.* (2010) 6:99–105. doi: 10.1038/nrrheum.2009.260
2. Ramot R, Kachhawa G, Kulshreshtha V, Varshney S, Sankar MJ, Devasenathipathy K, et al. Bone mass in newborns assessed by DXA—a systematic review and meta-analysis. *Indian J Endocrinol Metab.* (2019) 23:198–205. doi: 10.4103/ijem.IJEM_681_18
3. Godfrey KM. Maternal regulation of fetal development and health in adult life. *Eur J Obstet Gynecol Reprod Biol.* (1998) 78:141–50. doi: 10.1016/S0301-2115(98)00060-8
4. Minton SD, Steichen JJ, Tsang RC. Bone mineral content in term and preterm appropriate-for-gestational-age infants. *J Pediatr.* (1979) 95:1037–42. doi: 10.1016/S0022-3476(79)80305-4
5. Namgung R, Tsang RC. Factors affecting newborn bone mineral content: *In utero* effects on newborn bone mineralization. *Proc Nutr Soc.* (2000) 59:55–63. doi: 10.1017/S0029665100000070
6. Rupich RC, Specker BL, Lieuw-A-Fa M, Ho M. Gender and race differences in bone mass during infancy. *Calcif Tissue Int.* (1996) 58:395–7. doi: 10.1007/BF02509436
7. Horlick M, Thornton J, Wang J, Levine LS, Fedun B, Pierson RN. Bone mineral in prepubertal children: Gender and ethnicity. *J Bone Miner Res.* (2000) 15:1393–7. doi: 10.1359/jbmr.2000.15.7.1393
8. Kruger MC, Todd JM, Schollum LM, Kuhn-Sherlock B, McLean DW, Wylie K. Bone health comparison in seven Asian countries using calcaneal ultrasound. *BMC Musculoskelet Disord.* (2013) 14:1–9. doi: 10.1186/1471-2474-14-81
9. Wibowo N, Bardosono S, Irwinda R, Syafitri I, Putri AS, Prameswari N. Assessment of the nutrient intake and micronutrient status in the first trimester of pregnant women in Jakarta. *Med J Indones.* (2017) 26:109–15. doi: 10.13181/mji.v26i2.1617
10. Aji AS, Erwinda E, Yusrawati Y, Malik SG, Lipoeto NI. Vitamin D deficiency status and its related risk factors during early pregnancy: A cross-sectional study of pregnant Minangkabau women, Indonesia. *BMC Pregnancy Childbirth.* (2019) 19:1–10. doi: 10.1186/s12884-019-2341-4
11. RISKESDAS. *Indonesia Basic Health Survey*. Jakarta (2018). Available online at: http://www.yankes.kemkes.go.id/assets/downloads/PMK No. 57 Tahun 2013 tentang PTRM.pdf (accessed November 01, 2020).
12. Institute of Medicine and National Research Council. In: Rasmussen KM, Yaktine AL, editors. *Weight Gain During Pregnancy: Reexamining the Guidelines*. Washington, DC: National Academies Press (2009).
13. Hamaguchi Y, Kondo T, Nakai R, Ochi Y, Okazaki T, Uchihashi K, et al. Introduction of products over view and features of the automated hematology analyzer XN-L series. *Sysmex J Int.* (2015) 25:1–12.
14. WHO, UNICEF, UNU. *Iron Deficiency Anaemia: Assessment, Prevention, and Control, A Guide for Programme Manager.*. Geneva: WHO, UNICEF, UNU (2001).
15. Chou JH, Roumiantsev S, Singh R. PediTools electronic growth chart calculators: applications in clinical care, research, and quality improvement. *J Med Internet Res.* (2020) 22:e16204. doi: 10.2196/16204
16. Beltrand J, Alison M, Nicolescu R, Verkauskiene R, Deghmoun S, Sibony O, et al. Bone mineral content at birth is determined both by birth weight and fetal growth pattern. *Pediatr Res.* (2008) 64:86–90. doi: 10.1203/PDR.0b013e318174e6d8
17. Cooper C, Harvey N, Javaid K, Hanson M, Dennison E. Growth and bone development. *Nestle Nutr Work Ser Pediatr Progr.* (2008) 61:53–66. doi: 10.1159/000113170
18. Mickey RM, Greenland S. The impact of confounder selection criteria on effect estimation. *Am J Epidemiol.* (1989) 130:1066. doi: 10.1093/oxfordjournals.aje.a115409
19. Gordon CM, Bachrach LK, Carpenter TO, Crabtree N, El-Hajj Fuleihan G, Kutilek S, et al. Dual energy X-ray absorptiometry interpretation and reporting in children and adolescents: The 2007 ISCD pediatric official positions. *J Clin Densitom.* (2008) 11:43–58. doi: 10.1016/j.jocd.2007.12.005
20. Gallo S, Vanstone CA, Weiler HA. Normative data for bone mass in healthy term infants from birth to 1 year of age. *J Osteoporos.* (2012) 2012:672403. doi: 10.1155/2012/672403

21. Harvey NC, Holroyd C, Ntani G, Javaid K, Cooper P, Moon R, et al. Vitamin D supplementation in pregnancy: a systematic review. *Health Technol Assess.* (2014) 18:1–189. doi: 10.3310/hta18450
22. Ioannou C, Javaid MK, Mahon P, Yaqub MK, Harvey NC, Godfrey KM, et al. The effect of maternal vitamin D concentration on fetal bone. *J Clin Endocrinol Metab.* (2012) 97:E2070–7. doi: 10.1210/jc.2012-2538
23. Viljakainen HT, Saarnio E, Hytinantti T, Miettinen M, Surcel H, Mäkitie O, et al. Maternal Vitamin D Status Determines Bone Variables in the Newborn. *J Clin Endocrinol Metab.* (2010) 95:1749–57. doi: 10.1210/jc.2009-1391
24. Oktaria V, Graham SM, Triasih R, Soenarto Y, Bines JE, Ponsonby A, et al. The prevalence and determinants of vitamin D deficiency in Indonesian infants at birth and six months of age. *PLoS ONE.* (2020) 15:e0239603. doi: 10.1371/journal.pone.0239603
25. Namgung R, Tsang RC, Lee C, Han DG, Ho ML, Sierra RI. Low total body bone mineral content and high bone resorption in Korean winter-born versus summer-born newborn infants. *J Pediatr.* (1998) 132:421–5. doi: 10.1016/S0022-3476(98)70013-7
26. Cooper C, Harvey NC, Bishop NJ, Kennedy S, Papageorghiou AT, Schoenmakers I, et al. Maternal gestational vitamin D supplementation and offspring bone health (MAVIDOS): A multicentre, double-blind, randomised placebo-controlled trial. *Lancet Diabetes Endocrinol.* (2016) 4:393–402. doi: 10.1016/S2213-8587(16)00044-9
27. Thomas AK, McVie R, Levine SN. Disorders of maternal calcium metabolism implicated by abnormal calcium metabolism in the neonate. *Am J Perinatol.* (1999) 16:515–20. doi: 10.1055/s-1999-7280
28. Setiarsih D, Wirjatmadi B, Adriani M. Bone density status and vitamin D and calcium concentrations in pregnant and non-pregnant women. *Makara J Heal Res.* (2016) 20:63–8. doi: 10.7454/msk.v20i3.3540
29. Aji AS, Yerizel E, Desmawati D, Lipoeto NI. Low maternal vitamin d and calcium food intake during pregnancy associated with place of residence: a cross-sectional study in west sumatran women, Indonesia. *Open Access Maced J Med Sci.* (2019) 7:2879–85. doi: 10.3889/oamjms.2019.659
30. Venkataraman PS, Duke JC. Bone mineral content of healthy, full-term neonates: effect of race, gender, and maternal cigarette smoking. *Am J Dis Child.* (1991) 145:1310–2. doi: 10.1001/archpedi.1991.02160110102030
31. Mølgaard C, Thomsen BL, Michaelsen KF. Whole body bone mineral accretion in healthy children and adolescents. *Arch Dis Child.* (1999) 81:10–15. doi: 10.1136/adc.81.1.10
32. Cooper C, Harvey N, Cole Z, Hanson M, Dennison E. Developmental origins of osteoporosis: the role of maternal nutrition. *Adv Exp Med Biol.* (2009) 646:31–9. doi: 10.1007/978-1-4020-9173-5_3
33. Namgung R, Tsang RC. Bone in the pregnant mother and newborn at birth. *Clin Chim Acta.* (2003) 333:1–11. doi: 10.1016/S0009-8981(02)00025-6
34. Abrams SA. Normal acquisition and loss of bone mass. *Horm Res.* (2003) 60:71–6. doi: 10.1159/000074505
35. Godfrey K, Walker-Bone K, Robinson S, Taylor P, Shore S, Wheeler T, et al. Neonatal bone mass: Influence of parental birthweight, maternal smoking, body composition, and activity during pregnancy. *J Bone Miner Res.* (2001) 16:1694–703. doi: 10.1359/jbmr.2001.16.9.1694
36. Simon L, Borrego P, Darmaun D, Legrand A, Rozé JC, Chauty-Frondas A. Effect of sex and gestational age on neonatal body composition. *Br J Nutr.* (2013) 109:1105–8. doi: 10.1017/S0007114512002991
37. Holroyd CR, Harvey NC, Crozier SR, Winder NR, Mahon PA, Ntani G, et al. Placental size at 19 weeks predicts offspring bone mass at birth: Findings from the Southampton Women's Survey. *Placenta.* (2012) 33:623–9. doi: 10.1016/j.placenta.2012.04.007
38. Harvey NC, Javaid MK, Arden NK, Poole JR, Crozier SR, Robinson SM, et al. Maternal predictors of neonatal bone size and geometry: The Southampton Women's Survey. *J Dev Orig Health Dis.* (2010) 1:35–41. doi: 10.1017/S2040174409990055
39. Javaid MK, Godfrey KM, Taylor P, Robinson SM, Crozier SR, Dennison EM, et al. Umbilical cord leptin predicts neonatal bone mass. *Calcif Tissue Int.* (2005) 76:341–7. doi: 10.1007/s00223-004-1128-3
40. Gilsanz V, Kovanlikaya A, Costin G, Roe TF, Sayre J, Kaufman F. Differential Effect of Gender on the Sizes of the Bones in the Axial and Appendicular Skeletons*. *J Clin Endocrinol Metab.* (1997) 82:1603–7. doi: 10.1210/jc.82.5.1603
41. Juonala M, Pitkanen N, Tolonen S, Laaksonen M, Sievanen H, Jokinen E, et al. Childhood exposure to passive smoking and bone health in adulthood: The cardiovascular risk in young finns study. *J Clin Endocrinol Metab.* (2019) 104:2403–11. doi: 10.1210/jc.2018-02501
42. Cho GJ, Sim JY, Kim SE, Hong H-R, Ahn K-H, Hong S-C, et al. Maternal Smoke during Pregnancy Programs for Bone Disturbance in Offspring. *Perinatology.* (2018) 29:114. doi: 10.14734/PN.2018.29.3.114
43. Kovacs CS. Bone development and mineral homeostasis in the fetus and neonate: roles of the calciotropic and phosphotropic hormones. *Physiol Rev.* (2014) 94:1143–218. doi: 10.1152/physrev.00014.2014
44. Brand JS, Hiyoshi A, Cao Y, Lawlor DA, Cnattingius S, Montgomery S. Maternal smoking during pregnancy and fractures in offspring: National register based sibling comparison study. *BMJ.* (2020) 368:1–10. doi: 10.1136/bmj.l7057
45. Binkovitz LA, Henwood MJ. Pediatric DXA: Technique and interpretation. *Pediatr Radiol.* (2007) 37:21–31. doi: 10.1007/s00247-006-0153-y
46. Baradaran Mahdavi S, Daniali SS, Farajzadegan Z, Bahreynian M, Riahi R, Kelishadi R. Association between maternal smoking and child bone mineral density: a systematic review and meta-analysis. *Environ Sci Pollut Res.* (2020) 27:23538–49. doi: 10.1007/s11356-020-08740-1

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Pediatrics

ORIGINAL RESEARCH
published: 14 June 2021
doi: 10.3389/fped.2021.683510

Predictive Indicators for Necrotizing Enterocolitis With the Presence of Portal Venous Gas and Outcomes of Surgical Interventions

Xin Lin[1], *Hui-Ping Zeng*[2], *Yi-Fan Fang*[2], *Ying-Ying Lin*[3] *and Chang-Yi Yang*[1]*

[1] Department of Neonatology, Fujian Maternity and Child Health Hospital, Affiliated Hospital of Fujian Medical University, Fuzhou, China, [2] Department of Pediatric Surgery, Fujian Maternity and Child Health Hospital, Affiliated Hospital of Fujian Medical University, Fuzhou, China, [3] Department of Healthcare, Fujian Maternity and Child Health Hospital, Affiliated Hospital of Fujian Medical University, Fuzhou, China

Objectives: Portal venous gas (PVG) was an important clinical sign in stage II or III necrotizing enterocolitis (NEC) in preterm neonates. Not a proper predictive indicator was found to predict the diseases (NEC with the presence of PVG) up to now. There is a need to put forward predictive indicators and compare the predictive effects among them.

Methods: We conducted a retrospective study of preterm neonates with NEC-PVG ($n = 61$) or NEC-non PVG ($n = 62$) from 2014 to 2021. Predictive indicators were put forward and determined by receiver operating characteristic curve analysis. An analysis of the surgical interventions and their outcomes was performed.

Results: The incidence rate of NEC among preterm neonates was 4.99%; surgical and conservative interventions accounted for 20.47 and 75.07%, and the mortality rate was 0.03%. The composition ratio of shock in the NEC-PVG group increased 13.2% ($P = 0.029$). C-reactive protein, fibrinogen degradation product, and blood glucose had better predictive effects in the predictive indicators ($P < 0.05$). Intestinal necrosis and subependymal hemorrhage in the outcomes of surgical interventions had a strong relationship with the presence of PVG in NEC II/III ($P < 0.05$).

Conclusion: Early and reasonable use of antibiotics, improvement of coagulation function, rectification of acidosis, and decreased blood glucose could cut down the occurrence of the disease (NEC with the presence of PVG). Except for subependymal hemorrhage and intestinal necrosis, NEC with the presence of PVG did not increase the occurrence of other outcomes after surgery.

Keywords: predictive indicators, outcomes, necrotizing enterocolitis, portal venous gas, surgical intervention

OPEN ACCESS

Edited by:
Lynette Kay Rogers, The Research Institute at Nationwide Children's Hospital, United States

Reviewed by:
Sanjay Keshav Patole, KEM Hospital for Women, Australia
Gianluca Terrin, University of Rome La Sapienza, Italy

***Correspondence:**
Chang-Yi Yang
cyyang998@126.com;
cyyang996@qq.com

Specialty section:
This article was submitted to Neonatology, a section of the journal Frontiers in Pediatrics

Received: *21 March 2021*
Accepted: *07 May 2021*
Published: *14 June 2021*

Citation:
Lin X, Zeng H-P, Fang Y-F, Lin Y-Y and Yang C-Y (2021) Predictive Indicators for Necrotizing Enterocolitis With the Presence of Portal Venous Gas and Outcomes of Surgical Interventions. Front. Pediatr. 9:683510. doi: 10.3389/fped.2021.683510

INTRODUCTION

Necrotizing enterocolitis (NEC) is a serious intestinal disease caused by the combined effects of immaturity, infection, ingestion, ischemia injury, insufficient oxygenation, and immunological factors, which threatens the life of neonates (1). In the neonatal intensive care unit (NICU), the incidence rate of NEC is 2–5% overall and 4.5–8.7% among very low birth weight (VLBW) neonates, and the mortality rate is 20–30% (2, 3). The absolute indication for NEC surgery is intestinal perforation (4, 5), while conservative treatment failure and worsening of the condition are also relative indications for surgery (5).

Almost 30–50% of neonates with NEC eventually require surgical intervention. The stricture, short bowel syndrome (SBS) and neurodevelopmental impairment are sequelae for NEC (6). Portal venous gas (PVG) was an important clinical sign in stage II or III NEC in preterm neonates. The detection of PVG on abdominal ultrasound (AUS) (as opposed to on X-ray examination) was proven to be as early sign of impending NEC in VLBW neonates (7), and sonographic findings of PVG (OR = 3.9) were significantly associated with surgery and/or death (8). Moreover, PVG was detected by the AUS with high specificity (86–100%) and proper sensitivity (3–45%) in the diagnostic value of NEC in preterm neonates (9). Although the signs of PVG could be detected by X-ray in the severe NEC as well, repeating the examinations of X-ray increased exposure to radiation and did harm to preterm neonates. Thus, X-ray was not chosen as a predictive indicator in the study. Of the mechanism of NEC (10), changes in inflammation indicators were used at the early diagnosis of infections (11, 12); procoagulant status of coagulation function indicators was found at the start of NEC in prematurity (13); abnormity of blood lactate concentrations in the preoperative period carries a poor prognosis in neonates with NEC (14). However, whether these can be used as the predictive indicators on the disease (NEC with the presence of PVG) is still uncertain. There is an urgent need to find predictive indicators with both high sensitivities and specificity and to compare the predictive effects among them.

This retrospective study not only put forward the proper predictive indicators for the disease but also analyzed the outcomes of surgical interventions whether or not they had the relationship with the presence of PVG in NEC II/III.

MATERIALS AND METHODS

Study Population

The Criteria of Inclusion and Exclusion

Preterm neonates diagnosed with stage II or III NEC with a gestational age (GA) of 25–36 weeks were included in NICU from January 2014 to January 2021, while neonates diagnosed with stage I were excluded. According to the classification of Bell's criterial (15), stage II NEC was diagnosed as neonates with the following symptoms: ①the systemic symptoms: temperature instability, apnea, irritability, or lethargy; ②the gastrointestinal symptoms: abdominal distension, abdominal mass, blood in stools, or abdominal rumbling sound weakening; ③the results of iconography: intestinal obstruction, gas in the bowel wall, PVG, or ascites. Systemic symptoms (persistent low blood pressure, bradycardia, and shock), intestinal perforation, and acute diffuse peritonitis were added to stage III NEC. Stage I NEC was determined as neonates with blood in stools and mild gastrointestinal symptoms that did not reach the criterial of stage II.

Because of congenital intestinal malrotation, deformity of the digestive system, or inherited metabolic diseases, some preterm neonates among them were excluded. As a result, the included neonates with complete clinical data were divided into two groups according to conservative and surgical interventions, as shown in **Figure 1**.

The Demographic Characteristics

The demographic characteristics of neonates were recorded: the stage of NEC, GA and birth weight (BW), small for gestational age (SGA), male, asphyxia, and last volume of milk. Simultaneously, the characteristics of mothers were recorded: mothers' age, mode of delivery, premature rupture of membranes, turbid amniotic fluid, multiple pregnancies, prenatal use of antibiotics, and main maternal pregnancy diseases (e.g., gestational diabetes mellitus (GDM), intrauterine infection (IAI), hypertension during pregnancy, and anemia in pregnancy).

The characteristics of neonates when NEC happened were recorded, including apnea, temperature instability, shock, ascites, acute peritonitis, and length of stay in hospital. Acute peritonitis was indicated by a triad of peritoneal irritation (tenderness, abdominal muscle tension, and rebound tenderness). Ascites were detected by AUC when NEC occurred. Breathing that stopped for more than 20 s was accompanied by slowing of the heart rate to <100 beats/min, and decreased blood oxygen saturation was diagnosed as apnea. Neonatal shock was defined as changes in skin color, slower skin circulation, decreased limb temperature, weakened femoral artery fluctuations, and decreased blood pressure (16). Temperature instability was diagnosed as rectal temperature lower than 36.0°C or higher than 37.5°C when NEC happened.

Parameter Variables

The Examination of AUS

A total of 123 preterm neonates were divided into two groups: a NEC-PVG group ($n = 61$) and a NEC-non PVG group ($n = 62$). PVG refers to the imaging signs indicating the accumulation of gas in the portal vein and its branches in the liver and can easily be identified under AUS with the low echogenic flat background noise of the ordinary bloodstream (17). All preterm neonates were examined by AUS (Volumson S8, General Electric Healthcare ultrasound) to determine whether they had signs of PVG or ascites, once the suspicious systemic or gastrointestinal symptoms of NEC appeared.

The Examination of Laboratory Parameters

Percentage of neutrophils (NEUT%) refers to the percentage of neutrophils in all blood cells. The neutrophil/lymphocyte (N/L) ratio is determined as absolute neutrophil counts (NE) divided by absolute lymphocyte counts (LY). When suspicious symptoms of NEC appeared, routine blood indicators [white blood cells (WBC) count, NEUT%, NE, LY, N/L ratio, platelet count (PLT), and C-reactive protein (CRP)] were measured in venous blood samples (1 ml) using an automatic blood cell analyzer (BC-5390CRP, Mindray Co), and prothrombin time (PT), activated partial thromboplastin time (APTT), D-dimer, and fibrinogen degradation product (FDP) were measured in venous blood samples (2 ml) using an automatic blood coagulation analyzer (CS-5100, SYSMEX Co). Neonates received blood gas analysis (pH value, lactate levels, and blood glucose) with venous blood samples (0.5 ml) using an automatic blood gas analyzer (GEM Premier 4000, Instrumentation Laboratory Co.).

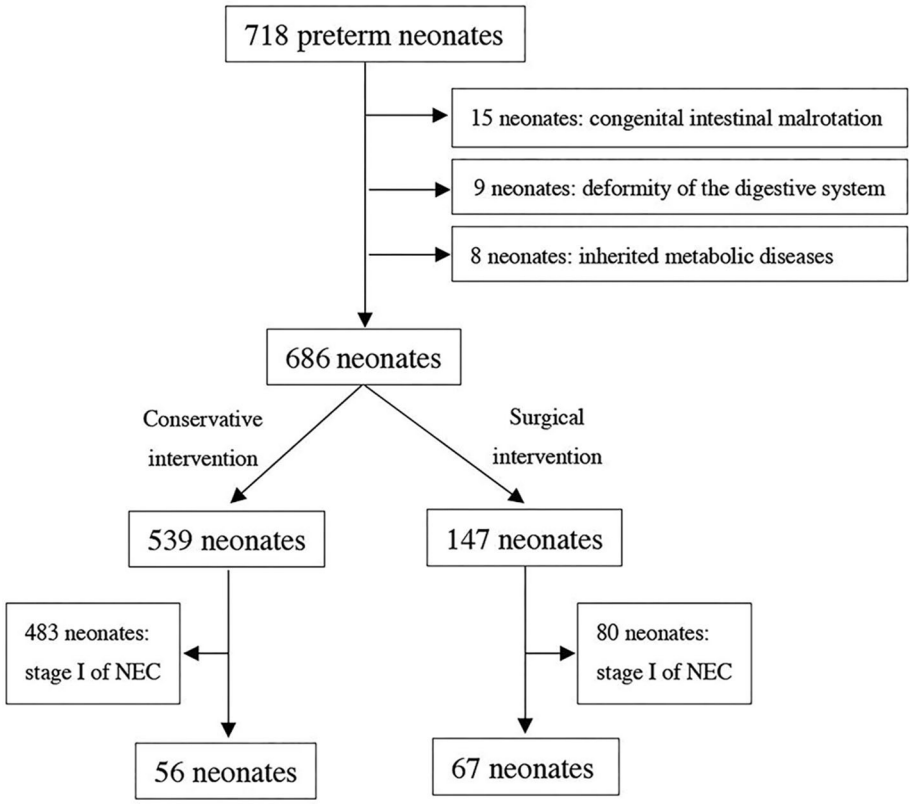


FIGURE 1 | Flow diagram for patient population of necrotizing exterocolitis.

Surgical Interventions and Outcomes

According to the area of necrosis, whether having intestinal perforations or not and clinical symptoms, four kinds of surgical interventions were carried out by surgeon, including laparotomy, enterostomy, intestinal resection and enterostomy, and intestinal resection and anastomosis.

Intraoperative bowel morphology and postoperative complications were recorded for outcomes in both the NEC-PVG group and NEC-non PVG group among neonates with surgery. Intestinal perforation, bleeding, and necrosis were confirmed through the first intraoperative bowel morphology and postoperative pathological results. Intestinal stricture and obstruction were diagnosed in the second intraoperative bowel morphology or postoperative pathological results. If the remaining small intestine was <50% of the length among neonates of the same age and compounded by malabsorption and disordered motility after surgery, SBS was indicated (18). Subependymal hemorrhage was diagnosed by transcranial ultrasound as a sequela after the first surgery of NEC.

Statistical Analysis

The statistical analyses were performed using SPSS Statistics version 26.0 (IBM Corp, Armonk, NY, USA). The demographic characteristics were expressed as the mean ± SD (standard deviation) or the median (P25, P75) for continuous data and number (%) for categories variables. In analyses of continuous data, if fit to a normal distribution, the variables were analyzed by *t*-tests; if not, they were analyzed by the Mann–Whitney U test. Chi-square tests or Fisher's exact tests were used in the analyses of categorical variables. A receiver operating characteristic curve (ROC curve) was generated to compare the area under the curve (AUC), sensitivity, specificity, and cutoff values. Results with $P < 0.05$ were considered statistically significant.

RESULTS

Study Population

From January 2014 to January 2021, 718 of 14,387 preterm neonates diagnosed with stage II or III NEC with a GA of 25–36 weeks were recorded. After 15 neonates of congenital intestinal malrotation, 9 neonates of deformity of the digestive system, and 8 neonates of inherited metabolic diseases were excluded, 686 preterm neonates were selected primarily. Among them, 147 underwent surgical intervention and 539 underwent conservative intervention. After excluding the neonates diagnosed with stage I NEC (483 neonates of conservative intervention and 80 neonates of surgical intervention), a total of 123 preterm neonates were eventually included in the study, as shown in **Figure 1**.

In NICU, the incidence rate of NEC among preterm neonates was 4.99% (718/14,387) within 7 years; among these neonates'

surgical interventions were performed in 20.47% (147/718) and conservative interventions accounting for 75.07% (539/718). The mortality rate of NEC in preterm neonates was 0.03% (4/14,387). In the NEC-PVG group, the mean GA was 30.5 ± 2.1 weeks, and the mean BW was 1408.7 ± 329.2 g. In the NEC-non PVG group, the mean GA was 29.9 ± 2.2 weeks, and the mean BW was 1350.7 ± 331.9 g. The demographic characteristics of the neonates among two groups were reported in **Table 1**.

The composition ratio of shock in the NEC-PVG group increased 13.2% compared to the NEC-non PVG group ($P = 0.029$). There was significant difference of acute peritonitis between two groups ($P < 0.01$). The composition ratios of apnea, temperature instability, or ascites were similar among two groups ($P > 0.05$). Compared to neonates (46 days) in the NEC-non PVG group, the median length of stay of the NEC-PVG group was 48 days ($P = 0.990$).

The Predictive Effects of Laboratory Parameters

The Inflammatory Indicators

An analysis comparing the inflammatory indicators between the two groups of neonates yielded a significant relationship between NEUT% and the disease (NEC with the presence of PVG) ($P = 0.001$, **Table 2**). The average value of NEUT% in the NEC-PVG group was much higher than that in the NEC-non PVG group (55.5 ± 18.0 vs. 44.5 ± 16.1), while there was no significant difference in the WBC count between them ($P = 0.633$). The median LY in the NEC-PVG group ($1.84\times10^9/L$) was significantly lower than that ($2.56\times10^9/L$) in the NEC-non PVG group ($P = 0.013$), while there were no differences of the median NE ($P = 0.373$) between them. The N/L ratio in the NEC-PVG group was significantly higher than that in the NEC-non PVG group ($P = 0.048$, **Table 2**). CRP was a significantly different between two groups (6.59 vs. 0.82, $P < 0.01$). CRP showed the largest AUC (0.694), and its specificity (0.86) was larger than those for the N/L ratio and NEUT% (shown in **Table 3** and **Figure 2**).

The Coagulation Function Indicators

D-dimer and FDP could also be used to predict the disease (NEC with the presence of PVG) among the coagulation function indicators ($P < 0.05$). Although the sensitivities for D-dimer and FDP were similar (0.36 vs. 0.38), the AUC (0.649) and specificity (0.92) of FDP were larger, as shown in **Table 3** and **Figure 2**. No significant differences of PLT, PT, and APTT were found in the study ($P > 0.05$).

The Blood Gas Analysis Indicators

Compared to the NEC-non PVG group, lactate levels and blood glucose in the blood gas analysis indicators were both higher in the NEC-PVG group ($P < 0.05$). The median of PH value in the NEC-PVG group was significantly lower than that in the NEC-non PVG group (7.35 vs. 7.39, $P = 0.011$). The AUC (0.703) of blood glucose was higher than those of lactate levels, and we eventually chose it as a better predictive indicator (specificity: 0.67; sensitivity: 0.76), as shown in **Table 3** and **Figure 2**.

The Surgical Interventions and Outcomes

Sixty-seven neonates reached the criterial of surgical intervention (5) and underwent surgical intervention within 24 h, and their demographic characteristics were reported in **Supplementary Table 1**. The majority of the surgical interventions in the NEC-non PVG group were laparotomy 48.0%, while the majority of them in the NEC-PVG group were enterostomy, intestinal resection, and anastomosis, which accounted for 90.5% (**Table 4**).

The composition ratio of intestinal necrosis was 69.0% in the NEC-PVG group and was just 36.0% in the NEC-non PVG group ($P < 0.05$). Intestinal perforation and bleeding of both groups had no significant differences in the intraoperative bowel morphology ($P > 0.05$). The composition ratio of subependymal hemorrhage in the NEC-PVG group was higher than that in the NEC-non PVG group (35.7% vs. 8%). Three neonates of NEC-PVG group died after surgery, and only one neonate of NEC-non PVG group died.

DISCUSSION

The incidence rate of NEC, which was 4.99% (718/14,387) in NICU over the 7-year study period, was in accordance with previous reports (2, 3), while the mortality rate was much lower than the reported (0.03%). PVG is associated with more severe NEC and sepsis (19), and it was found to be associated with more acute peritonitis and shock in our study.

In the inflammatory indicators, CRP provided a largest predictive effect than the NEUT% and N/L ratio with the sensitivity of 0.86. Persistently elevated CRP in neonates with NEC suggested associated complications, which required surgical intervention (20). The specificity of CRP levels >4.18 mg/dl for the disease (NEC with the presence of PVG) was 86%, and the sensitivity was 56%. As gram-negative germs produced significantly higher CRP levels than gram-positive germs in VLBW neonates (21), we found that more gram-negative germs (*Klebsiella pneumoniae* and *Escherichia coli*) played an important role in increasing CRP levels. NEUT% provided a second predictive effect with a sensitivity of 0.46 and a specificity of 0.84. A significant increase in NEUT% means that neonates with NEC have serious bacterial infections, and the transcriptional regulator C/EBP-α serves a central function (22). A NEUT% higher than 60.4% probably prompted a significantly increased occurrence of the disease, and it was invalid to only study WBC count. Although Maheshwar (23) found a decrease of NEUT% in severe or late period of NEC, we did not find it in the study because of early examination of laboratory parameters for neonates. de Jager et al. (24) pointed out that the N/L ratio was closely related to the inhibition of the body's immune function; additionally, the absolute lymphocyte counts decreased as disease worsened and the body's immune suppression was aggravated in patients with sepsis. This phenomenon also appeared in our study. When the median of absolute neutrophil counts was similar and the median of absolute lymphocyte counts was lower, there was a significant difference of the N/L ratio between two groups. A 2.03 of N/L ratio was considered as

TABLE 1 | The demographic characteristics of NEC-PVG group and NEC-non PVG group.

Variables	NEC-PVG	NEC-non PVG	*P*
	(*n* = 61)	(*n* = 62)	
NEC stage III	30 (49.2%)	21 (33.9%)	0.085
GA (week)	30.5 ± 2.1	29.9 ± 2.2	0.182
BW (g)	1408.7 ± 329.2	1350.7 ± 331.9	0.333
Male	36 (59.0%)	42 (67.7%)	0.315
SGA	12 (19.7%)	8 (12.9%)	0.309
Asphyxia	10 (16.4%)	12 (19.4%)	0.668
Last volume of milk	23 (19, 30)	25 (17, 33)	0.334
Mother's age	30.0 ± 4.6	30.5 ± 5.0	0.591
Vaginal delivery	26 (42.6%)	34 (54.8%)	0.175
Premature rupture of membranes	16 (26.2%)	20 (32.3%)	0.463
Turbid amniotic fluid	12 (19.7%)	14 (22.6%)	0.693
Multiple pregnancy	15 (24.6%)	13 (21.0%)	0.632
GDM	13 (21.3%)	15 (24.2%)	0.703
Hypertension during pregnancy	12 (19.7%)	13 (21.0%)	0.858
Anemia in pregnancy	23 (37.7%)	24 (38.7%)	0.909
IAI	16 (26.2%)	15 (24.2%)	0.795
Apnea	46 (75.4%)	45 (72.6%)	0.721
Temperature instability	26 (42.6%)	28 (45.2%)	0.777
Shock	12 (19.7%)	4 (6.5%)	0.029
Ascites	25 (41.0%)	23 (37.1%)	0.659
Acute peritonitis	31 (50.8%)	11 (17.7%)	<0.01
Length of stay in hospital	48 (33, 59)	46 (37, 59)	0.990

NEC, necrotizing enterocolitis; GA, gestational age; BW, birth weight; SGA, small for gestational age; GDM, gestational diabetes mellitus; IAI, intrauterine infection.

TABLE 2 | Laboratory parameters of NEC-PVG group and NEC-non PVG group.

Variables	NEC-PVG	NEC-non PVG	*P*
	(*n* = 61)	(*n* = 62)	
Inflammatory indicators			
WBC count ($\times 10^9/L$)	6.87 (4.14, 10.78)	7.42 (5.64, 10.32)	0.633
NEUT (%)	55.50 ± 18.03	44.51 ± 16.08	0.001
NE ($\times 10^9/L$)	3.39 (2.18, 5.74)	3.38 (1.75, 4.81)	0.373
LY ($\times 10^9/L$)	1.84 (1.04, 2.93)	2.56 (1.50, 3.84)	0.013
N/L ratio	1.79 (1.02, 3.41)	1.24 (0.68, 2.03)	0.048
CRP (mg/L)	6.59 (0.54, 16.80)	0.82 (0.50, 2.47)	<0.01
Coagulation function indicators			
PLT ($\times 10^9/L$)	258.0 (174.5, 314.5)	216.0 (162.8, 272.5)	0.115
PT (s)	14.80 (13.53, 16.80)	14.45 (13.40, 16.63)	0.557
APTT (s)	57.00 (46.50, 79.55)	53.85 (41.90, 67.65)	0.106
D-dimer (g/L)	1.70 (0.93, 4.45)	1.28 (0.83, 2.14)	0.039
FDP (mg/L)	5.98 (4.24, 12.89)	5.16 (3.12, 6.87)	0.004
Blood gas analysis indicators			
PH value	7.35 (7.30, 7.40)	7.39 (7.33, 7.45)	0.011
Lactate levels (mmol/L)	1.7 (1.2, 2.9)	1.4 (1.2, 2.0)	0.041
Blood glucose (mmol/L)	6.6 (5.6, 7.6)	5.2 (4.3, 6.2)	<0.01

WBC count, white blood cell count; NEUT %, percentage of neutrophils; NE, absolute neutrophil count; LY, absolute lymphocyte count; N/L ratio, neutrophil to lymphocyte ratio; CRP, C-reactive protein; PLT, platelet count; PT, prothrombin time; APTT, activated partial thromboplastin time; FDP, fibrinogen degradation products.

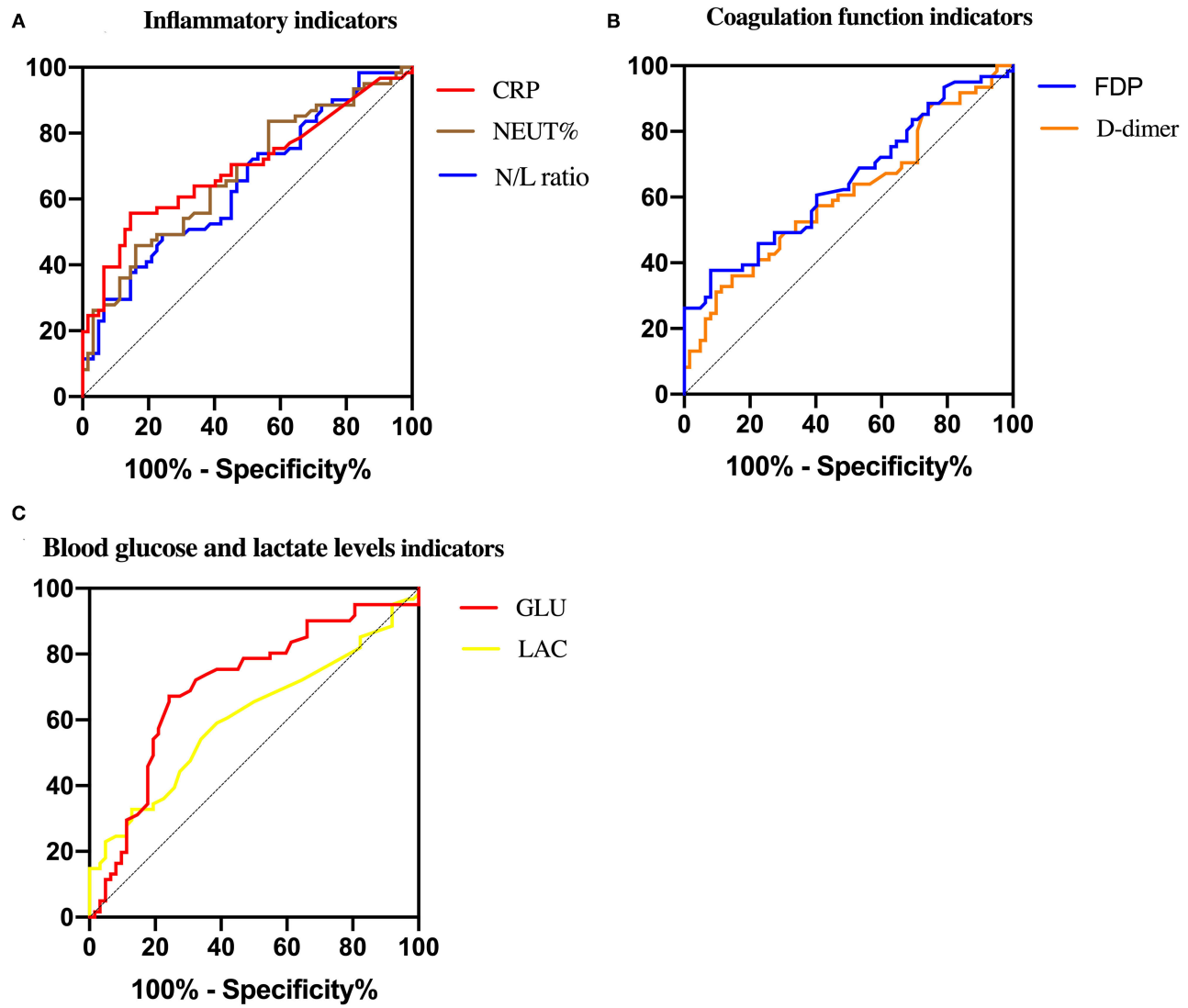


FIGURE 2 | Roc curve of predictive indicators between NEC-PVG group and NEC-non PVG group.

TABLE 3 | ROC values of inflammatory indicators, coagulation function indicators, and blood glucose and lactate level indicators between comparisons of two groups.

Variables	AUC	*P*	95%CI	Sen	Spe	Cutoff
Inflammatory indicators						
NEUT%	0.671	0.001	0.577–0.766	0.46	0.84	60.4
N/L ratio	0.646	0.005	0.549–0.742	0.49	0.76	2.03
CRP	0.694	0	0.600–0.789	0.56	0.86	4.18
Coagulation function indicators						
D-dimer	0.608	0.039	0.508–0.708	0.36	0.78	2.53
FDP	0.649	0.004	0.552–0.746	0.38	0.92	9.15
Blood glucose and lactate levels indicators						
Lactate levels	0.606	0.042	0.506–0.707	0.59	0.61	1.6
Blood glucose	0.703	0	0.608–0.798	0.67	0.76	6.2

NEUT %, percentage of neutrophils; N/L ratio, neutrophil to lymphocyte ratio; CRP, C-reactive protein; FDP, fibrinogen degradation products; AUC, area under the curve; Sen, sensitivity; Spe, specificity.

TABLE 4 | Surgical interventions and outcomes in neonates with surgery between both groups.

Variables	NEC-PVG	NEC-non PVG	*P*
	(*n* = 42)	(*n* = 25)	
Surgical interventions			
Laparotomy	4 (9.5%)	12 (48.0%)	0.005
Enterostomy	10 (23.8%)	4 (16.0%)	
Intestinal resection and enterostomy	22 (52.4%)	7 (28.0%)	
Intestinal resection and anastomosis	6 (14.3%)	2 (8.0%)	
Intraoperative bowel morphology			
Intestinal perforation	8 (19.0%)	5 (20.0%)	0.924
Intestinal bleeding	15 (35.7%)	9 (36.0%)	0.981
Intestinal necrosis	29 (69.0%)	9 (36.0%)	0.008
Postoperative complications			
Intestinal stricture	5 (11.9%)	5 (20.0%)	0.368
Intestinal obstruction	7 (16.7%)	2 (8.0%)	0.525
Short bowel syndrome	3 (7.1%)	1 (4.0%)	0.600
Death	3 (7.1%)	1 (4.0%)	0.600
Subependymal hemorrhage	15 (35.7%)	2 (8.0%)	0.026

the cutoff value for the disease with a sensitivity of 0.49 and a specificity of 0.76.

Giuliani et al. (13) mentioned that neutrophil elastase, CD63, PROS1, HGF, and F12 were upregulated with an overall procoagulant effect, and MFGE8, factor II (thrombin) receptor-like 1 (F2RL1), FGL2, PLAT, PROCR, SERPIND1, and HNF4A were downregulated with a reduction in fibrinolysis and endothelial regeneration in the early period of NEC. As a result, FDP and D-dimer increased and PT and APTT were prolonged. As diseases deteriorated, D-dimer and FDP increased and disseminated intravascular coagulation (DIC) eventually occurred. Similarly, we found that D-dimer and FDP were significantly increased in the NEC-PVG group. FDP increased above 9.15 mg/L, which was in line with the procoagulant effect, and it was selected as a better predictive indicator on account of high specificity (0.92). High levels of FDP and D-dimer increased the risk of thrombosis and bleeding, and we found a higher occurrence of subependymal hemorrhage after surgery in the NEC-PVG group.

Blood lactate levels reflected inadequate global tissue oxygen delivery rather than a local disease process, and there was a significant difference between neonates who had a necrotic bowel and those who had a healthy bowel (14). Lactate levels >1.6 mmol/L, which was associated with more severe illness, predicted a high occurrence of the disease (NEC with the presence of PVG) (sensitivity: 0.59). García et al. (25) pointed out that neonates requiring surgical intervention presented higher values of glycemia at the diagnosis of NEC, and we also found it in the study. Blood glucose was used as a better predictive indicator with both higher sensitivity of 0.67 and specificity of 0.76. The probable explanation for hyperglycemia appearing more often in the NEC-PVG group was that more severe infectious diseases inhibited the release of insulin, cytokines, or endotoxins, reduced glucose utilization, and increased cortisol or catecholamines.

A loss of integrity of the ischemic and necrotic bowel wall may be the mechanism of PVG (26), which allows intraluminal gas to go through the layers of the wall, translocate into the ischemic tissue, or pass directly into the microvasculature. We found that intestinal necrosis was more likely to appear in the neonates of the NEC-PVG group in the intraoperative bowel morphology. A meta-analysis (27) mentioned that PVG was not associated with surgical intervention or severe complications, and we found that except for subependymal hemorrhage, NEC with the presence of PVG did not increase the occurrence of other outcomes after surgery.

Although this was the first study to put forward the predictive indicators of the disease (NEC with the presence of PVG) in preterm neonates following conservative and surgical interventions, because of the strict selected inclusion applied, only 123 of neonates were included. Additionally, the long-term follow-up throughout childhood is necessary, and we will track it continuously.

CONCLUSIONS

CRP, FDP, and blood glucose had better predictive effects to be used as the predictive indicators for the disease (NEC with the presence of PVG). Intestinal necrosis and subependymal hemorrhage in the outcomes of surgical interventions had a strong relationship with the presence of PVG in NEC II/III. Therefore, early and reasonable use of antibiotics, improvement of coagulation function, rectification of acidosis, and a decrease of blood glucose are suggested.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding authors.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the ethics committee of the Fujian Maternal and Child Health Hospital (Ethics approval number: 2020YJ236). Written informed consent from the participants' legal guardian/next of kin was not required to participate in this study in accordance with the national legislation and the institutional requirements. Written informed consent was not obtained from the individuals, nor the minors' legal guardian/next of kin, for the publication of any potentially identifiable images or data included in this article.

AUTHOR CONTRIBUTIONS

XL and H-PZ wrote the main manuscript text. Y-YL prepared **Tables 1**–**4**. Y-FF and C-YY amended the manuscript text. All authors reviewed the manuscript.

FUNDING

This study was supported by the Leading project of Science and Technology Department of Fujian Province (No. 2019Y0058).

ACKNOWLEDGMENTS

The authors want to thank the hospital Heads of the Neonatal Department and Pediatric Surgery Department, Fujian Maternity and Child Health Hospital for their permission and also the nurses and medical officers who were working in the NICU and Pediatric Surgery Department, Fujian Maternal and Child Health Hospital during the study for their support. This study was carried out without grant.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fped.2021.683510/full#supplementary-material

REFERENCES

1. Eaton S, Rees CM, Hall NJ. Current research on the epidemiology, pathogenesis, and management of necrotizing enterocolitis. *Neonatology.* (2017) 111:423–30. doi: 10.1159/000458462
2. Jiang S, Yan W, Li S, Zhang L, Zhang Y, Shah PS, et al. Mortality and morbidity in infants <34 weeks' gestation in 25 NICUs in China: a prospective cohort study. *Front Pediatr.* (2020) 8:33. doi: 10.3389/fped.2020.00033
3. Jones IH, Hall NJ. Contemporary outcomes for infants with necrotizing enterocolitis-a systematic review. *J Pediatr.* (2020) 220:86–92. e3. doi: 10.1016/j.jpeds.2019.11.011
4. De Bernardo G, Sordino D, De Chiara C, Riccitelli M, Esposito F, Giordano M, et al. Management of NEC: surgical treatment and role of traditional X-ray versus ultrasound imaging, experience of a single centre. *Curr Pediatr Rev.* (2019) 15:125–30. doi: 10.2174/1573396314666181102122626
5. Munaco AJ, Veenstra MA, Brownie E, Danielson LA, Nagappala KB, Klein MD. Timing of optimal surgical intervention for neonates with necrotizing enterocolitis. *Am Surg.* (2015) 81:438–43. doi: 10.1177/000313481508100521
6. Carr BD, Gadepalli SK. Does surgical management alter outcome in necrotizing enterocolitis? *Clin Perinatol.* (2019) 46:89–100. doi: 10.1016/j.clp.2018.09.008
7. Yue G, Yang H, Jin M, Deng Y, Ju R. Portal venous gas by ultrasound in advance of impending necrotizing enterocolitis of a very low birth weight infant. *J Clin Ultrasound.* (2020) 48:178–80. doi: 10.1002/jcu.22774
8. Garbi-Goutel A, Brévaut-Malaty V, Panuel M, Michel F, Merrot T, Gire C. Prognostic value of abdominal sonography in necrotizing enterocolitis of premature infants born before 33 weeks gestational age. *J Pediatr Surg.* (2014) 49:508–13. doi: 10.1016/j.jpedsurg.2013.11.057
9. Janssen Lok M, Miyake H, Hock A, Daneman A, Pierro A, Offringa M. Value of abdominal ultrasound in management of necrotizing enterocolitis: a systematic review and meta-analysis. *Pediatr Surg Int.* (2018) 34:589–612. doi: 10.1007/s00383-018-4259-8
10. Neu J, Pammi M. Pathogenesis of NEC: impact of an altered intestinal microbiome. *Semin Perinatol.* (2017) 41:29–35. doi: 10.1053/j.semperi.2016.09.015
11. Y Yu M, Liu G, Feng Z, Huang L. Combination of plasma white blood cell count, platelet count and C-reactive protein level for identifying surgical necrotizing enterocolitis in preterm infants without pneumoperitoneum. *Pediatr Surg Int.* (2018) 34:945–50. doi: 10.1007/s00383-018-4305-6
12. Yang Y, Cao ZL, Zhou XY, Chen XQ, Pan JJ, Cheng R. Does neutrophil/lymphocyte ratio have good diagnostic value in neonatal necrotizing colitis? *J Matern Fetal Neonatal Med.* (2019) 32:3026–33. doi: 10.1080/14767058.2018.1455182
13. Giuliani S, Tan YW, Zheng D, Petropoulou E, Sohail A, Bradley S, et al. Coagulation gene expression profiling in infants with necrotizing enterocolitis. *J Pediatr Gastroenterol Nutr.* (2016) 63:e169–75. doi: 10.1097/MPG.0000000000001215
14. Abubacker M, Yoxall CW, Lamont G. Peri-operative blood lactate concentrations in pre-term babies with necrotizing enterocolitis. *Eur J Pediatr Surg.* (2003) 13:35–9. doi: 10.1055/s-2003-38298
15. Bell MJ. Neonatal necrotizing enterocolitis. *N Engl J Med.* (1978) 298:281–2. doi: 10.1056/NEJM197802022980519
16. Morin L, Ray S, Wilson C, Remy S, Benissa MR, Jansen NJG, et al. Refractory septic shock in children: a European society of paediatric and neonatal intensive care definition. *Intensive Care Med.* (2016) 42:1948–57. doi: 10.1007/s00134-016-4574-2
17. Bohnhorst B. Usefulness of abdominal ultrasound in diagnosing necrotising enterocolitis. *Arch Dis Child Fetal Neonatal Ed.* (2013) 98:F445–50. doi: 10.1136/archdischild-2012-302848
18. Gutierrez IM, Kang KH, Jaksic T. Neonatal short bowel syndrome. *Semin Fetal Neonatal Med.* (2011) 16:157–63. doi: 10.1016/j.siny.2011.02.001
19. Tooke L, Alexander A, Horn A. Extensive portal venous gas without obvious pneumatosis intestinalis in a preterm infant with necrotizing enterocolitis. *J Pediatr Surg.* (2012) 47:1463–5. doi: 10.1016/j.jpedsurg.2012.03.064
20. Pourcyrous M, Korones SB, Yang W, Boulden TF, Bada HS. C-reactive protein in the diagnosis, management, and prognosis of neonatal necrotizing enterocolitis. *Pediatrics.* (2005) 116:1064–9. doi: 10.1542/peds.2004-1806
21. Dritsakou K, Liosis G, Gioni M, Glynou E, Avdeliodi K, Papagaroufalis K. CRP levels in extremely low birth weight (ELBW) septic infants. *J Matern Fetal Neonatal Med.* (2015) 28:237–9. doi: 10.3109/14767058.2014.908842
22. Lawrence SM, Corriden R, Nizet V. The ontogeny of a neutrophil: mechanisms of granulopoiesis and homeostasis. *Microbiol Mol Biol Rev.* (2018) 82:e00057–17. doi: 10.1128/MMBR.00057-17
23. Maheshwari A. Immunologic and hematological abnormalities in necrotizing enterocolitis. *Clin Perinatol.* (2015) 42:567–85. doi: 10.1016/j.clp.2015.04.014
24. de Jager CP, van Wijk PT, Mathoera RB, de Jongh-Leuvenink J, van der Poll T, Wever PC. Lymphocytopenia and neutrophil-lymphocyte count ratio predict

bacteremia better than conventional infection markers in an emergency care unit. *Crit Care.* (2010) 14:R192. doi: 10.1186/cc9309
25. García M, Pértega S, González C, Casal I. Surgical treatment for necrotizing enterocolitis; a new score to extreme surveillance. *Minerva Pediatr*. (2019). doi: 10.23736/S0026-4946.19.05465-3
26. Cui M, Lu X. Portal venous gas. *N Engl J Med.* (2018) 379:e37. doi: 10.1056/NEJMicm1806082
27. Cuna AC, Reddy N, Robinson AL, Chan SS. Bowel ultrasound for predicting surgical management of necrotizing enterocolitis: a systematic review and meta-analysis. *Pediatr Radiol.* (2018) 48:658–66. doi: 10.1007/s00247-017-4056-x

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Pediatrics

ORIGINAL RESEARCH
published: 16 June 2021
doi: 10.3389/fped.2021.685016

Maternal and Perinatal Determinants of Late Hospital Discharge Among Late Preterm Infants; A 5-Year Cross-Sectional Analysis

Wasim Khasawneh, Rahaf Alyousef, Zuhour Akawi, Areen Al-Dhoon and Ahlam Odat*

Department of Pediatrics and Neonatology, Faculty of Medicine, Jordan University of Science and Technology, Irbid, Jordan

OPEN ACCESS

Edited by:
Lynette Kay Rogers, The Research Institute at Nationwide Children's Hospital, United States

Reviewed by:
Gianluca Terrin, University of Rome La Sapienza, Rome, Italy
Wambui Waruingi, Anthem Inc., United States
Manar Al-lawama, The University of Jordan, Jordan
Hercília Guimarães, University of Porto, Portugal

****Correspondence:***
Wasim Khasawneh
wakhasawneh@just.edu.jo

Specialty section:
This article was submitted to Neonatology, a section of the journal Frontiers in Pediatrics

Received: *24 March 2021*
Accepted: *19 May 2021*
Published: *16 June 2021*

Citation:
Khasawneh W, Alyousef R, Akawi Z, Al-Dhoon A and Odat A (2021) Maternal and Perinatal Determinants of Late Hospital Discharge Among Late Preterm Infants; A 5-Year Cross-Sectional Analysis. Front. Pediatr. 9:685016. doi: 10.3389/fped.2021.685016

Background: Although late preterm infants (LPIs) account for the majority of preterm births, they are mistakenly labelled and treated as "near term." Whether longer initial hospital stay improves their outcomes and lowers readmission is controversial. The aim of this study is to identify maternal and perinatal factors associated with longer hospital stay and to assess the rate of readmission.

Methods: The medical records of LPIs delivered at an academic center in Jordan over a 5-year period were reviewed. They were divided according to their initial hospital stay into: Early discharge group (ED, $\leq$ 3 days) and late discharge group (LD, $>$ 3 days). Maternal and perinatal factors associated with $>$ 3-day hospital stay were reported. The rate of readmission was compared between both groups.

Results: 2236 LPIs were included in the analysis representing 13% of total births and 81% of premature births. LD group constituted 54%. A thousand two hundred forty three (56%) required admission to NICU. Factors associated with longer hospital stay included maternal prolonged rupture of membranes (AOR 1.9, 95% C.I 1.5, 2.4, *p* 0.000), C-section delivery (AOR 2.4, 95% C.I 1.9, 3, *p* 0.001), $<$ 35-week gestation (AOR 3.8, 95% C.I 2.6, 5, *p* 0.000), small-for-gestational age (AOR 1.9, 95% C.I 1.1, 3.8, *p* 0.03), birthweight $<$ 2,500 g (AOR 1.3, 95% C.I 1.1, 1.6, *p* 0.02), NICU admission (AOR 6.3, 95% C.I 3.4, 11.5, *p* 0.000), RDS (AOR 2.3, 95% C.I 1.5, 3.6, *p* 0.005), surfactant therapy (AOR 5, 95% C.I 1.9, 13.5, *p* 0.001), use of CPAP (AOR 1.7, 95% C.I 1.2, 2.2, *p* 0.001), jaundice (AOR 11.2, 95% C.I 7.7, 16.2, *p* 0.000), and sepsis (AOR 10.3, 95% C.I 4.8, 22, *p* 0.000). Readmission rate was 19% among the LD group and 13% among the ED group.

Conclusion: LPIs are at high risk for developing prematurity-related morbidities and the duration of their initial hospital stay can be anticipated based on certain predisposing maternal and perinatal factors. Late discharge of LPIs does not lower the rate of readmission.

Keywords: late preterm, length of stay, perinatal factors, readmission, Jordan

INTRODUCTION

Prematurity remains the leading cause of morbidity and mortality during the neonatal period and early childhood (1). The overall rate of prematurity varies between countries with rates reported between 7 and 20%. Late preterm infants (LPIs) delivered at gestational age (GA) of 34 to 36 6/7 weeks represent the most mature and the fast-growing category of prematurity (1, 2).

The rate of late preterm (LP) delivery has substantially increased over the past decade. Several reports have shown that LPIs constituted about 7–12% of all births and nearly 75% of all premature infants (3). The majority of LPIs are delivered to mothers who present with preterm labor or with premature rupture of membranes (4). Still, some LPIs are delivered due to other obstetric medical indications related to the mother or the fetus including antepartum hemorrhage, hypertension, gestational diabetes mellitus, multiple gestations, and intrauterine growth restriction (5). The increasing rates of assisted pregnancy, labor induction, and elective Cesarean section (CS) delivery have contributed to the increase in the rate of LPI delivery as well (4, 6).

Although LPIs are sometimes called near term infants, this is a misconception as this group of premature babies poses a much higher risk than term babies due to structural and functional immaturity (7). Studies have clearly documented that LPIs are at higher risk of respiratory morbidities, neonatal jaundice, feeding difficulties, hypoglycemia, temperature instability, and sepsis (8, 9). Similarly, the mortality rate and long-term neurodevelopmental outcomes are worse than term infants (10).

The outcome of LPIs has been extensively studied and reported in literature. Several reports have studied the maternal and fetal risk factors associated LPI delivery. Other reports focused on the perinatal outcomes of LPIs according to indication for delivery (5, 11).

Therapeutic and preventive interventions have been targeted toward preterm babies delivered before 34 weeks. This factor together with the falsified assumption that LPIs act like small term infants led to an underestimate of these babies' needs (7). In most practices, LPIs are admitted after delivery to the postpartum mother's room or to the well-baby nursery where they receive routine newborn care. Furthermore, in resource-limited countries like Jordan, most women tend to request discharge from the hospital within 12 h following vaginal birth and before 36 h following C-section, and they request to have their newborn babies discharged with them unless they are getting extra support in the neonatal ICU.

Several studies concluded that shorter postnatal stay is not associated with an increased rate of readmission if proper outpatient follow-up is provided (12, 13). On the other hand, short-term morbidities, readmission rates, and neonatal mortality have been linked to shorter initial hospital stay of newborns in several other reports (14). In the US, a state legislation was implemented in 1996 mandating an in-hospital observation of all newborns for a minimum of 48 h after normal vaginal delivery (15).

Besides the exact gestational age, multiple perinatal and neonatal variables are expected to affect the LOS among LPIs. Most of the reports that studied the LOS have focused on the effect of LOS on the readmission rate (16, 17). While analyzing such a potential association, it should be emphasized that infants with longer LOS often have serious health conditions that warrant higher readmission (12).

In Jordan and nearby countries, data is very scarce about LPIs care practice and outcomes. We, therefore, conducted this review to assess the maternal and perinatal factors associated with > 3-day initial LOS among LPIs who are delivered at King Abdullah University Hospital (KAUH) in North Jordan in the period 2015–2020 and to determine the effect of the initial LOS on readmission.

MATERIALS AND METHODS

We conducted a retrospective single-center analysis of all LPIs delivered at KAUH in the period 2015–2020. KAUH is the only tertiary academic center in North of Jordan that provides healthcare access for more than two million Jordanian population with an annual number of deliveries around 3,500.

Our local hospital protocol is to keep all healthy term infants and LPIs at the hospital for a minimum of 24 h after vaginal birth and 48 h after CS unless parents request earlier discharge and sign a form against medical advice. LPIs born at a GA of 34 to 34 6/7 weeks or with a birth weight of <2,000 g are initially admitted to the NICU for 48 h. Discharge criteria include stable vital signs for 24 h, stable respiratory status without requiring any oxygen support, and reasonably tolerating enteral feeding. Close follow up of serum bilirubin within 24–48 h is scheduled for all LPIs and an appointment at the outpatient clinic is scheduled within 5–7 days.

All LPIs who were delivered during the study period were included in the data collection. A list of the included infants was obtained from the hospital electronic database. Excluded from the analysis were LPIs with major congenital anomalies, chromosomal abnormalities, or with <1,500-g birthweight as these conditions are expected to require longer NICU stay and might contribute to skewness of the results.

Data collection was conducted by well-trained interns and medical students using pretested variables and documented in an excel spreadsheet.

Collected maternal variables include age, parity, employment, previous CS delivery, assisted pregnancy, antenatal care attendance, antenatal steroid, and pregnancy complications. Delivery related variables include gestational age, induction of labor, prolonged premature rupture of membranes (PPROM), mode of delivery, and type of anesthesia.

Neonatal variables include gender, birth weight, weight for GA status, and whether a singleton or multiple gestation.

We also collected data about neonatal outcomes including Apgar score, admission to NICU, morbidities like respiratory morbidity and support, hypoglycemia, hypothermia, neonatal jaundice, sepsis, congenital anomalies, discharge weight,

duration of hospitalization, and readmission within 28 days after discharge.

Respiratory distress syndrome (RDS) and transient tachypnea of the newborn (TTN) were diagnosed in babies with signs of respiratory distress and typical CXR findings (18). Neonatal jaundice was diagnosed and treated according to the AAP guidelines (19). The term small-for-gestational age (SGA) was used for any baby with a birth weight below the 3rd centile for age based on Fenton growth chart, while large-for-gestational age (LGA) was used if it falls above the 97th centile (20).

LPIs were divided into two groups, the "early discharge" group (ED) refers to those who were discharged from the hospital within 3 days from the time of birth. The "late discharge" group (LD) refers to those who had an initial LOS of more than 3 days. We chose 3 days as the cutoff since the median length of stay among our cohort was 3 days. Both groups were compared in terms of maternal and perinatal characteristics and neonatal outcomes including readmission rate.

The primary outcome was to assess maternal and perinatal factors associated with longer hospital stay or late discharge beyond 3 days. The secondary outcomes were the rates of morbidities such as RDS, respiratory support, hypothermia, hypoglycemia, jaundice, sepsis, and readmission rate.

Statistical analysis was performed using IBM SPSS Statistics Version 25 (Armonk, NY, IBM Corp). Data were presented as frequency distributions and percentages for categorical variables and mean ± standard deviation (SD) for continuous variables. Pearson $\chi 2$ test was used to evaluate the significance of association between categorical variables, while student's *t*-test and one-way ANOVA were applied to examine the significance level for continuous normally distributed variables. A *P*-value ≤ 0.05 was considered statically significant. If a significant relationship was observed, a *post-hoc* residual analysis for categorical variables and a Fisher's test for continuous variables were applied to determine the exact significance between groups for each variable. After identification of maternal, neonatal, and perinatal factors with significant association with a LOS of > 3 days based on *p*-values of < 0.05, a binary logistic regression model was performed to study the multiple effects of different variables on the LOS. Adjusted OR with 95% C.I were accordingly reported.

RESULTS

A total of 2,265 LPIs were delivered at KAUH during the 5-year study period representing a rate of 13% of the total number of births (17,420) and 81% of the total premature births (2,796). Twenty-nine LPIs were excluded (four with chromosomal anomalies, five with neural tube defects, one with congenital diaphragmatic hernia, two with intestinal atresia, two with complex congenital heart disease, and 15 VLBW < 1,500 g). Of the 2,236 LPIs included in analysis, 1,137 (51%) were males and 1,915 (86%) were born after 35-week gestation. The majority (88%) of LPIs were appropriate-for-gestational age with a mean birth weight of 2,575 (± 485) g while only 185 (8.3%) were SGA.

TABLE 1 | Maternal and neonatal characteristics.

Maternal		***N* = 2,236**	**%**
Age (years)	≤20	52	2.3
	21–35	1,642	73.4
	>35	542	24.2
Parity	Primiparous	521	23.3
	Multiparous	1,715	76.7
Employment		444	20
Assisted pregnancy		322	14.4
Adequate antenatal care		989	44.2
Previous Cesarean section		1,010	45.2
Antenatal steroids		740	33
Pregnancy complications	None	1,654	74
	Pre-eclampsia	127	5.7
	Gestational DM	93	4.2
	Placenta Previa	95	4.2
	Placental abruption	20	1
	Chorioamnionitis	12	0.5
	Others	235	10.5
Induction of labor	Yes	282	13
PPROM	Yes	535	24
Mode	Vaginal	517	23
	Elective CS	1,003	45
	Emergency CS	716	32
Anesthesia	None	517	23
	General	507	23
	Spinal/epidural	1,212	54
Neonatal			
Gender	Male	1,137	51
	Female	1,099	49
Gestational age	<35	321	14
(weeks)	≥35	1,915	86
	34	321	14
	35	551	25
	36	1,364	61
Birth weight	≤2,500	1,052	47
(grams)	>2,500	1,184	53
Weight/gestational age	AGA	1,975	88.3
	SGA	185	8.3
	LGA	76	3.4
Multiple gestation	yes	522	23

DM, diabetes mellitus; CS, cesarean section; PPROM, prolonged premature rupture of membranes; AGA, appropriate for gestational age; SGA, small for gestational age; LGA, large for gestational age.

Mothers of the included LPIs were mostly 21–35 years old (1,642, 73%), multiparous (1,715, 76%), and non-employed (1792, 80%). Antenatal care attendance was ascertained in 989 (44%) and antenatal steroids were administered for 740 (33%). Five hundred thirty-five (24%) presented with PPROM, 282 (13%) required induction of labor for medical reasons while 1,719 (77%) delivered by Cesarean section (CS) of whom 716 had an emergency procedure (**Table 1**).

More than half (54%, 1,216) stayed at the hospital for > 3 days (LD group) and 1,243 (56%) required NICU admission. Compared with the ED group, LPIs in the LD group were more likely to have a GA of <35 weeks (22 vs. 6%, $p < 0.005$), a birth weight below 2,500 g (54 vs. 39%, $p < 0.005$), be SGA (11 vs. 5%, $p < 0.005$), and be a product of multiple gestation pregnancy (26 vs. 20%, $p = 0.001$). Maternal and perinatal factors of significant association with LD included non-employment (83 vs. 77%, $p = 0.001$), assisted pregnancy (18 vs. 10%, $p < 0.005$), antenatal steroids (39 vs. 26%, $p < 0.005$), pregnancy complications (30 vs. 22%, $p < 0.005$), maternal PPROM (28 vs. 19%, $p < 0.005$), CS delivery (83 vs. 70%, $p < 0.005$), and the use of general anesthesia (25 vs. 20%, $p < 0.005$) (**Table 2**).

Table 3 shows the clinical outcomes of the included infants. One hundred and six (5%) had a 5-min Apgar score of <7, 509 (23%) were diagnosed with RDS or TTN, 137 (7%) received

TABLE 2 | A comparison of maternal and perinatal factors according to the length of stay.

Maternal		**ED group *N* = 1,020** ***N* (%)**	**LD group hours *N* = 1,216** ***N* (%)**	***P*-value**
Age (years)	≤20	28 (3)	24 (2)	ns
	21–35	746 (73)	896 (74)	
	>35	246 (24)	295 (24)	
Parity	Primiparous	219 (21)	302 (25)	ns
	Multiparous	801 (79)	914 (75)	
Employment		235 (23)	209 (17)	0.001
Assisted pregnancy		107 (10)	215 (18)	0.000
Adequate antenatal care		467 (46)	522 (43)	ns
Previous Cesarean section		438 (43)	572 (47)	ns
Antenatal steroids		265 (26)	475 (39)	0.000
Pregnancy complications	None	797 (78)	857 (70)	0.000
	Pre-eclampsia	46 (5)	81 (7)	
	Gestational DM	30 (3)	63 (5)	
	Placenta Previa	30 (3)	65 (5)	
	Placental abruption	6 (<1)	14 (1)	
	Chorioamnionitis	5 (<1)	7 (<1)	
	Others	106 (10)	129 (11)	
Induction of labor		139 (14)	143 (12)	ns
PPROM		197 (19)	338 (28)	0.000
Mode of delivery	Vaginal	312 (30)	205 (17)	0.000
	Elective CS	415 (41)	588 (48)	
	Emergency CS	293 (29)	423 (35)	
Anesthesia	None	314 (31)	203 (17)	0.000
	General	201 (20)	306 (25)	
	Spinal/epidural	507 (49)	707 (58)	
Neonatal				
Gender	Male	520 (51)	617 (51)	ns
	Female	500 (49)	599 (49)	
Gestational age	<35	59 (6)	262 (22)	0.000
(weeks)	≥35	961 (94)	954 (78)	
	34	59 (6)	262 (22)	0.000
	35	228 (22)	323 (26)	
	36	733 (72)	631 (52)	
Birth weight	≤2,500	398 (39)	654 (54)	0.000
	>2,500	622 (61)	562 (46)	
Weight/gestational age	AGA	939 (92)	1036 (86)	0.000
	SGA	46 (5)	139 (11)	
	LGA	35 (3)	41 (3)	
Multiple gestation		205 (29)	317 (26)	0.001

ED, early discharge; LD, late discharge; DM, diabetes mellitus; CS, cesarean section; PPROM, prolonged premature rupture of membranes; AGA, appropriate for gestational age; SGA, small for gestational age; LGA, large for gestational age.

TABLE 3 | Neonatal outcomes of late preterm infants.

Outcome		*N* = 2,236 *N* (%)	ED group *N* = 1,020 *N* (%)	LD group hours *N* = 1,216 *N* (%)	*P*-value
APGAR	<7	106 (5)	22 (2)	84 (7)	0.000
	≥7	2,130 (95)	998 (98)	1,132 (93)	
NICU admission		1243 (56)	297 (29)	946 (78)	0.000
RDS		390 (17)	45 (4)	345 (28)	0.000
TTN		119 (5)	26 (2)	93 (8)	0.000
Surfactant therapy	1 dose	80 (4)	6 (<1)	74 (6)	0.000
	≥ 2 doses	57 (3)	0 (0)	57 (5)	
Invasive ventilation		9 (<1)	5 (<1)	4 (<1)	
CPAP		700 (31)	119 (12)	581 (48)	0.000
Hypoglycemia		40 (2)	12 (1)	28 (2)	0.04
Hypothermia		10 (<1)	3 (<1)	7 (<1)	
Jaundice		373 (17)	33 (3)	340 (28)	0.000
Sepsis	Presumed	83 (4)	7 (<1)	76 (6)	0.000
	Confirmed	19 (1)	0 (0)	19 (2)	
Readmission		362 (16)	132 (13)	230 (19)	0.000

ED, early discharge; LD, late discharge; NICU, neonatal intensive care unit; RDS, respiratory distress syndrome; TTN, transient tachypnea of newborn; CPAP, continuous positive airway pressure.

surfactant, 373 (17%) developed jaundice requiring therapy, 102 (5%) had sepsis, 40 (22%) had hypoglycemia, and only 10 (<1%) had hypothermia. Of the 362 (16%) LPIs who were readmitted to the hospital within 28 days after discharge, 230 belong to the LD group accounting for a rate of 19% compared with 132 (13%) among the ED group (*p*-value 0.001). The main indications for readmission were neonatal jaundice and suspected sepsis. The median age at readmission was 10 (25–75% IQR 5, 16).

After identification of maternal, neonatal, and perinatal factors with significant association with a LOS of > 3 days based on *p*-values of <0.05, a binary logistic regression model was performed and the factors with persistent significance were reported. Determinants of a > 3-day LOS included maternal PPROM (AOR 1.9, 95% C.I 1.5, 2.4, *p* 0.000), CS delivery (AOR 2.4, 95% C.I 1.9, 3, *p* 0.001), GA < 35 weeks (AOR 3.8, 95% C.I 2.6, 5, *p* 0.000), SGA (AOR 1.9, 95% C.I 1.1, 3.8, *p* 0.03), birth weight < 2,500 g (AOR 1.3, 95% C.I 1.1, 1.6, *p* 0.02), NICU admission (AOR 6.3, 95% C.I 3.4, 11.5, *p* 0.000), RDS (AOR 2.3, 95% C.I 1.5, 3.6, *p* 0.005), surfactant therapy (AOR 5, 95% C.I 1.9, 13.5, *p* 0.001), use of CPAP (AOR 1.7, 95% C.I 1.2, 2.2, *p* 0.001), jaundice (AOR 11.2, 95% C.I 7.7, 16.2, *p* 0.000), and sepsis (AOR 10.3, 95% C.I 4.8, 22, *p* 0.000) (**Table 4**).

DISCUSSION

In this single-center 5-year retrospective analysis from a major tertiary center in Jordan, we reported that LPIs accounted for 13% of the total births and 81% of the premature births. This study analyzed the maternal and perinatal factors associated with late hospital discharge > 3 days and its effect on readmission. We identified maternal PROM, C-section delivery, < 35-week gestation, SGA, birthweight < 2,500 g, NICU admission, RDS, surfactant therapy, use of CPAP, jaundice, and sepsis as major factors associated with late discharge and concluded that a longer initial hospital stay does not necessarily lower the incidence of readmission.

Globally, the rate of prematurity has not been significantly decreased despite the advance in neonatal care. However, there has been a shift in the level of prematurity with a substantial rise in the number of LP births over the past 5–10 years (1), (2). In 2019, the US rate of LP birth was reported to be about 7.5% among all births and 73% among premature births (2, 3). In Saudi Arabia, Al-Qurashi et al. reported a prematurity rate of 6.5% with 75% being LPIs (21). In Jordan, the rate of LP birth was previously reported in 2010 from a single-center review over one-year period and found to be 7.8% (22). The higher rate of 13% reported in our analysis could be attributed to the fact that our center is the only tertiary center in North Jordan where most women with high-risk pregnancies including preterm labor, PPROM, and multiple gestations are transferred for delivery.

Our study showed that LPIs delivered before 35-week gestation or with low birth weight (LBW; <2,500 g) particularly small for gestational age (SGA) infants were more likely to stay at the hospital for more than 3 days. These factors remained significant after correction for confounders in binary logistic regression model. Factors affecting the LOS have been previously studied, but most studies focused mainly on comparing LPIs with very preterm infants or LPIs with early term infants and concluded that LOS increases by decreasing GA (12, 23). In a national US study about factors affecting LOS among LPIs, Aly et al. reported that <35-week gestation and LBW were significantly associated with a longer LOS (24). The prediction of longer LOS among this high-risk group of premature infants based on certain clinical and demographic factors might be utilized as a core element to establish healthcare guidelines that focus on provision of high-quality care, modification of discharge

TABLE 4 | Binary logistic regression of maternal and perinatal factors associated with late discharge > 72 hours among late preterm infants.

Factor		AOR	95% C.I.	*P*-value
Maternal PPROM	Yes	1.9	1.5, 2.4	0.000
	No	Ref		
Mode of delivery	C-section	2.4	1.9, 3	0.001
	Vaginal	Ref		
Gestational age	<35	3.8	2.6, 5	0.000
	≥35	Ref		
Weight/gestational age	SGA	1.9	1.1, 3.8	0.03
	AGA	Ref		
Birth weight	<2500	1.3	1.1, 1.6	0.02
	≥2500	Ref		
NICU admission	Yes	6.3	3.4, 11.5	0.000
	No	Ref		
RDS	Yes	2.3	1.5, 3.6	0.005
	No	Ref		
Surfactant therapy	yes	5	1.9, 13.5	0.001
	No	Ref		
CPAP	Yes	1.7	1.2, 2.2	0.001
	No	Ref		
Jaundice	Yes	11.2	7.7, 16.2	0.000
	No	Ref		
Sepsis	Yes	10.3	4.8, 22	0.000
	No	Ref		

AOR, adjusted odds ratio; PPROM, prolonged premature rupture of membranes; AGA, appropriate for gestational age; SGA, small for gestational age; NICU, neonatal intensive care unit; RDS, respiratory distress syndrome; CPAP, continuous positive airway pressure.

criteria, and improving the antenatal counseling of pregnant mothers with potential delivery before term gestation considering their cultural background and preferences.

Our study highlighted that maternal PPROM and NICU admission were associated with late discharge of LPIs. Although PPROM is an indication for NICU admission in our hospital protocol, it should be clarified that our local policy is to admit all infants delivered after PPROM to the NICU for observation immediately after delivery and to be discharged after 48 h if they meet the discharge criteria. The overall rate of NICU admission among the LPIs included in our cohort was 56% which is quite similar to the rate reported from USA (56%)(25) and from Egypt (53%) (26), a nearby Arab country, compared with 30% in Turkey (27). In a previous report, we found that maternal PPROM was the major indication of NICU admission among term infants (28).

The rate of Cesarean section delivery in the current study was 77%. This very high rate can be explained by the high percentage of high-risk pregnant women who are referred to our center for delivery including women with a previous CS (1,010, 45.2%). We found CS delivery to be a major determinant of longer LOS. This finding is in line with what has been previously reported in many studies (24, 29). For example, in the USA, Aly et al. reported that CS was associated with longer LOS among LPIs regardless of their GA category. Besides the increase in maternal request, many obstetric care providers tend to perform early CS in high-risk pregnancies mainly in multiple gestations and after assisted reproduction (30). The compliance with the American College of Obstetrics and Gynecologists' (ACOG) recommendation regarding avoiding elective CS delivery at late preterm and early term infants should be always emphasized in obstetric centers to avoid preventable prematurity-related morbidities among this high-risk group of infants in order to decrease their LOS and improve their outcomes (31).

The present study highlighted the main morbidities identified in this high-risk group. RDS, TTN, hyperbilirubinemia, and sepsis were the main complications encountered during NICU stay. Most LPIs who developed these complications stayed at the NICU beyond 3 days. This finding is not different from the mounting reports published previously from all over the world which indicated that LPIs have more complications than term infants (5, 32, 33). The rates of hypoglycemia (1.8%) and hypothermia (<1%) among our cohort were much lower than other studies (7, 8). This emphasizes the importance of local hospital policies and protocols of handling of LPIs, implementing skin-to-skin care with the mother, and early initiation of breastfeeding and frequent enteral feedings in stable infants. In the US, Baker compared the LPIs' outcomes between 2008 and 2013 at an urban Medical Center and concluded that the implementation of clinical practice guidelines in caring for LPIs had reduced the overall LOS and decreased the rates of hypoglycemia and hypothermia (34).

The high risk of readmission among LPIs has been well studied and potentially explained by the underestimate of their needs in the immediate postnatal period (14, 16, 17). Evidence about safe early newborn discharge is inconclusive. In infants who meet the criteria for discharge, it is not clear if longer length of stay (LOS) to provide longer observation of the newborns and maternal counseling would improve their outcomes. In our cohort, the mean and median initial LOS were 4.6 (±6) and 3 (IQR 2,5) days respectively with a readmission rate of 16%. In a smaller review from Lebanon, the rate of readmission was 7.9% following early discharge before 48 h (35). The main indications were consistent with most other reports worldwide and included hyperbilirubinemia and sepsis evaluation due to feeding difficulties (14), (36). Several studies addressed the rate of readmission among LPIs and considered it as a main indicator about the quality of care provided to this high-risk group. Escobar et al. reported a three-fold increase in the rate of readmission among LPIs compared with term infants and concluded that infants who were not admitted to the NICU or those with short NICU stay were more likely to be readmitted within 2 weeks This was more obvious in the more mature 36-week infants (17). Despite that, there is a conflicting evidence about the effect of initial LOS on readmission rates (37). Although longer initial hospitalization might theoretically prevent readmission, discharge from the hospital should not be postponed for infants who meet the discharge criteria. In the present study, the rate of readmission was 19% among the "LD" group compared with 13% among the "ED" group. This could be explained by the fact that LPIs who stayed longer were sicker and so posed a higher risk of having more serious

complications. Same finding was highlighted in a national study from UK about all neonatal admissions at National Health Service hospitals reporting a rate of 10.6% among LPIs with a higher risk reported among those with a longer initial LOS (37). The risk of rehospitalization among LPIs extends beyond the neonatal period (38). McLaurin et al. studied the rate of rehospitalization in the first year of life and reported a rate of 15.2% with an increased chance of readmission in neonates with a >4-day initial hospital stay (39). Since most LPIs' readmissions reported by different studies are due to worsening jaundice and suspected sepsis with poor feeding, it is vital to council mothers about the proper breastfeeding technique and frequency of feeding and to arrange for close follow up at the outpatient clinic after discharge (40).

Although the number of LPIs included in our analysis exceeds the number included in many other reports, this study is not without limitations. The main limitation is being a single-center retrospective review where the accuracy of the documented data could not be validated in all cases. The poor generalizability of our results is another limitation for the same reason. Stratification of LPIs according to different gestational ages and reporting the LOS determinants, clinical morbidities, and readmission rates accordingly might give a more valid insight to their outcomes given the innate difference in physiology and functional maturity encountered with each week change in gestational age.

In conclusion, late preterm birth constitutes the main category of prematurity and LPIs remain at risk for prematurity-related morbidities and subsequent readmission. Although certain maternal and perinatal factors can be utilized to anticipate the length of initial hospitalization, a longer initial hospital stay does not necessarily lower the incidence of readmission.

Implications and Suggestions

Local hospital protocols should be adjusted to deliver an ongoing assessment of LPIs to identify their inherent risks in order to provide them with appropriate care that meets their actual needs. Implementing policies to avoid elective deliveries at late preterm and early term gestation is essential. Quality improvement initiatives addressing the discharge timing and close outpatient follow up are key points to improve the overall outcome and to minimize the readmission rates. A multi-center future research is needed to better understand the outcome of LPIs at a national level.

DATA AVAILABILITY STATEMENT

All data collected is available from the corresponding author upon reasonable request.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by The Institutional Review Board at Jordan University of Science and Technology. Written informed consent for participation was not provided by the participants' legal guardians/next of kin because: this work involves chart review and no direct contact with patients. Deidentified data were used all through.

AUTHOR CONTRIBUTIONS

WK made substantial contributions to conception and design, analysis and interpretation of data, and involved in drafting the manuscript or revising it critically for important intellectual content. RA, ZA, AA-D, and AO made substantial contribution to the acquisition and interpretation of data and were involved in drafting the manuscript. All authors gave final approval of the version to be published and agreed to be accountable for all aspects of the work.

ACKNOWLEDGMENTS

We would like to extend deep thanks to all healthcare workers at the neonatal ICU for their great efforts taking care of our precious neonates.

REFERENCES

1. WHO. *Preterm birth*. Available online at: https://www.who.int/news-room/fact-sheets/detail/preterm-birth (accessed December 17, 2020).
2. CDC. *Products—National Vital Statistics Reports—Homepage*. Available online at: https://www.cdc.gov/nchs/products/nvsr.htm (accessed December 17, 2020).
3. Martin JA, Hamilton BE, Osterman MJK. *Births in the United States* 2018. *Key findings Data from the National Vital Statistics System*. (2018). Available online at: https://www.cdc.gov/nchs/products/index.htm (accessed December 17, 2020).
4. Laughon SK, Reddy UM, Sun L, Zhang J. Precursors for late preterm birth in singleton gestations. *Obstet Gynecol.* (2010) 116:1047–55. doi: 10.1097/AOG.0b013e3181f73f97
5. Trilla CC, Medina MC, Ginovart G, Betancourt J, Armengol JA. Perinatal outcomes of spontaneous and medically-indicated late preterm infants. *Clin Obstet Gynecol Reprod Med.* (2019) 5:3278–87. doi: 10.15761/COGRM.1000249
6. Gyamfi-Bannerman C. Obstetric decision-making and the late and moderately preterm infant. *Semin Fetal Neonatal Med.* (2012) 17:132–7. doi: 10.1016/j.siny.2012.01.014
7. Demestre Guasch X, Raspall Torrent F, Martínez-Nadal S, Vila Cerén C, Elizari Saco MJ, Sala Castellví P. Late preterm infants: a population at underestimated risk. *An Pediatr.* (2009) 71:291–8. doi: 10.1016/j.anpedi.2009.06.011
8. B. Medoff Cooper et al. Newborn clinical outcomes of the awhonn late preterm infant research-based practice project. *JOGNN.* (2012) 41:774–85. doi: 10.1111/j.1552-6909.2012.01401.x
9. Natile M, Ventura ML, Colombo M, Bernasconi D, Locatelli A, Plevani C, et al. Short-term respiratory outcomes in late preterm infants. *Ital J Pediatr.* (2014) 40:52. doi: 10.1186/1824-7288-40-52
10. Petrini JR, Dias T, McCormick MC, Massolo ML, Green NS, Escobar GJ. Increased risk of adverse neurological development for late preterm infants. *J Pediatr.* (2009) 154:169–76. doi: 10.1016/j.jpeds.2008.08.020
11. Vanin LK, Zatti H, Soncini T, Nunes RD, Staudt de Siqueira LB. Maternal and fetal risk factors associated with late preterm infants. *Rev Paul Pediatr.* (2020) 38:e2018136. doi: 10.1590/1984-0462/2020/38/2018136

12. Goyal N, Zubizarreta JR, Small DS, Lorch SA. Length of stay and readmission among late preterm infants: an instrumental variable approach. *Hosp Pediatr.* (2013) 3:7–15. doi: 10.1542/hpeds.2012-0027
13. Kotagal UR, Atherton HD, Eshett R, Schoettker PJ, Perlstein PH. Safety of early discharge for medicaid newborns. *J Am Med Assoc.* (1999) 282:1150–6. doi: 10.1001/jama.282.12.1150
14. Bayoumi YA, Bassiouny YA, Hassan AA, Gouda HM, Zaki SS, Abdelrazek AA. Is there a difference in the maternal and neonatal outcomes between patients discharged after 24 h versus 72 h following cesarean section? A prospective randomized observational study on 2998 patients. *J Matern Neonatal Med.* (2016) 29:1339–43. doi: 10.3109/14767058.2015.1048678
15. State sennate S. *Rept. 104-326—NEWBORNS' AND MOTHERS' HEALTH PROTECTION ACT OF 1996*. Available online at: https://www.congress.gov/congressional-report/104th-congress/senate-report/326/1 (accessed December 18, 2020).
16. Pezzati M. Hospital readmissions in late preterm infants. *Ital J Pediatr.* (2014) 40 (S2):A29. doi: 10.1186/1824-7288-40-S2-A29
17. Escobar GJ, Greene JD, Hulac P, Kincannon E, Bischoff K, Gardner MN, et al. Rehospitalisation after birth hospitalisation: Patterns among infants of all gestations. *Arch Dis Child.* (2005) 90:125–31. doi: 10.1136/adc.2003.039974
18. Reuter S, Moser C, Baack M. Respiratory distress in the newborn. *Pediatr Rev.* (2014) 35:417–28. doi: 10.1542/pir.35-10-417
19. AAP Subcommittee. Management of hyperbilirubinemia in the newborn infant 35 or more weeks of gestation. *Pediatrics.* (2004) 114:297–316. doi: 10.1542/peds.114.1.297
20. Fenton TR, Kim JH. A systematic review and meta-analysis to revise the Fenton growth chart for preterm infants. *BMC Pediatr.* (2013) 13:59. doi: 10.1186/1471-2431-13-59
21. Al-Qurashi FO, Yousef AA, Awary BH. Epidemiological aspects of prematurity in the Eastern region of Saudi Arabia. *Saudi Med J.* (2016) 37:414–9. doi: 10.15537/smj.2016.4.14309
22. Abu-Salah O. Unfavourable outcomes associated with late preterm birth: observations from Jordan. *J Pak Med Assoc.* (2011) 61:769–72.
23. Tomashek KM, Shapiro-Mendoza CK, Weiss J, Kotelchuck M, Barfield W, Evans S, et al. Early discharge among late preterm and term newborns and risk of neonatal morbidity. *Semin Perinatol.* (2006) 30:61–8. doi: 10.1053/j.semperi.2006.02.003
24. Aly H, Hoffman H, El-Dib M, Said L, Mohamed M. Factor affecting length of stay in late preterm infants: an US national database study. *J Matern Neonatal Med.* (2015) 28:598–604. doi: 10.3109/14767058.2014.927428
25. Lubow JM, How HY, Habli M, Maxwell R, Sibai BM. Indications for delivery and short-term neonatal outcomes in late preterm as compared with term births. *Am J Obstet Gynecol.* (2009) 200:e30–3. doi: 10.1016/j.ajog.2008.09.022
26. Algameel A, Elhawary M, Amin S, Abd Elmenem M. Outcome of late preterm newborns in Upper Egypt. *Egypt Pediatr Assoc Gaz.* (2020) 68:11. doi: 10.1186/s43054-020-00023-1
27. Celik IH, Demirel G, Canpolat FE, Dilmen U. A common problem for neonatal intensive care units: Late preterm infants, a prospective study with term controls in a large perinatal center. *J Matern Neonatal Med.* (2013) 26:459–462. doi: 10.3109/14767058.2012.735994
28. Khasawneh W, Sindiani A, Rawabdeh SA, Aleshawi A, Kanaan D. Indications and clinical profile of neonatal admissions: a cross-sectional descriptive analysis from a single academic center in Jordan. *J Multidiscip Healthc.* (2020) 13:997–1006. doi: 10.2147/JMDH.S275267
29. Engle WA, Kominiarek MA. Late preterm infants. early term infants, and timing of elective deliveries. *Clin Perinatol.* (2008) 35:325–341. doi: 10.1016/j.clp.2008.03.003
30. Betrán AP, Ye J, Moller A-B, Zhang J, Gülmezoglu AM, Torloni MR. The increasing trend in caesarean section rates: global. regional and national estimates: 1990–2014. *PLoS ONE.* (2016) 11:e0148343. doi: 10.1371/journal.pone.0148343
31. *Avoidance of Nonmedically Indicated Early-Term Deliveries and Associated Neonatal Morbidities ACOG*. Available online at: https://www.acog.org/clinical/clinical-guidance/committee-opinion/articles/2019/02 (accessed December 18, 2020).
32. Darcy AE. Complications of the late preterm infant. *J Perinat Neonat Nurs.* (2009) 23:78–86. doi: 10.1097/JPN.0b013e31819685b6
33. MTsai ML, Lien R, Chiang M-C, Hsu J-F, Fu R-H, Chu S-M, et al. Prevalence and morbidity of late preterm infants: Current status in a medical center of Northern Taiwan. *Pediatr Neonatol.* (2012) 53:171–177. doi: 10.1016/j.pedneo.2012.04.003
34. Baker B, Woodruff NH, Woodruff School H. JOGNN I N F O C U S evidence-based practice to improve outcomes for late preterm infants. *JOGNN.* (2015) 44:127–34. doi: 10.1111/1552-6909.12533
35. Farhat R, Rajab M. Length of postnatal hospital stay in healthy newborns and re-hospitalization following early discharge. *N Am J Med Sci.* (2011) 3:146–51. doi: 10.4297/najms.2011.3146
36. Isayama T, Lewis-Mikhael AM, O'Reilly D, Beyene J, McDonald SD. Health services use by late preterm and term infants from infancy to adulthood: a meta-analysis. *Pediatrics.* (2017) 140:e20170266. doi: 10.1542/peds.2017-0266
37. Harron K, Gilbert R, Cromwell D, Oddie S, van der Meulen J. Newborn length of stay and risk of readmission. *Paediatr Perinat Epidemiol.* (2017) 31:221–32. doi: 10.1111/ppe.12359
38. Ray KN, Lorch SA. Hospitalization of early preterm. late preterm, and term infants during the first year of life by gestational age. *Hosp Pediatr.* (2013) 3:194–203. doi: 10.1542/hpeds.2012-0063
39. McLaurin KK, Hall CB, Jackson EA, Owens OV, Mahadevia PJ. Persistence of morbidity and cost differences between late-preterm and term infants during the first year of life. *Pediatrics.* (2009) 123:653–9. doi: 10.1542/peds.2008-1439
40. Phillips RM, Goldstein M, Hougland K, Nandyal R, Pizzica A, Santa-Donato A, et al. Multidisciplinary guidelines for the care of late preterm infants. *J Perinatol.* (2013) 33 (Suppl 2) S5–S22. doi: 10.1038/jp.2013.52

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Pediatrics

ORIGINAL RESEARCH
published: 21 June 2021
doi: 10.3389/fped.2021.690788

Extrauterine Growth Restriction in Very Low Birth Weight Infants: Concordance Between Fenton 2013 and INTERGROWTH-21st Growth Charts

*Lara González-García, Enrique García-López, Belén Fernández-Colomer, Laura Mantecón-Fernández, Sonia Lareu-Vidal, Marta Suárez-Rodríguez, Rosa Patricia Arias-Llorente and Gonzalo Solís-Sánchez**

Central University Hospital of Asturias, Oviedo, Spain

OPEN ACCESS

Edited by:
Jonathan L. Slaughter, The Research Institute at Nationwide Children's Hospital, United States

Reviewed by:
Jonathan Michael Davis, Tufts University, United States
Sam Ebenezer Athikarisamy, University of Western Australia, Australia

***Correspondence:**
Gonzalo Solís-Sánchez
solisgonzalo@uniovi.es

Specialty section:
This article was submitted to Neonatology, a section of the journal Frontiers in Pediatrics

Received: *04 April 2021*
Accepted: *20 May 2021*
Published: *21 June 2021*

Citation:
González-García L, García-López E, Fernández-Colomer B, Mantecón-Fernández L, Lareu-Vidal S, Suárez-Rodríguez M, Arias-Llorente RP and Solís-Sánchez G (2021) Extrauterine Growth Restriction in Very Low Birth Weight Infants: Concordance Between Fenton 2013 and INTERGROWTH-21st Growth Charts. Front. Pediatr. 9:690788. doi: 10.3389/fped.2021.690788

Postnatal growth restriction has high prevalence in very low birth weight (VLBW) preterm neonates, and this could affect their long-term prognosis. Nowadays, there is no consensus on how to monitor growth in these neonates.

Objective: This study aimed to compare prevalence of intra- and extrauterine growth restriction (IUGR and EUGR) in a sample of VLBW infants according to the Fenton 2013 charts and INTERGROWTH-21st (IW-21) standards and to analyze concordance between both in the different EUGR definitions criteria (cross-sectional, dynamic, and true).

Patients and Methods: An observational retrospective study of 635 VLBW preterm was performed. The study was carried out in Central University Hospital of Asturias. Body measurements (weight, length, and head circumference) were collected at birth and at hospital discharge and expressed in z-scores for the two references (Fenton 2010 and IW-21). Kappa concordance was calculated.

Results: Kappa concordance between Fenton and IW-21 was 0.887 for IUGR and 0.580 for static EUGR. Prevalence was higher according to Fenton in IUGR (36.5 vs. 35.1%), in static EUGR (73.8 vs. 59.3%), and in dynamic EUGR (44.3 vs. 29.3%). Despite observing low prevalence of EUGR when IW-21 was used to define EUGR, a statistical association between neonatal morbidity and diagnosis of EUGR was observed.

Conclusion: The Fenton and IW-21 concordance for IUGR is good. IW-21 is more restrictive than Fenton in EUGR. Patients diagnosed by IW-21 as EUGR are more likely to have neonatal morbidity, especially if we use EUGR dynamic definition. In our study, we cannot conclude that one graph is better than the other.

Keywords: newborn, extrauterine growth restriction, intrauterine growth restriction, very low birth weight, nutrition

INTRODUCTION

Preterm infants are at risk of extrauterine growth restriction (EUGR) as a consequence of their own intrauterine growth restriction (IUGR), immaturity, and related morbidities, usually associated with food intolerance, inadequate nutrition, and elevated metabolic needs during their hospital admission (1, 2). These changes in growth could have short- and long-term consequences such as growth failure, cardiovascular risk, and developmental disabilities (3–6).

Today, there is no international consensus regarding how to monitor growth of premature infants, especially in those who are very low birth weight (VLBW) infants (7, 8), which in turn are the group of premature babies with the highest risk of growth disturbance. Doubts arise in two directions: what graphs or standards to use as normality reference and what criteria to use to classify IUGR and EUGR.

Since 1977, the American Academy of Pediatrics (AAP) has recommended the use of fetal growth charts during pregnancy to monitor postnatal growth. However, it is not usual for a premature infant to present the same postnatal growth pattern once born. With these references, a high percentage of VLBW infants will be classified as EUGR at discharge (9, 10). VLBW infants usually often fail to gain weight as expected based on intrauterine growth charts (7, 11).

By now, Fenton growth charts have been the most used references to monitor postnatal growth. They were made with somatometric data obtained at birth from fetus according to gestational age (GA) and sex from almost 4 million births from different countries, and they were updated back in 2013. The Fenton charts continue with the World Health Organization (WHO) growth charts at 50 weeks postmenstrual age (PMA). These charts have variability in the measurement methods and do not take into account the physiological loss of weight that occurs after delivery (12).

In recent years, a paradigm shift has emerged. It is preferred to use growth standards of healthy preterm infants than graphics based on cross-sectional somatometric data from fetus at birth (8). Based on these recommendations, the INTERGROWTH-21st Proyect (IW-21) was made prospectively with postnatal growth standards. IW-21 charts include patients from eight countries and overlap WHO growth charts at 64 PMA and are universally applicable (8, 13). In IW-21 growth standards, whose data were prospectively collected between 2009 and 2014, low-risk healthy women who conceived spontaneously with a reliable estimate GA from first trimester without IUGR were eligible to participate. Standardized anthropometric measurements were made in preterm births from this cohort (13).

Although IW-21 charts seem to be better than Fenton, their use is not widespread in daily practice. Besides, the growth chart we use (Fenton vs. IW-21) will influence the prevalence of IUGR and EUGR because this varies widely according to the standards used to monitor postnatal growth (14, 15).

On the other hand, there is no consensus in how to define EUGR. It can be defined in two ways: transversal (cross-sectional) or longitudinal (dynamical). The cross-sectional definition uses a specific time (typically at time of discharge or at 36 weeks PMA) and includes those patients having a weight below the 10th percentile. The longitudinal (dynamical) definition includes those patients with a weight loss of more than 1 or 2 standard deviation (SD) from birth to discharge or at 36 weeks PMA. Some studies point out a better prognostic utility when dynamic definition is used (14). Recently, a new concept of "true EUGR" has started to be discussed: non-IUGR patients at birth are EUGR at 36 weeks or discharge. This new "true EUGR" avoids EUGR patients with IUGR at birth who probably does not have a growth problem of postnatal origin, maybe as a result of a continuation of impaired growth that began at fetal time (16).

Given the high incidence of EUGR during neonatal intensive care unit (NICU) stay in VLBW infants and its possible effect in long-term growth (17) and neurological development (5, 6), it is very important to define which growth chart must be used and how to better define EUGR to monitor the postnatal growth. For this reason, the objective of our study was to compare IUGR and EUGR (static, dynamic, and true) prevalence according to the Fenton and IW-21 standards, looking for concordance between both and analyzing neonatal factors associated with these classifications.

MATERIALS AND METHODS

A clinical retrospective study was designed. A total of 792 VLBW preterm neonates who weighed <1,500 g at birth were eligible to participate in the study during a period of 16 years, from January 2002 to December 2017. The study was carried out in the Neonatology Unit of the Central University Hospital of Asturias (Oviedo, Spain), a third-level hospital that is reference for a population of 1 million inhabitants and with about 5,000 deliveries a year. From the initial population, 635 patients were finally studied (**Figure 1** shows the flowchart of the included patients).

All patients were included at birth in a perinatal morbidity database (SEN1500) after an informed consent was given their parents or legal guardian (18). Exclusion criteria were as follows: GA <24 weeks, death before hospital discharge, major congenital malformations, chromosomopathies, and congenital embryopathies with growth impairment (such as congenital infection by cytomegalovirus).

The study has been carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) and was approved by the Research Ethics Committee of Principado de Asturias (CEIm PA, SPAIN).

Abbreviations: AAP, American Academy of Pediatrics; BMI, body mass index; BPD, bronchopulmonary dysplasia; CRIB, Clinical Risk Index for Babies; EUGR, extrauterine growth restriction; GA, gestational age; HC, head circumference; IUGR, intrauterine growth restriction; IVH, intraventricular hemorrhage; IW-21, INTERGROWTH-21st; L, length; MV, mechanical ventilation; NEC, necrotizing enterocolitis; non-IUGR, non-intrauterine growth restriction; PDA, patent ductus arteriosus; PMA, postmenstrual age; PVL, periventricular leukomalacia; RDS, respiratory distress syndrome; ROP, retinopathy of prematurity; SD, standard deviation; VLBW, very low birth weight; W, weight; WHO, World Health Organization.

The recommendations of the Spanish Society of Neonatology were followed at all times regarding the early introduction of trophic and parenteral nutrition. It should be noted that the study took place in a period of 16 years, so nutrition recommendations have been changing, adapting to current international recommendations. Parenteral nutrition and trophic enteral nutrition were introduced in the first 24 h of life, fortifying breastfeeding when an enteral feeding volume reached 100 ml/kg/day.

Weight (W), length (L), and head circumference (HC) were expressed in mean and SDs (z-scores) using the Fenton 2013 and IW-21 references at birth, at 28 days, and at hospital discharge. In one male patient, the z-score for Fenton at discharge could not be calculated because he was discharged at 60 weeks GA (z-score unavailable). Neonates were classified as IUGR if their birth weight was below the 10th percentile. Body mass index (BMI) was calculated using the weight and length data using the following formula: (g/cm^2) * 10. Subsequently, the z-score BMI was calculated using the Olsen references (19). Values were expressed as mean and SD.

EUGR was defined in a transverse-static way (weight at discharge below the 10th percentile using the Fenton and IW-21 references) and longitudinally dynamically (decrease >1 SD between birth and hospital discharge using Fenton and IW-21). "True EUGR" was defined as the EUGR (static or dynamic criteria) in non-IUGR patients.

Statistical Analysis

Data were analyzed using IBM SPSS statistical software, version 22.0 (IBM®). Quantitative variables were expressed as mean with SD, and qualitative variables as absolute number and percentage. Chi-square test was used for the comparison of qualitative variables, while for the quantitative variables, parametric tests (Student's *t*-test) were used when the sample followed a normal distribution. Kappa coefficient was used to see the concordance between the IW-21 and Fenton references.

Receiver operating characteristic (ROC) curves were used to compare the discriminatory power of the decrease in z-score in the first 28 days of life in EUGR prediction. Area under the curve (AUC) was calculated for each decrease in z-score to diagnose the outcome variable (EUGR in its different definitions) in both graphs.

Multivariate logistic regression (enter method) analysis for dynamic and static "true EUGR" in weight was conducted in each growth chart to determine factors influencing "true EUGR." The significance level adopted was 5%.

RESULTS

Mean GA was 30.2 ± 2.5 weeks. Mean W, L, and HC at birth were 1,173 ± 239 g, 38.1 ± 3 cm, and 26.4 ± 2.1 cm, respectively. Two hundred eight cases (32.8%) came from multiple births and 107 (16.9%) from *in vitro* fecundation gestation. A proportion of 57.8% (367) of the mothers completed a full dose of prenatal corticosteroids, and 72.9% (463) delivered by cesarean section. Morbidities during the neonatal period can be seen in **Table 1**.

TABLE 1 | Characteristics of the study population.

		Value
Gestational age	At birth (weeks)	30.2 ± 2.5
	<30 weeks at birth	284 (44.7)
	PMA at discharge (weeks)	38.7 ± 2.7
Somatometry at birth	Weight (g)	1,173 ± 239
	Length (cm)	38.1 ± 3
	Head circumference (cm)	26.4 ± 2.1
	BMI (g/cm^2)	7.97 ± 0.91
Somatometry at discharge	Weight (g)	2,416.2 ± 307.5
	Length (cm)	45.6 ± 2.1
	Head circumference (cm)	33.2 ± 1.5
	BMI (g/cm^2)	11.56 ± 1.09
Perinatal data	Male gender	306 (48.2)
	Prenatal corticosteroids (complete)	367 (57.8)
	Multiple gestation	208 (32.8)
	Cesarean section	463 (72.9)
	Apgar score 5 min <5	20 (3.1)
	Crib 1 score median (RIQ)	1 (1, 2)
	Intubation resuscitation	224 (35.4)
Neonatal pathology	RDS	307 (48.3)
	MV	345 (54.3)
	Pneumothorax	20 (3.1)
	Early-onset sepsis	24 (3.8)
	Late-onset sepsis	201 (31.7)
	Anemia (transfusion)	176 (33.6)
	NEC	21 (3.3)
	PDA	144 (22.7)
	Hypotension (inotropic support)	45 (7.1)
	Acute kidney injury	14 (2.2)
	Parenteral nutrition at 28 days of life	54 (8.5)
	ROP ≥ 2 stage	62 (9.8)
	BPD	125 (19.7)
	PVL	58 (9.1)
	HIV grade 3–4	27 (4.3)

Values are expressed as number (%) or mean ± standard deviation.
BMI, body mass index; CRIB, Clinical Risk Index for Babies; RDS, respiratory distress syndrome; MV, mechanical ventilation; NEC, necrotizing enterocolitis; PDA, patent ductus arteriosus; ROP, retinopathy of prematurity; BPD, bronchopulmonary dysplasia; PMA, postmenstrual age; PVL, periventricular leukomalacia; HIV, intraventricular hemorrhage.

The proportion of children identified as IUGR using the 10th percentile of the IW-21 references for W, L, and HC was 35.1, 29.8, and 34.8%, respectively, and was 36.5, 26.8, and 34.8%, respectively, using Fenton. Kappa concordance between both was 0.887 for W, 0.856 for L, and 0.806 for HC (**Table 2**).

Patients identified by Fenton and IW-21 (both) as IUGR compared with the rest of the patients (non-IUGR in both) had a significantly higher GA and presented fewer complications associated with prematurity [lower risk of respiratory distress syndrome (RDS), intubation in resuscitation at birth, bronchopulmonary dysplasia (BPD), need for parenteral nutrition at 28 days, intraventricular hemorrhage (IVH), and sepsis] (**Table 3**). Comparing the patients who were only

TABLE 2 | IUGR comparison using Fenton and IW-21 (weight, length, and head circumference).

			INTERGROWTH-21st			Kappa
			IUGR	Non-IUGR	Total	
Fenton 2013	Weight	IUGR	211 (33.2)	21 (3.3)	232 (36.5)	0.887
		Non-IUGR	12 (1.9)	391 (61.3)	403 (63.5)	
		Total	223 (35.1)	412 (64.9)	635	
	Length	IUGR	161 (25.4)	9 (1.4)	170 (26.8)	0.856
		Non-IUGR	28 (4.4)	436 (68.8)	464 (73.2)	
		Total	189 (29.8)	445 (70.2)	634	
	HC	IUGR	193 (30.4)	28 (4.4)	221 (34.8)	0.806
		Non-IUGR	28 (4.4)	386 (60.8)	414 (65.2)	
		Total	221 (34.8)	414 (65.2)	635	

Values are expressed as number (%).
IUGR, intrauterine growth restriction; HC, head circumference.

identified by Fenton with those who were only identified by IW-21, there were no significant differences except a predominance of male and a significantly lower W at birth in IW-21.

If we focus on IUGR, IUGR patients met the static EUGR criteria in 97% of cases using the Fenton graphs and in 87.9% using IW-21. However, IUGR was a protective factor for the development of dynamic EUGR, occurring only in 29% using Fenton and in 10.8% using IW-21.

Static Extrauterine Growth Restriction (<10th Percentile at Hospital Discharge)

With a cross-sectional cutoff point, the proportion of infants identified as EUGR according to Fenton and IW-21 with respect to W, L, and HC was 73.7 and 53.9%, 63.5 and 57.6%, and 23.5 and 25.9%, respectively. Furthermore, 20% (127 children) identified by Fenton as EUGR had no IW-21 EUGR. Kappa concordance between both classifications was 0.58 in W, 0.803 in L, and 0.852 in HC.

A third of all static EUGR patients (30.9% in IW-21 vs. 35.5% in Fenton) had previous history of IUGR.

Analyzing the subgroup of patients with birth weight <1,000 g ($N = 161$), we observed that only 23.6% (38) achieved a discharge weight above the 10th percentile using IW 21 vs. 13% (20) using Fenton, maintaining good concordance between both classifications (Kappa = 0.613) (**Table 4**).

Dynamic Extrauterine Growth Restriction (Decrease > −1 SD at Hospital Discharge)

Dynamic EUGR (decrease in more than 1 SD between birth and hospital discharge) according to Fenton and IW-21 for W, L, and HC was 44.3 and 29.3%, 58.3 and 43.8%, and 13.7 and 12.6%, respectively. We observed that 15.3% of children diagnosed as dynamic EUGR in the Fenton charts for W did not have dynamic EUGR according to IW-21. Kappa concordance in dynamic EUGR diagnosis was 0.672 for W, 0.619 for L, and 0.704 for HC.

TABLE 3 | Comparison of neonatal morbidity in IUGR and non-IUGR patients for both charts.

	IUGR for both charts ($n = 211$)	Non-IUGR in both charts ($n = 424$)	*p*
Male sex	103 (48.8)	203 (47.9)	NS
Gestational age (weeks)	32.3 ± 2.2	29.1 ± 2	<0.0001
Birth weight (g)	1,139 ± 266	1,189 ± 223	0.02
Weight 28 days (g)	1,569 ± 434	1,444 ± 363	<0.0001
Weight at discharge (g)	2,312 ± 168	2,468 ± 345	<0.0001
Length of stay (days)	52.7 ± 23.5	63.63 ± 27.2	<0.0001
Apgar score 5 min < 5	6 (2.8)	14 (3.3)	NS
Intubation resuscitation	50 (23.7)	175 (41.4)	<0.0001
RDS	49 (7.7)	258 (40.6)	<0.0001
MV	63 (29.9)	283 (66.7)	<0.0001
Pneumothorax	2 (0.9)	18 (4.2)	0.025
Early-onset sepsis	2 (0.9)	22 (5.2)	0.008
Late-onset sepsis	51 (24.2)	150 (35.4)	0.004
Anemia (transfusion)	46 (26.1)	130 (37.4)	0.01
NEC	5 (2.4)	16 (3.8)	NS
PDA	16 (7.6)	128 (30.2)	<0.0001
Hypotension (inotropic use)	10 (4.7)	37 (8.7)	NS
AKI	5 (2.8)	9 (2.6)	NS
Parenteral nutrition at 28 days	12 (5.7)	42 (10)	0.001
ROP ≥ stage 2	16 (9.5)	46 (11.5)	NS
BPD	21 (10.1)	104 (24.5)	<0.0001
PVL	17 (8.1)	41 (9.7)	NS
HIV grade 3–4	2 (0.9)	25 (5.9)	0.004

Values are expressed as number (%) or mean ± standard deviation.
IUGR, intrauterine growth restriction; RDS, respiratory distress syndrome; MV, mechanical ventilation; NEC, necrotizing enterocolitis; PDA, patent ductus arteriosus; AKI, acute kidney injure; ROP, retinopathy of prematurity; BPD, bronchopulmonary dysplasia; PVL, periventricular leukomalacia; HIV, intraventricular hemorrhage.

TABLE 4 | Static EUGR in weight (<10th percentile at hospital discharge) in <1,000 g.

		INTERGROWTH-21st			Kappa
Fenton 2013		EUGR	Non-EUGR	Total	
	EUGR	122 (75.8)	18 (11.2)	140 (87)	0.613
	Non-EUGR	1 (0.6)	20 (12.4)	21 (13)	
	Total	123 (76.4)	38 (23.6)	161	

Value is expressed as number (%).
EUGR, extrauterine growth restriction.

Table 5 shows the prevalence of IUGR, EUGR (static and dynamic), and "true EUGR" (static and dynamic), expressing Kappa concordance.

True Extrauterine Growth Restriction

True static EUGR (EUGR prevalence in non-IUGR VLBW) was 35.7% using IW-21 standards and 60.4% using the Fenton curves.

Patients who experienced static true EUGR according to IW-21, in comparison with non-true EUGR, were more frequently male; had lower GA and lower birth weight;

TABLE 5 | Prevalence of IUGR, EUGR (static and dynamic), and "true EUGR" according to Fenton 2013 and INTERGROWTH-21st and Kappa concordance between both classifications.

	IUGR	EUGR			
		Static	Dynamic	True* static	True* dynamic
Weight					
Fenton	36.5	73.8	44.3	59.2	52.8
IW-21	35.1	53.9	29.3	34.9	41
Kappa	0.887	0.58	0.672	0.539	0.746
Length					
Fenton	26.8	63.6	58.6	47.9	66.2
IW-21	29.8	57.6	43.8	42.8	45.6
Kappa	0.856	0.803	0.619	0.804	0.571
Head circumference					
Fenton	34.8	23.5	13.7	14.4	19.2
IW-21	34.8	25.9	12.6	15.9	15.4
Kappa	0.806	0.852	0.704	0.86	0.723

IUGR, intrauterine growth restriction; EUGR, Extrauterine growth restriction; IW-21, INTERGROWTH-21st; Fenton, Fenton 2013.

**True EUGR excludes IUGR (denominator IW-21 and Fenton are n = 412 and n = 402, respectively).*

had longer hospitalization stays; and frequently suffered from more retinopathy of prematurity (ROP) ≥stage 2, necrotizing enterocolitis (NEC), mechanical ventilation, BPD, late-onset sepsis, hypotension, anemia requiring transfusion, acute kidney injury (AKI), patent ductus arteriosus (PDA), and RDS; and had more parenteral use at 28 days of life; and their BMI was significantly lower. Patients who experienced static true EUGR using Fenton had experienced similar comorbidities, although no association was seen with hypotension, AKI, and parenteral nutrition use at 28 days of life (**Table 6**).

In dynamic true EUGR (decrease of more than 1 SD at discharge in non-IUGR patients), prevalence was 39.6% in IW-21 and 52.7% in Fenton. Patients with dynamic true EUGR using IW-21 have a higher risk of having comorbidities than when we used true static EUGR. In dynamic true EUGR, relative risks of presenting each complication are higher, and the relationship between the development of periventricular leukomalacia (PVL) and dynamic true EUGR is added.

Dynamic true EUGR according to IW-21 showed a history of significantly higher incidence of sex male, intubation during resuscitation at birth, RDS, mechanical ventilation, late sepsis, anemia requiring transfusion, NEC, PDA, hypotension, parenteral nutrition at 28 days, ROP ≥stage 2, BPD, PVL, and grade 3–4 IVH. In dynamic true EUGR according to Fenton, there was no association with male sex, hypotension, leukomalacia, and grade 3–4 IVH, but there was association with the rest of the morbidity levels.

Logistic regression analysis with the risk factors for the development of true EUGR (static and dynamic) is presented in **Table 7**. Independent variables related to the development of static "true EUGR" using IW-21 were GA, birth weight, male sex, RDS, anemia, ROP ≥stage 2, and NEC. Independent variables related with static "true EUGR" according to Fenton were GA, birth weight, male sex, RDS, and anemia. Independent variables related to the development of dynamic "true EUGR" using IW-21 were lower GA, male sex, RDS, and anemia. In dynamic "true EUGR" according to Fenton, independent variables related were male sex, RDS, and ROP ≥stage 2.

Influence of the First 28 Days of life

Greater decrease in W z-score (IW-21 and Fenton) in the first 28 days of life was directly related with greater risk of static and dynamic EUGR at hospital discharge, more important with dynamic criteria and with IW-21 standards (**Figure 2**). In IW-21, ROC-AUC in dynamic EUGR was 0.849 (95% CI 0.816–0.882), and ROC-AUC in static EUGR was 0.610 (95% CI 0.566–0.654). In Fenton, ROC-AUC in dynamic EUGR was 0.805% (95% CI 0.764–0.835), and ROC-AUC in static EUGR was 0.566 (95% CI 0.514–0.617).

Changes Over Time

Dividing the period into octens (2002–2009 vs. 2010–2017), we observed that there were no significant differences in the prevalence of newborns <28 weeks (21.5 vs. 17%); neither in IUGR (Fenton 34.2 vs. 39%, IW-21 32.7 vs. 37.7%) nor in static EUGR (Fenton 75.2 vs. 72.4%, IW-21 56.7 vs. 51.1%).

However, we found a significantly lower prevalence of dynamic EUGR (Fenton 51.5 vs. 36.5%, IW-21 37.1 vs. 21%) ($p < 0.0001$).

DISCUSSION

In this retrospective study, we analyzed the prevalence of IUGR at birth and EUGR at discharge in VLBW infants using two different growth charts (IW-21 and Fenton 2013) and in EUGR using three different criteria. The use of these different classifications is important because of the medium- and long-term consequences that they can define. In our series, with the use of both graphs and a cutoff point in the 10th percentile, IUGR frequency was 33.2, 25.4, and 30.4% for W, L, and HC, respectively.

When we analyzed IUGR data independently with each growth chart, we observed that IW-21 and Fenton classify them similarly (IW-21: 35.1, 28.8, and 34.8% vs. Fenton: 36.5, 26.8, and 34.8%, for W, L, and HC, respectively) with a high level of agreement (Kappa > 0.8). When we compared the morbidity between IUGR for both vs. non-IUGR for both charts, we observed that IUGRs for both had less frequency of morbidities, probably related to having a higher GA (32.3 ± 2.2 vs. 29.2 ± 2.2 weeks, $p < 0.000$). No significant differences were observed in morbidities of patients in whom both graphs differ when defining IUGR. IUGR frequency in our series is higher than that of other studies (10–20%) (15, 20, 21), probably due in large part to the use of birth weight instead of GA as a selection criterion. This frequency is similar to the total of the SEN1500 network (33.3%) (22).

TABLE 6 | Static and dynamic "true* EUGR" compared with "true* non-EUGR" and their comorbidities for INTERGROWTH-21st and Fenton 2013.

	Static true EUGR		Dynamic true EUGR	
	IW-21 ***N* = 147 (35.7)**	**Fenton** ***N* = 243 (60.4)**	**IW-21** ***N* = 163 (39.6)**	**Fenton** ***N* = 212 (52.7)**
Male sex	86 (58.5)	129 (53.1)	97 (59.5)	114 (53.8)
Gestational age (weeks)	28.76 ± 2.20	29.08 ± 2.1	28.21 ± 1.99	28.38 ± 1.96
Birth weight (g)	1,104 ± 236	1,144 ± 232	1,118.6 ± 230.3	1,121.46 ± 225.19
z-score BMI at birth	−0.56 ± 0.84	−0.42 ± 1.02	−0.08 ± 0.9	−0.15 ± 0.94
z-score BMI at discharge	−1.18 ± 0.87	−1.06 ± 0.69	−1.1 ± 0.76	−1.03 ± 0.75
Length of stay (days)	78.7 ± 34.3	69.3 ± 28.9	79.37 ± 31.59	74.46 ± 28.03
Intubation resuscitation	80 (54.4)	117 (48.1)	103 (63.2)	123 (58)
RDS	106 (72.1)	171 (70.4)	132 (81)	163 (76.9)
MV	117 (79.6)	178 (73.3)	144 (88.3)	177 (83.5)
Early-onset sepsis	7 (4.8)	14 (5.8)	10 (6.1)	11 (5.2)
Late-onset sepsis	71 (48.3)	102 (42)	74 (45.5)	91 (42.9)
Anemia (transfusion)	72 (59.5)	92 (45.8)	79 (59.8)	95 (54.3)
NEC	10 (6.8)	14 (5.8)	13 (8)	13 (6.1)
PDA	59 (40.1)	84 (34.6)	75 (46)	82 (38.7)
Hypotension (inotropic support)	19 (12.9)	24 (9.9)	23 (14.1)	22 (10.4)
Acute kidney injury	8 (6.7)	6 (3)	6 (4.5)	5 (2.9)
Parenteral nutrition at 28 days	24 (16.4)	30 (12.4)	33 (20.4)	36 (17.1)
ROP ≥ stage 2	30 (20.8)	34 (14.6)	32 (19.8)	39 (18.7)
BPD	53 (36.1)	75 (30.9)	66 (40.5)	78 (36.8)
PVL	16 (10.9)	23 (9.5)	22 (13.5)	25 (11.8)
HIV grade 3–4	12 (8.2)	14 (5.8)	16 (9.8)	17 (8)

Values are expressed as number (%) or mean ± standard deviation.

IW-21, INTERGROWTH-21st; Fenton, Fenton 2013. BMI, body mass index; EUGR, extrauterine growth restriction; RDS, respiratory distress syndrome; MV, mechanical ventilation; NEC, necrotizing enterocolitis; PDA, patent ductus arteriosus; ROP, retinopathy of prematurity; BPD, bronchopulmonary dysplasia; PVL, periventricular leukomalacia; HIV, intraventricular hemorrhage.

**True EUGR excludes IUGR (denominator IW-21 and Fenton are n = 412 and n = 402 respectively).*

Lebrao et al. (23) in a retrospective study with 26–33 weeks preterms ($n = 173$) showed that IW-21 and Fenton were similar for classifying IUGR by weight (35.2 vs. 39.2%). In Barreto's series, which included 2,489 newborns between 34 and 41 weeks, fewer patients were only identified as IUGR using IW-21 (13 vs. 8.7%) (21). However, Tuzun et al. (17), using newborns under 32 weeks GA, identified more IUGR (15 vs. 12%) with IW-21.

Today, there is great controversy about which classification to use to define EUGR in VLBW newborns and which one best defines their long-term prognosis. Classically, static definition has been used to define EUGR, which includes all patients below the 10th percentile at hospital discharge after neonatal admission. Recently, there has been talk of dynamic EUGR, including patients who, from birth to discharge, experienced a decrease in weight >1 SD. In addition, in order to isolate the confounding effect of IUGR, whose postnatal growth may be influenced by prenatal factors, the concept of true EUGR was introduced, defining patients EUGR without history of IUGR.

When we analyzed the frequency of static EUGR (less than the 10th percentile at discharge), using the IW-21 and Fenton charts, we observed a high disparity in weight but a good match in length and HC (IW-21: 53.9, 57.6, and 25.9% vs. Fenton: 73.8, 63.6, and 23.5% W, L, and HC, respectively). These data are influenced by IUGR frequency, so that in our case, only 3 and 12% of them (Fenton and IW-21, respectively) reached a W 10th percentile at discharge.

Dynamic analysis of postnatal growth reflects better how this has been, isolating the positive effect of IUGRs and the negative effect of non-IUGRs. Thus, 69.3 and 89.2% of the IUGR, according to Fenton and IW-21, respectively, reached a weight >1 SD at discharge. Overall, 29.0, 41.4, and 9.8% of IUGRs had dynamic EUGRs for W, L, and HC, respectively, for both charts. The disparity between both charts was lower than with the static method (IW-21: 29.3, 43.8, and 12.6% vs. Fenton: 44.3, 58.5, and 13.7% for W, L, and HC, respectively), with IW-21 being more restrictive for all three measures. The percentage of misclassified cases was lower with IW-21 than with Fenton for all three: 0.3, 2.4, and 2.8% vs. 15.3, 17.1, and 3.9%, IW-21 vs. Fenton for W, L, and HC, respectively.

Ávila-Álvarez (24) used the Fenton curves in a cohort of 130 VLBW and obtained an 59.2% EUGR prevalence. Figueras-Aloy (16) obtained 50% EUGR prevalence (W below the 10th percentile in weeks 34–36) using IW-21 references in a cohort of 479 children under 32 weeks born between 2003 and 2014. In our series, with the use of these criteria, it would be 73.8% (Fenton)

TABLE 7 | Logistic regression analysis of risk factor for static and dynamic true EUGR with Fenton and IW-21.

	B	Error standard	*p* value	OR	95% CI
Static true EUGR for INTERGROWTH-21st					
Gestational age (weeks)	0.944	0.162	<0.0001	2.57	1.87–3.52
Birth weight (g)	−0.008	0.001	<0.0001	0.992	0.989–0.994
Male sex	1.32	0.3	<0.0001	3.77	2.08–6.81
RDS	0.768	0.318	0.016	2.15	1.15–4.02
Anemia	1.34	0.322	<0.0001	3.83	2.04–7.2
ROP ≥ 2	0.951	0.473	0.045	2.58	1.02–6.54
NEC	2.62	1.15	0.023	13.85	1.43–133.96
Static true EUGR for Fenton 2013					
Gestational age (weeks)	1.16	0.16	<0.0001	3.21	2.33–4.43
Birth weight (g)	−0.01	0.001	<0.0001	0.99	0.987–0.993
Male sex	0.632	0.283	0.025	1.88	1.08–3.27
RDS	1.27	0.30	<0.0001	3.56	1.94–6.53
Anemia	0.87	0.328	0.008	2.38	1.25–4.53
ROP ≥ 2	0.201	0.501	0.688	1.22	0.45–3.26
NEC	1.68	1.12	0.134	5.37	0.59–48.36
Dynamic true EUGR for INTERGROWTH-21st					
Gestational age (weeks)	−0.45	0.13	0.001	0.63	0.49–0.828
Birth weight (g)	0.002	0.001	0.06	1.002	1–1.004
Male sex	0.983	0.271	<0.0001	2.67	1.57–4.54
RDS	0.848	0.304	0.005	2.33	1.28–4.23
Anemia	0.819	0.288	0.004	2.26	1.29–3.98
ROP ≥ 2	0.668	0.45	0.14	1.95	0.79–4.76
NEC	21.18	12,879.7	0.999	1.5E8	0.00–
Dynamic true EUGR for Fenton 2013					
Gestational age (weeks)	−0.23	0.12	0.059	0.791	0.62–1.009
Birth weight (g)	0.000	0.001	0.9	1	0.998–1.002
Male sex	0.518	0.259	0.045	1.67	1.01–2.78
RDS	0.79	0.27	0.003	2.22	1.3–3.79
Anemia	0.802	0.28	0.005	2.22	1.26–3.92
ROP ≥ 2	1.11	0.54	0.042	3.05	1.04–8.95
NEC	20.5	12,827.69	0.99	8.0E8	0.00–

RDS, respiratory distress syndrome; NEC, necrotizing enterocolitis; ROP, retinopathy of prematurity.

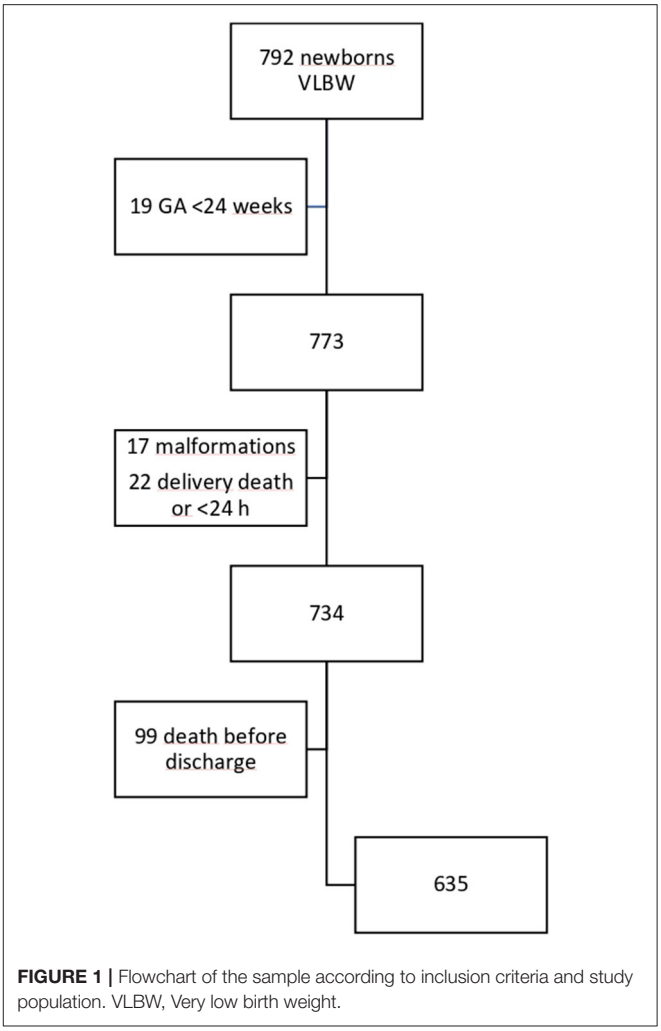


FIGURE 1 | Flowchart of the sample according to inclusion criteria and study population. VLBW, Very low birth weight.

and 53.9% (IW-21) due to the high proportion of IUGR patients in our series.

If we focus on <1,000 g birth weight infants ($N = 161$), only 23.6% achieved a discharge W above the 10th percentile using IW-21 and 13% using Fenton. These results are very similar to those that had already been reported in another publication (25). We observed a good concordance between both classifications in this group (Kappa > 0.6).

When we exclude IUGR, static and dynamic true EUGR prevalence for W was, respectively, 35.7 and 39.6% for IW-21 and 60.4 and 52.7% for Fenton. The percentage of misclassified cases as non-EUGR by IW-21 was much lower than that by Fenton (1 vs. 98%). The agreement of both graphs for L and HC was much better, especially for the static calculation. In any case, our data are lower than those of Figueras-Aloy (16), who obtained a true EUGR prevalence of 42.7% by IW-21, and higher than those of Tuzun et al. (17), who published 24 and 37.7% for IW-21 and Fenton, respectively.

Patients who experienced true EUGR have had more frequent perinatal morbidity during their admission (late-onset sepsis, RDS, anemia requiring transfusion, hypotension, BPD, ROP ≥ stage 2, PDA, parenteral nutrition at 28 days of life, and NEC) and longer neonatal admission. A greater relative risk is observed for having had these pathologies if we use dynamic criteria vs. static criteria to define EUGR, and in both cases greater statistical association using IW-21. Therefore, dynamic EUGR defined with IW-21 references is associated with greater complications in neonatal period and may better represent EUGR.

Among independent risk factors for the development of true EUGR, we found, as Figueras-Aloy did, male sex, birth weight, GA, and RDS (16). In our series, BPD was not found to be an independent risk factor.

In our study, greater decrease in the z-score in W (IW-21 and Fenton) in the first 28 days of life was directly related to a greater risk of static and dynamic EUGR at hospital discharge, but with

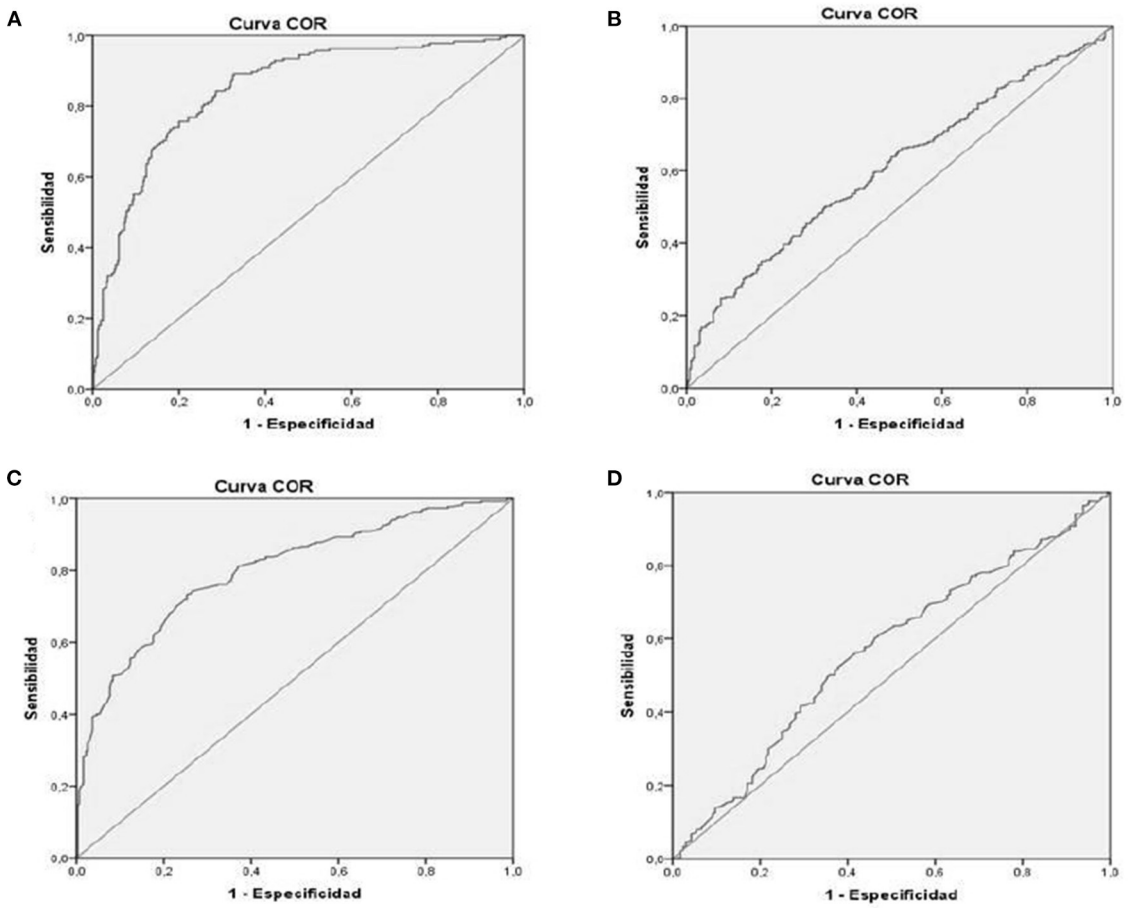


FIGURE 2 | ROC cube: relationship between decrease Z score in the first 28 days of life and risk of EUGR **(A)** INTERGROWTH-21st dynamic-EUGR. **(B)** INTERGROWTH-21st static-EUGR. **(C)** Fenton dynamic-EUGR. **(D)** Fenton static-EUGR). EUGR, Extrauterine growth restriction.

greater power of relation with dynamic criteria and with IW-21 standards. This implies that the first weeks of life of VLBW represent a critical period of growth, with a high probability of morbidity and difficulties in growth, and the repercussion on growth will continue at hospital discharge. Prioritizing and emphasizing nutrition in the first 28 days of life are essential.

According to the criteria and chart we use, EUGR prevalence in VLBW varies substantially within the same series: from 73.8% of patients using static criteria in the Fenton graphs to 29.3% using dynamic criteria with IW-21 standards. Therefore, up to twice as many patients can be diagnosed with EUGR according to the criteria we use; hence, the importance of determining which classification is better.

In our series, IW-21 seems stricter than Fenton for classifying EUGR (static, dynamic, and true EUGR). Furthermore, patients diagnosed by IW-21 as EUGR for W had more frequent neonatal morbidity during their admission.

Kim et al. (26) obtained a similar result after comparing the dynamic EUGR and static EUGR in both graphs in a cohort of 1,356 preterm infants with GA <28 weeks. Tuzun et al. (17) also used static criteria to define EUGR and made a comparison between the Fenton and IW-21 curves, including 248 children under 32 weeks with an IUGR percentage of 12%, observing a lower prevalence of EUGR using IW-21 (31.5 vs. 40%). Reddy et al. (15) also obtained a lower incidence of EUGR with IW-21 compared with Fenton (48 vs. 55%), after analyzing 603 under 32 weeks with a proportion of IUGR of 15%. In a European multicountry cohort, EUGR (discharge weight <10th percentile) using the Fenton charts varied from 24% (Sweden) to 60% (Portugal) and using IW-21 from 13% (Sweden) to 43% (Portugal) (27). A wide variation in rate of EUGR is related to heterogeneity of inclusion criteria and used definition in different reports.

This study and other previous ones highlight the need to standardize criteria and the evaluation method of EUGR, which allow to compare results and to generate hypotheses to improve nutrition in neonatal units and to perform studies on its long-term implication.

Several studies link long-term EUGR with growth retardation and adverse neurodevelopmental, although this last association is not clearly demonstrated (28). Although morbidity associated with prematurity contributes to EUGR, nutrition is the most

significant determinant. Adequate nutritional support during the hospital stay is critical to reduce EUGR rate. However, if it is excessive, this can lead to an increase in fat mass and a higher future risk of non-communicable diseases such as obesity or metabolic syndrome (29).

Although more studies are necessary, we believe that IW-21 could be the best standard for assessing postnatal growth, in terms of both the method of construction and its correlation with EUGR risk factors. We also consider that the change in z-score reflects postnatal growth better than static analysis at discharge.

Among the limitations of our study, we should note the long-term recruiting time in order to include as much low VLBW infants as possible, having in count the low VLBW incidence we have in our unit. In this period of time, there were changes in neonatal management according to the latest recommendations. However, this study analyzes a very large sample of VLBW infants to evaluate IUGR and EUGR prevalence according to classic curves (Fenton) and new ones, although little used in daily practice (IW-21), including a comparison between different ways of defining EUGR (static and dynamic) and associated factors.

CONCLUSIONS

Concordance between the Fenton and IW-21 graphs for IUGR is good, but there is less agreement in EUGR, with IW-21 being more restrictive. However, patients diagnosed by IW-21 as EUGR are more likely to have had neonatal morbidities, especially if we use the dynamic definition of EUGR. Greater decrease in z-score in W in first 28 days predicts EUGR (all types) risk at discharge in both growth charts. In our study, we cannot conclude that one graph is better than the other.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Research Ethics Committee of Principado de Asturias (CEIm PA, SPAIN). Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin.

AUTHOR CONTRIBUTIONS

GS-S, LG-G, and EG-L contributed to conception and design of the study. BF-C organized the database. LM-F and GS-S performed the statistical analysis. LG-G wrote the first draft of the manuscript. SL-V, MS-R, and RA-L wrote sections of the manuscript. All authors contributed to manuscript revision, read, and approved the submitted version.

REFERENCES

1. Ruth VA. Extrauterine growth restriction: a review of the literature. *Neonatal Netw*. (2008) 27:177–84. doi: 10.1891/0730-0832.27.3.177
2. Clark RH, Thomas P, Peabody J. Extrauterine growth restriction remains a serious problem in prematurely born neonates. *Pediatrics*. (2003) 111(5 Pt 1):986–90. doi: 10.1542/peds.111.5.986
3. Blencowe H, Cousens S, Chou D, Oestergaard M, Say L, Moller A-B, et al. Born too soon: the global epidemiology of 15 million preterm births. *Reprod Health*. (2013) 10(Suppl 1):S2. doi: 10.1186/1742-4755-10-S1-S2
4. Pampanini V, Boiani A, De Marchis C, Giacomozzi C, Navas R, Agostino R, et al. Preterm infants with severe extrauterine growth retardation (EUGR) are at high risk of growth impairment during childhood. *Eur J Pediatr*. (2015) 174:33–41. doi: 10.1007/s00431-014-2361-z
5. Ramel SE, Demerath EW, Gray HL, Younge N, Boys C, Georgieff MK. The relationship of poor linear growth velocity with neonatal illness and two-year neurodevelopment in preterm infants. *Neonatology*. (2012) 102:19–24. doi: 10.1159/000336127
6. Hsu C-T, Chen C-H, Lin M-C, Wang T-M, Hsu Y-C. Post-discharge body weight and neurodevelopmental outcomes among very low birth weight infants in Taiwan: a nationwide cohort study. *PLoS ONE*. (2018) 13:e0192574. doi: 10.1371/journal.pone.0192574
7. Horbar JD, Ehrenkranz RA, Badger GJ, Edwards EM, Morrow KA, Soll RF, et al. Weight growth velocity and postnatal growth failure in infants 501 to 1500 grams: 2000-2013. *Pediatrics*. (2015) 136:e84–92. doi: 10.1542/peds.2015-0129
8. Villar J, Giuliani F, Barros F, Roggero P, Coronado Zarco IA, Rego MAS, et al. Monitoring the postnatal growth of preterm infants: a paradigm change. *Pediatrics*. (2018) 141: e20172467. doi: 10.1542/peds.2017-2467
9. American Academy of Pediatrics, Committee on Nutrition. Nutritional needs of low-birth-weight infants. *Pediatrics*. (1977) 60:519–30.
10. Ehrenkranz RA, Younes N, Lemons JA, Fanaroff AA, Donovan EF, Wright LL, et al. Longitudinal growth of hospitalized very low birth weight infants. *Pediatrics*. (1999) 104(2 Pt 1):280–9. doi: 10.1542/peds.104.2.280
11. Griffin IJ, Tancredi DJ, Bertino E, Lee HC, Profit J. Postnatal growth failure in very low birthweight infants born between 2005 and 2012. *Arch Dis Child Fetal Neonatal Ed*. (2016) 101:F50–5. doi: 10.1136/archdischild-2014-308095
12. Fenton TR, Kim JH. A systematic review and meta-analysis to revise the Fenton growth chart for preterm infants. *BMC Pediatr*. (2013) 13:59. doi: 10.1186/1471-2431-13-59
13. Villar J, Giuliani F, Bhutta ZA, Bertino E, Ohuma EO, Ismail LC, et al. Postnatal growth standards for preterm infants: the preterm postnatal follow-up study of the INTERGROWTH-21(st) project. *Lancet Glob Health*. (2015) 3:e681–91. doi: 10.1016/S2214-109X(15)00163-1
14. Izquierdo Renau M, Aldecoa-Bilbao V, Balcells Esponera C, Del Rey Hurtado de Mendoza B, Iriondo Sanz M, et al. Applying methods for postnatal growth assessment in the clinical setting: evaluation in a longitudinal cohort of very preterm infants. *Nutrients*. (2019) 11:2772. doi: 10.3390/nu11112772
15. Reddy KV, Sharma D, Vardhelli V, Bashir T, Deshbotla SK, Murki S. Comparison of Fenton 2013 growth curves and Intergrowth-21 growth standards to assess the incidence of intrauterine growth restriction and extrauterine growth restriction in preterm neonates ≤32 weeks. *J Matern Fetal Neonatal Med*. (2019) 1—8. doi: 10.1080/14767058.2019.1670795
16. Figueras-Aloy J, Palet-Trujols C, Matas-Barceló I, Botet-Mussons F, Carbonell-Estrany X. Extrauterine growth restriction in very preterm infant: etiology, diagnosis, and 2-year follow-up. *Eur J Pediatr*. (2020) 179:1469–79 doi: 10.1007/s00431-020-03628-1
17. Tuzun F, Yucesoy E, Baysal B, Kumral A, Duman N, Ozkan H. Comparison of INTERGROWTH-21 and Fenton growth standards to assess size at birth and extrauterine growth in very preterm infants. *J Matern Fetal Neonatal Med*. (2017) 31:1–6. doi: 10.1080/14767058.2017.1339270
18. Moro Serrano M, Fernández Pérez C, Figueras Alloy J, Pérez Rodríguez J, Coll E, Doménech Martínez E, et al. SEN1500: diseño y desarrollo del

registro de niños de menos de 1.500 g al nacer en España. *An Pediatr*. (2008) 68:181–8. doi: 10.1157/13116235
19. Olsen IE, Lawson ML, Ferguson AN, Cantrell R, Grabich SC, Zemel BS, et al. BMI curves for preterm infants. *Pediatrics*. (2015) 135:e572–81. doi: 10.1542/peds.2014-2777
20. Pritchard NL, Hiscock RJ, Lockie E, Permezel M, McGauren MFG, Kennedy AL, et al. Identification of the optimal growth charts for use in a preterm population: an Australian state-wide retrospective cohort study. *PLoS Med*. (2019) 16:e1002923. doi: 10.1371/journal.pmed.1002923
21. Barreto CM, Pereira MAL, Rolim ACB, Abbas SA, Langhi Junior DM, Santos AMND. Incidence of small for gestational age neonates, according to the Fenton and Intergrowth-21st curves in a level ii maternity. *Rev Paul Pediatr*. (2021) 39:e2019245. doi: 10.1590/1984-0462/2021/39/2019245
22. García P, San Feliciano L, Benito F, García R, Guzmán J, Salas S, et al. Evolución a los 2 años de edad corregida de una cohorte de recién nacidos con peso inferior o igual a 1.500 g de los hospitales pertenecientes a la red neonatal SEN1500. *An Pediatr*. (2013) 79:279–87. doi: 10.1016/j.anpedi.2013.03.017
23. Lebrão CW, Suano-Souza FI, Sarni ROS. Is the intrauterine INTERGROWTH-21 growth curve better than fenton's for the classification at birth and prediction of postnatal growth in preterm infants? *Matern Child Health J*. (2020) 24:1446–53. doi: 10.1007/s10995-020-02988-2
24. Avila-Alvarez A, Solar Boga A, Bermúdez-Hormigo C, Fuentes Carballal J. Restricción del crecimiento extrauterino en recién nacidos de menos de 1.500 gramos de peso al nacer?. *An Pediatr*. (2018) 89:325–32. doi: 10.1016/j.anpedi.2018.02.004
25. Mabhandi T, Ramdin T, Ballot DE. Growth of extremely low birth weight infants at a tertiary hospital in a middle-income country. *BMC Pediatr*. (2019) 19:231. doi: 10.1186/s12887-019-1568-6
26. Kim Y-J, Shin SH, Cho H, Shin SH, Kim SH, Song IG, et al. Extrauterine growth restriction in extremely preterm infants based on the Intergrowth-21st project preterm postnatal follow-up study growth charts and the Fenton growth charts. *Eur J Pediatr*. (2021) 180:817–24. doi: 10.1007/s00431-020-03796-0
27. El Rafei R, Jarreau P-H, Norman M, Maier RF, Barros H, Van Reempts P, et al. Variation in very preterm extrauterine growth in a European multicountry cohort. *Arch Dis Child Fetal Neonatal Ed*. (2020) 106:316–23. doi: 10.1136/archdischild-2020-319946
28. Fenton TR, Nasser R, Creighton D, Tang S, Sauve R, Bilan D, et al. Weight, length, and head circumference at 36 weeks are not predictive of later cognitive impairment in very preterm infants. *J Perinatol*. (2021) 41:606–14. doi: 10.1038/s41372-020-00855-0
29. Moore BF, Harrall KK, Sauder KA, Glueck DH, Dabelea D. Neonatal adiposity and childhood obesity. *Pediatrics*. (2020) 146:e20200737. doi: 10.1542/peds.2020-0737

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Pediatrics

BRIEF RESEARCH REPORT
published: 10 August 2021
doi: 10.3389/fped.2021.711400

Small for Gestational Age Preterm Neonates Exhibit Defective GH/IGF1 Signaling Pathway

Emmanuelle Motte-Signoret[1,2,3]*, *Shivani Shankar-Aguilera*[1], *Sylvie Brailly-Tabard*[4], *Yohan Soreze*[1], *Valentina Dell Orto*[1], *Rafik Ben Ammar*[1], *Daniele De Luca*[1] and *Pascal Boileau*[2,5]

[1] Assistance Publique-Hôpitaux de Paris, Béclere Hospital, GH Paris Sud, Neonatal Intensive Care Unit, Clamart, France, [2] Poissy St Germain Hospital, Neonatal Intensive Care Unit, Poissy, France, [3] Paris-Saclay University, Université Versailles Saint Quentin, Institut National de Recherche pour l'Agriculture, l'Alimentation et l'Environnement, BREED, Jouy-en-Josas, France, [4] Assistance Publique-Hôpitaux de Paris, Bicêtre Hospital, Molecular genetics Pharmacogenetics and Hormonology, Le Kremlin-Bicêtre, France, [5] Institut National de la Santé et de la Recherche Médicale U1185, Le Kremlin-Bicêtre, France

Objective: To investigate the impact of fetal growth restriction (FGR) on hormonal regulation of post-natal growth and glucose metabolism [via insulin and growth hormone (GH)/Insulin-like Growth factor 1 (IGF1) axis pathways] in small for gestational age (SGA) neonates.

Methods: We conducted a monocentric observational prospective comparative study on 73 singleton babies born with a weight inferior to 2,000 g. We analyzed auxological (weight, height and head circumference), and hormonal (GH, IGF1, and insulin plasma concentrations) data comparing SGA and appropriate for gestational age (AGA) neonates, between day 1 and 60.

Results: One third (23/73) of the neonates were SGA. Twenty-five percent (18/73) required insulin for idiopathic hyperglycemia of prematurity and were smaller in weight and head circumference at discharge. In the SGA group compared with the AGA group, GH plasma concentrations were higher at day 3 (70.1 vs. 38.0 mIU/L) and IGF1 plasma concentrations were higher at day 10 (29.0 vs. 18.7 ng/ml).

Conclusions: SGA neonates displayed resistance to GH and IGF1, concomitant to insulin resistance. This could partially explain the initial defective catch-up growth and, later in life, the higher prevalence of metabolic syndrome in this population.

Keywords: small for gestational age, preterm infants, fetal growth restriction, post-natal growth, idiopathic hyperglycemia, hormone resistance, metabolic syndrome, fetal programming 3

OPEN ACCESS

Edited by:
Lynette Kay Rogers, The Research Institute at Nationwide Children's Hospital, United States

Reviewed by:
Anna Kistner, Karolinska University Hospital, Medical Radiation Physics and Nuclear Medicine, Sweden
Renata Stawerska, Polish Mother's Memorial Hospital Research Institute, Poland

***Correspondence:**
Emmanuelle Motte-Signoret
emmanuelle.mottesignoret@ght-yvelinesnord.fr

Specialty section:
This article was submitted to Neonatology, a section of the journal Frontiers in Pediatrics

Received: *18 May 2021*
Accepted: *14 July 2021*
Published: *10 August 2021*

Citation:
Motte-Signoret E, Shankar-Aguilera S, Brailly-Tabard S, Soreze Y, Dell Orto V, Ben Ammar R, De Luca D and Boileau P (2021) Small for Gestational Age Preterm Neonates Exhibit Defective GH/IGF1 Signaling Pathway. Front. Pediatr. 9:711400. doi: 10.3389/fped.2021.711400

INTRODUCTION

Preterm birth is a major risk factor of neonatal morbidity and mortality which is worsened with fetal growth restriction (FGR) (1–3). In the 90's, Barker's work revealed that small for gestational age (SGA) neonates, defined as a birthweight below the 10th percentile for the gestational age, had an increased risk to develop metabolic syndrome when adults. This was the beginning of the concepts of fetal programming and the thrifty phenotype hypothesis (4, 5). Long term alterations in energetic metabolism affect these children with some of them exhibiting early insulin resistance (6). In addition, idiopathic hyperglycemia is frequent among extremely preterm or very low birth

weight (VLBW) neonates (7, 8), and is usually explained by defects in glucose regulation with insulin resistance and lower insulin secretion (9, 10).

During fetal life and the first months after birth, growth is predominantly depending on nutrient intake and fetal insulin (11). The growth hormone (GH)/Insulin-like Growth factor 1 (IGF1) axis has a minor role in the regulation of growth during the first months of life, but is essential for glucose homeostasis (12). Maternal and placental GH are not known to impact fetal growth either (13). It is likely that insulin and GH/IGF1 endocrine systems share some of their intracellular signaling pathways and have synergistic anabolic and metabolic actions during the first weeks of extra-uterine life. Recently, murine models have emphasized the correlation between lack of catch-up growth in SGA rats and GH resistance (14) and the existence of cross-linked interactions between insulin and GH/IGF1 axis pathways (15).

Prematurity compounded with FGR expose the neonate to an higher risk of perinatal mortality (16), postnatal growth failure and ultimately metabolic syndrome in adult life (17). We hypothesized that FGR alters GH/IGF1 post-natal signalization with mechanisms similar to insulin signalization defects, and could thus affects post-natal growth of SGA neonates. We conducted a prospective study in 73 low birth-weight (LBW) neonates, describing auxological evolution and hormonal assay during their hospitalization in neonatal intensive care unit (NICU) in order to address our hypothesis.

METHODS

Patients

This non-interventional comparative prospective cohort study was conducted in a single tertiary academic NICU between December 2015 and July 2016, with no modification of routine clinical care. It was approved by the local institutional review board (Paris Saclay University) and followed all the relevant local regulations (French Advisory Committee for the Protection of People, and CNIL n°2051804). Consent has been obtained from each patient's parent after full explanation of the purpose and nature of all procedures used.

All singleton, inborn neonates with birth weight $< 2{,}000$ g admitted to the NICU were eligible for inclusion. Gestational age was determined by ultrasound assessment during the first trimester of pregnancy. Exclusion criteria were multiple pregnancies, uncertain gestational age at birth, maternal diabetes, maternal height lower than 150 cm, established chromosomal abnormality, genetic disease or skeletal dysplasia.

Patient management was standardized according to the ESPGHAN guidelines for enteral and parenteral nutrition (18). Glucose intakes were initially 6 to 8 g/kg/d, advanced by 1 to 2 g/kg/d and the goal was to reach 12–15 g/kg/d at the end of the first week. Glycaemia was measured on blood drops obtained by heel prick, and using point-of-care glucometer at the bedside. The administration of insulin for idiopathic neonatal hyperglycemia was initiated after three capillary glycaemia over 12 mmol/l, and introduced with intravenous continuous lispro insulin (initial posology 0.02 IU/kg/h secondarily adapted to capillary glycaemia sampled every 3 h).

Abbreviations: FGR, fetal growth restriction; SGA, small for gestational age; AGA, appropriate for gestational age; GH, growth hormone; IGF1, insulin-like growth factor 1; IGF2, insulin-like growth factor 2; IU, international unit; NICU, neonatal intensive care unit; EUGR, extra-uterine growth retardation; HC, head circumference; VLBW, very low birth-weight; LBW, low birth-weight; SRS, silver-russel syndrome; HOMA, homeostasis model assessment of insulin resistance.

Data Collection

We collected clinical data [sex, gestational age, antenatal corticosteroids, etiology of prematurity, mode of delivery, insulin treatment, weight, height and head circumference (HC) at birth, around day 10, day 30 and day 60], in real time during hospitalization until discharge. Small and appropriate for gestational age (SGA and AGA) neonates were differentiated according to Fenton 2013 growth charts (19) taking for threshold the 10th percentile for weight and/or height. We defined extra-uterine growth retardation (EUGR) as growth values $\leq$10th percentile of intrauterine growth expectation based on estimated postmenstrual age in premature neonates at the time they are discharged from the hospital (20).

Plasma concentrations of IGF1, GH and insulin were measured between day 2 and 3, between day 8 and 10, and between day 25 and 35 using Sandwich immunometric assays (Immulite 2,000 XPi, Siemens Healthcare Diagnostics Erlangen, Germany for GH (detection thresholds: 3–1,000 mUI/L) and IGF1 (detection thresholds: 2–1,000 ng/mL) and Liaison, DiaSorin, Saluggia, Italy for Insulin, which does not crossmatch with lispro (detection thresholds: 0.87–500 μIU/mL)]. Blood was drawn through indwelling central venous lines or when not the case, by venipuncture at the same time of blood sampling for routine clinical care of the neonate. In case of venipuncture, non-pharmacological sedation (nursing 30% glucose solution) was given orally according to our clinical protocol, classically used in NICU without significantly modifying glycaemia.

We defined insulin resistance as the need for insulin treatment despite appropriate glucose intakes, and GH resistance as the association of elevated GH levels and extremely decreased IGF1 levels (below assay threshold).

Statistical Analysis

Gaussian distributed data were expressed as mean +/- standard deviation. Differences in categorical variables were assessed by Chi2 or Fisher test, as appropriate. Continuous data were compared with Student test, correlation was tested with Pearson's coefficient.

We compared AGA vs. SGA groups, and patients requiring insulin therapy vs. the others (insulin and no insulin groups, respectively). The analysis was carried out with the statistical software GraphPad Prism 8.1. *P*-values < 0.05 were considered statistically significant.

RESULTS

Clinical Characteristics of the Study Population

Seventy-three LBW neonates were included in our study, 22 (30%) were born before 28 weeks GA, 23 (32%) were SGA. **Figure 1** describes the longitudinal evolution of the cohort: 60 babies (82%) were still hospitalized in our unit at day 10 (18 SGA), 39 (53%) at day 30 and only 17 (23%) at day 60. All SGA neonates had been discharged before day 60. Hormonal assays were performed in 51, 34, and 25 patients at day 3, 10, and 30, respectively.

Prematurity was medically induced in 49% (36/73) of all, and in 91% (21/23) of SGA neonates. Before 28 weeks of gestation, there was no preterm birth medically induced for fetal growth restriction (FGR). Conversely, preterm labor and preterm premature rupture of membranes rates were lower with a higher gestational age. Preterm birth was medically induced for FGR in 62% (14/23) of SGA neonates, whereas prematurity was spontaneous in 70% (35/50) of AGA neonates. Within the overall population, 26 neonates (36%) were extremely low birth weight (ELBW, <1,000 g), 22 (30%) and 55 (75%) were born before 28 and 32 weeks of gestation, respectively.

Birth auxological characteristics are described in **Table 1**. SGA neonates had a higher gestational age with a similar BW in comparison to AGA neonates. By definition, BW, BH and BHC in percentiles were significantly lower in SGA group. No difference was observed between the two groups for antenatal steroids. Eighteen (25%) neonates required insulin administration during their hospitalization, with a mean gestational age of 27.1 weeks and a mean BW of 881 g. Extremely preterm (<28 weeks) and ELBW infants had a significantly increased need for insulin treatment compared to the global study population ($p = 0.0005$ and $p < 0.0001$, respectively). There was no significant difference between AGA and SGA groups for need for insulin.

Post-natal Growth

Thirty eight percent (15/39) of the babies exhibited EUGR at day 30 (8 within 32 AGA neonates (25%) vs. 100% of SGA neonates – $p < 0.0001$). Seventy one percent (12/17) of them had EUGR at day 60 and among them 90% were those who required insulin in the first days of life.

The post-natal growth of the whole population and according to SGA/AGA and insulin/no insulin requirement status is illustrated in **Figure 2**. Weight and height in percentile of SGA neonates persisted to be significantly lower than the AGA neonates at birth, day 10 and day 30, while the HC was not significantly different between the two groups at day. Weight and HC of neonates requiring insulin were not significantly different than those without insulin at birth but were significantly lower at day 60.

Hormonal Regulation

GH, IGF1 and insulin plasma concentrations are shown in **Table 2** and **Supplementary Figure 1**. Fifty-one neonates had available measurements at day 3, 34 at day 10, and 25 at day 30 (respectively, 70, 47, and 34% of initial study population). We compared the results between AGA vs. SGA infants and between those who required insulin treatment vs. the others.

GH plasma concentrations during the first month of life exhibited a mean range of 50, 38, and 47 mIU/l at day 3, day 10 and day 30 respectively. Forty two neonates (82%) exhibited GH levels to 20 mIU/L at day 3, 31 (91%) at day 10 and 23 (92%) at day 30. On the contrary, 4 (8%) neonates exhibited GH levels lower to 10 mIU/ml at day 3, 1 (3%) at day 10 and none at day 30. GH plasma concentrations were higher at day 3 in SGA (70.1 vs. 38.0 mIU/L; $p = 0.004$). We found no correlation between GH at birth and GA nor BW and a low correlation between GH and BW Fenton percentile ($R^2 = 0.09$, $p = 0.03$).

IGF1 plasma concentrations exhibited a mean range of 7 ng/ml at birth, progressively increased during the first month (20 ng/ml at day 10 and 25 ng/ml at day 30) and were below the minimum threshold of the assay in 13 neonates (25%) at day 3, 2 (6%) at day 10 and 1 (4%) at day 30, regardless of weight, gestational age and weight for gestational age. At birth, IGF1 levels discretely correlated to GA ($R^2 = 0.08$, $p = 0.04$) and BW ($R^2 = 0.14$, $p = 0.007$) but not BW Fenton percentile. IGF1 plasma concentrations were higher at day 10 in SGA compared to AGA neonates (29 vs. 19 ng/mL; $p < 0.001$).

There was no significant difference of GH and IGF1 plasma concentrations during the first month of life between neonates needing insulin therapy and the others. Mean insulin levels were 15.5 mIU/ml at day 3 and decreased to 11.0 and 11.5 mIU/ml at day 10 and day 30. Low insulin levels (< 5 mIU/ml) were observed in 13 (25%), 7 (21%) and 3 (12%) neonates whereas 10 (20%), 2 (6%) and 1 (4%) neonates exhibited high insulin levels (> 40 mIU/ml) at day 3, day 10 and day 30, respectively. There were no significant differences in insulin plasma concentrations between AGA and SGA neonates. However, we found significantly higher insulin levels in babies requiring insulin treatment at day 10. There was no correlation between insulin plasma concentrations with either gestational age or birth weight.

At day 3, we found no correlation between GH and IGF1 plasma concentrations, for any gestational age or weight. However, 2 SGA neonates exhibited extremely high plasma concentrations of GH (208 and 171 mIU/L) with concomitant concentrations of IGF1 below the minimum threshold of the assay (<1 ng/ml). At day 30, GH plasma concentrations were inversely correlated to IGF1 levels ($R^2 = 0.38$, $p = 0.01$) in AGA babies.

DISCUSSION

Glucose metabolism ontogenesis and fetal and neonatal growth are mainly regulated by insulin and GH/IGF1 axis pathways (21, 22). Prematurity and fetal growth restriction expose the neonate to growth failure and metabolic syndrome in childhood and when adults (17, 23–25). We have found that post-natal growth was altered in babies who required insulin for idiopathic hyperglycemia of prematurity and that SGA neonates were likely resistant to GH and IGF1. These results support the existence of cross-linked interactions between insulin and GH/IGF1 axis

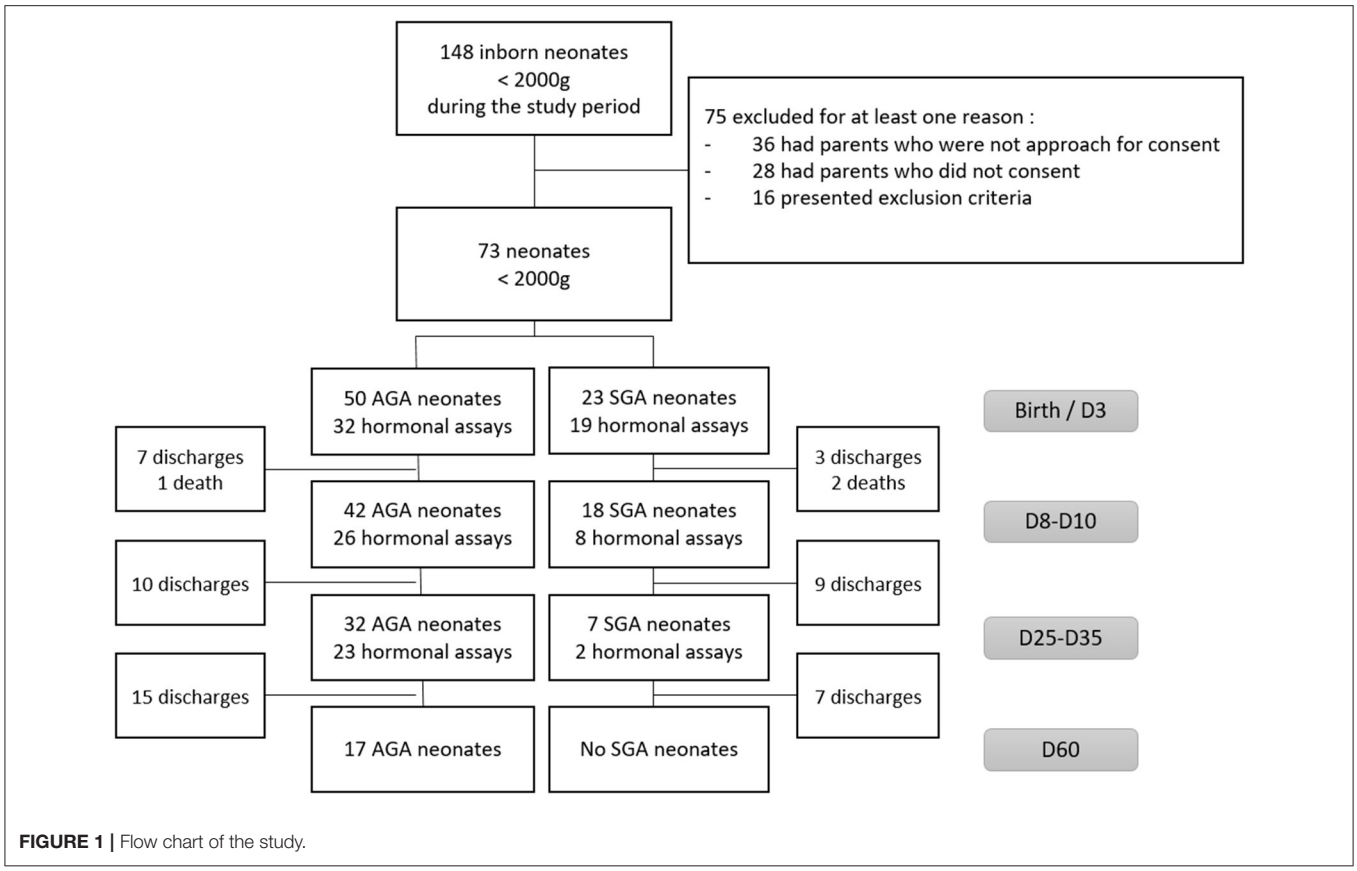


FIGURE 1 | Flow chart of the study.

TABLE 1 | Main characteristics of the study population.

	All	< 28 weeks GA	AGA	SGA
N	73	22	50	23
GA (weeks)	29.8 ± 3.0	26.5 ± 1.2	28.6 ± 2.2	32.6 ± 2.5***
< 28 wZeeks GA (%)	22 (30)	–	18 (36)	4 (17) ns
SGA (%)	23 (32)	4 (18)	–	–
BW (g)	1,153 ± 314	828 ± 156	1,118 ± 310	1,230 ± 315
BW (percentile)	33 ± 25	42 ± 21	45.6 ± 20.3	5.9 ± 4.6***
BH (cm)	37.0 ± 3.3	33.5 ± 2.0	36.6 ± 3.3	37.8 ± 3.2
BH (percentile)	34 ± 28	43 ± 20	47.5 ± 24	6.0 ± 6.3***
BHC (cm)	26.3 ± 3.6	23.5 ± 1.4	25.8 ± 2.6	27.2 ± 2.2*
BHC (percentile)	36 ± 27	40 ± 20	48.0 ± 24.5	10.4 ± 9.8***
Sex F/M	36/37	10/12	23/27	13/10
Antenatal steroids *n* (%)	57 (78)	18 (82)	39 (78)	18 (78) ns
Insulin therapy *n* (%)	18 (25)	12 (55)	15 (30)	3 (13) ns

*GA, gestational age; BW, BH, BHC, weight, height and head circumference at birth. Results expressed as mean ± standard deviation and percentage. ns non significant*p < 0.05 ***p < 0.001 for comparison between AGA vs. SGA groups.*

pathways, possibly contributing to a defect in growth and metabolic adaptation in this population of FGR babies.

There was several limitations in this prospective study conducted in 73 low birth weight preterm neonates, first prevalence of medically induced prematurity for FGR increases with the gestational age at birth due to obstetrical FGR management. Second, at day 60, an important number of patients has been lost to follow-up, including all the SGA neonates which had been discharged. Both limitations could have been source of significant bias in our study.

We first described post-natal growth and observed persistence in differences of auxological parameters between SGA and

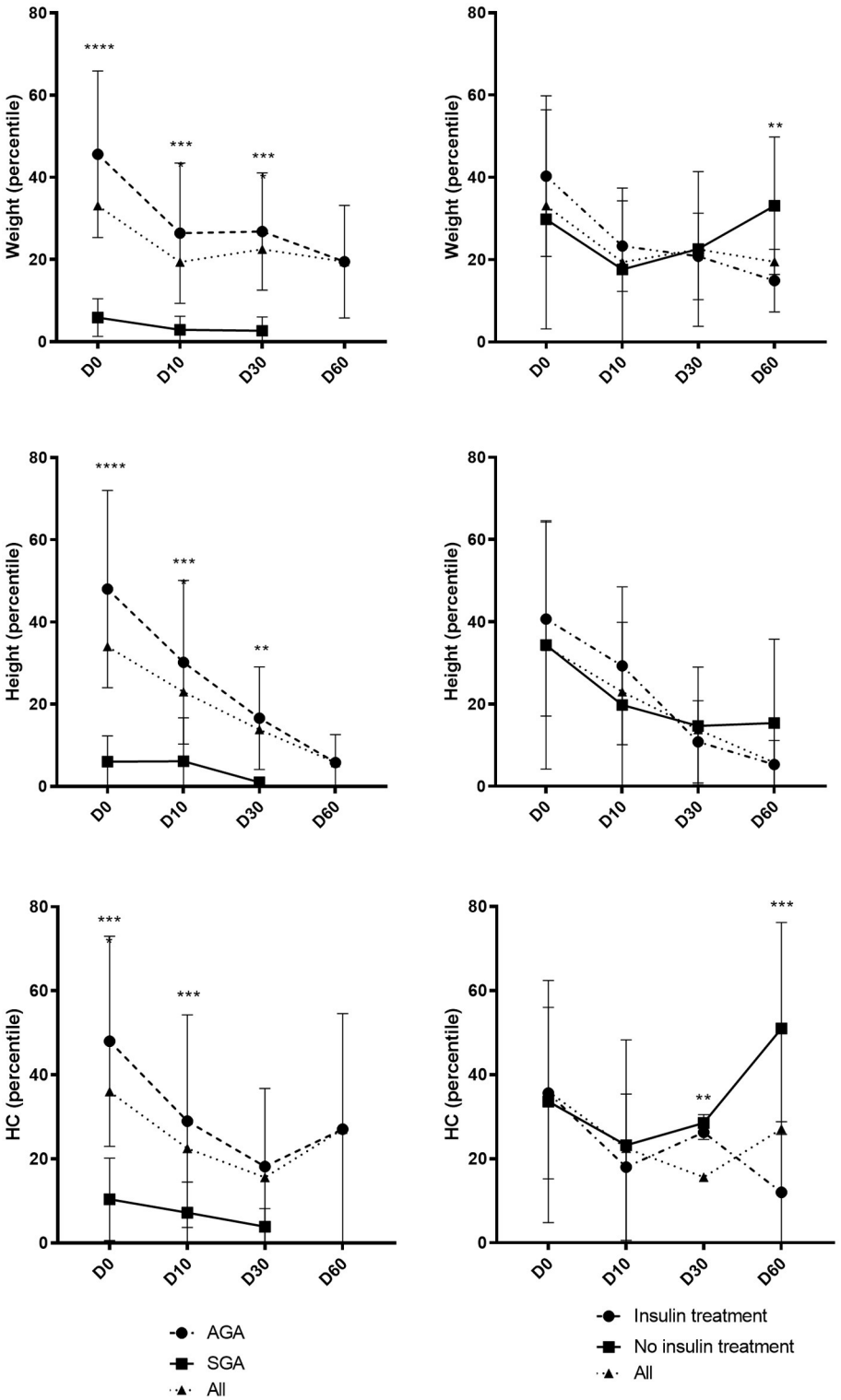


FIGURE 2 | Evolution of growth parameters during the first 2 months of life. Day 0 (D0): all $n = 73$/AGA $n = 50$ vs. SGA $n = 23$/insulin $n = 18$ vs. no insulin $n = 55$. Day 10 (D10): all $n = 60$/AGA $n = 42$ vs. SGA $n = 18$/insulin $n = 16$ vs. no insulin $n = 44$. Day 30 (D30): all $n = 39$/AGA $n = 32$ vs. SGA $n = 7$/insulin $n = 13$ vs. no insulin $n = 26$. Day 60 (D60): all $n = 17$/AGA $n = 17$ vs. SGA $n = 0$/insulin $n = 7$ vs. no insulin $n = 10$. Results expressed in mean ± standard deviation. $^{**}p < 0.01$ $^{***}p < 0.001$ $^{****}p < 0.0001$.

TABLE 2 | mean plasma concentration of GH, IGF1 and insulin at day 3, 10 and 30 [mean ± standard deviation (min-max)].

		GH (mIU/L)	IGF1 (ng/ml)	Insulin (mIU/L)
Day 3	**All ($n = 51$)**	**50 ± 41 (5–208)**	**9 ± 8 (1–29)**	**27 ± 46 (1–307)**
	< 28 weeks GA	42 ± 37 (11–110)	5.8 ± 6 (1–23)	34 ± 35 (2–131)
	AGA ($n = 32$)	38 ± 28 (6–110)	8 ± 8 (2–29)	25 ± 26 (1–131)
	SGA ($n = 19$)	70 ± 51 (5–208)**	10 ± 7 (1–25)	31 ± 74 (1–307)
	Insulin ($n = 14$)	44 ± 38 (5–110)	5 ± 7 (1–23)	56 ± 83 (5–208)
	No insulin ($n = 47$)	52 ± 42 (6–208)	10 ± 8 (1–29)	18 ± 20 (1–80)
Day 10	**All ($n = 34$)**	**38 ± 25 (5–148)**	**21 ± 12 (1–40)**	**16 ± 16 (1–80)**
	< 28 weeks GA	43 ± 44 (5–148)	11 ± 8 (1–25)	31 ± 25 (7–80)
	AGA ($n = 26$)	38 ± 28 (5–148)	19 ± 12 (1–40)	18 ± 19 (1–80)
	SGA ($n = 8$)	38 ± 10 (23–52)	29 ± 10 (13–39)*	11 ± 8 (1–23)
	Insulin ($n = 10$)	39 ± 42 (5–148)	16 ± 8 (1–25)	30 ± 23 (12–80)*
	No insulin ($n = 24$)	38 ± 16 (12–75)	24 ± 13 (1–40)	10 ± 9 (1–36)
Day 30	**All ($n = 25$)**	**47 ± 29 (14–144)**	**26 ± 13 (1–48)**	**14 ± 14 (1–61)**
	< 28 weeks GA	48 ± 26 (21–80)	18 ± 12 (1–48)	12 ± 6 (1–21)
	AGA ($n = 23$)	43 ± 20 (14–82)	25 ± 13 (1–48)	15 ± 14 (1–61)
	SGA ($n = 2$)	(35–144)	(37–40)	(2)
	Insulin ($n = 8$)	42 ± 27 (14–80)	23 ± 15 (13–48)	13 ± 5 (7–21)
	No insulin ($n = 17$)	50 ± 32 (17–144)	29 ± 11 (13–45)	15 ± 16 (1–61)

*$p < 0.05$ **$p < 0.01$ for comparison between AGA vs. SGA or insulin vs. no insulin groups.

AGA groups during hospitalization. However, the differences were reduced progressively, mainly due to extra-uterine growth retardation (EUGR) in AGA neonates. We also found that preterm babies requiring insulin for idiopathic hyperglycemia, who had a higher birth weight percentile had lower weight and HC at day 60. Two others studies have found similar results: Scheurer et al. described that hyperglycemic preterm neonates were discharged with lower HC and had lower height, weight, and HC at four months post-discharge (26); Ramel and colleagues published that height, weight, and HC of hyperglycemic preterm neonates were lower at 36 months (27). These results are in accordance with our hypothesis of a resistance to the anabolic effect of insulin and concomitantly a poor response on growth of GH/IGF1 axis in this population.

In the second part, we analyzed plasma levels of GH, IGF1 and insulin. It has been reported that random GH values are clinically useful within the first month of life, and that serum IGF1 levels reflect spontaneous 24 h GH secretion (28, 29). GH plasma concentrations were elevated during the first month of life, as previously reported (28). A large proportion of the neonates exhibited spontaneous GH levels superior to 20 mIU/L (usual threshold used to define deficiency during GH stimulation test) while very few exhibited GH levels inferior to 10 mIU/ml. On the contrary, IGF1 plasma concentrations were very low at birth and progressively increased during the first month. They were frequently below the minimum threshold of the assay at birth. Overall, this suggests a resistance to GH action during the first month of life in this population.

At birth, there was no correlation between GH and BW or GA while IGF1 was discretely correlated to BW. However, BW percentile was correlated with GH levels, but not with IGF1 levels. GH and IGF1 plasma levels were also higher in SGA neonates, respectively, at day 3 and 10. GH and IGF1 plasma levels were not correlated before day 30. This suggests a higher level of resistance to GH and IGF1 in the first days of life in SGA neonates.

There are no precedent studies on GH plasma levels in this population but our results are in concordance with the few studies on IGF1 plasma levels in preterm babies (30). It has also been showed that IGF1 plasma concentration at birth was lower in SGA neonates (31), that mRNA and IGF1 protein were less expressed and translated in the placenta of SGA neonates (32). While IGF1 plasma levels were lower at birth they were found higher at 1 and 3 years in SGA children compared with AGA children (33). In addition, if SGA neonates do not exhibit catch-up growth before 3 years of life, IGF1 plasma levels remain lower at day 30 and 60 (30, 34).

Fetal growth relies on insulin and nutrient intake, and GH does not play a significant role in fetal and neonatal growth. Consistently, children with pan-hypopituitarism are not SGA and have no growth failure in the first months of life but frequently are hypoglycemic with low IGF1 levels (35). Conversely, children with defects in IGF1 signalization are SGA (36), and many polymorphisms in *IGF1* and *IGF1R* genes have been described associated with FGR (37, 38). This dissociation in the role of GH and IGF1 in fetal growth suggests that IGF1 secretion is partially independent of GH during this period. Fetal growth is largely due to insulin and nutrient intake [example of gestational diabetes mellitus (39)] but also to Insulin-like Growth Factor 2 (IGF2). This latter is central in the pathophysiology of Silver-Russel syndrome [SRS, low IGF2 and very SGA neonates (40)]. IGF1, IGF2 and insulin (and their respective receptors) share structural analogy, and their signalizations have cross-linked interactions (41). We suggest that SGA neonates, similarly to SRS children, might have a defect in the IGF system with compensatory increase in GH levels.

In our study, 22% of the neonates required insulin therapy for idiopathic hyperglycemia (45% among <28 weeks). All were born before 32 weeks and/or with a birth weight <1,500 g, concordant with previous findings (7). There was no correlation between insulin plasma levels and birth weight or gestational age. We observed a great variability in the plasma concentrations of insulin, especially among those who required insulin therapy. This is in accordance with the hypothesis that idiopathic hyperglycemia of the preterm neonate is related to both insulinopenia (due to abnormality in insulin biosynthesis during post-translational stages with pro-insulin accumulation) and insulin resistance (9, 10, 42). Nevertheless, considering that we analyzed neither the impact of nutritional intake nor the correlation with glycaemia during the insulin assay, these results should be taken with caution, even if all the neonates were managed with standardized procedures of enteral and parenteral nutrition according to ESPGHAN guidelines.

SGA neonates displayed a risk to develop metabolic syndrome (5, 23, 25) and SGA children have insulin resistance (43, 44). Some studies even correlate Homeostasis Model Assessment of Insulin Resistance (HOMA) index and higher IGF1 levels during childhood (45). Conversely, the absence of insulin resistance and better insulin secretion is correlated with a better catch-up growth (46, 47). Thus, post-natal growth and insulin sensitivity seem to be associated. Murine models have emphasized the correlation between lack of catch-up growth in SGA rats and GH resistance (14) and the existence of cross-linked interactions between insulin and GH/IGF1 axis pathways (15).

Overall, our results suggest GH and IGF1 resistance in addition to insulin resistance during the first weeks of life in SGA neonates.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by CNIL n°2051804. Written informed consent from the participants' legal guardian/next of kin was not required to participate in this study in accordance with the national legislation and the institutional requirements.

AUTHOR CONTRIBUTIONS

EM-S, PB, and DD conceived and designed the study. EM-S, SS-A, YS, VD, and RB provided clinical data. SB-T performed the biochemical assays. EM-S and PB analyzed the data. EM-S drafted the article and SS-A, PB, and DD revised it critically for important intellectual content. All authors contributed to the article and approved the submitted version.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fped.2021.711400/full#supplementary-material

Supplemental Figure 1 | Correlation between GH/IGF1 plasma concentrations at birth (day 0 to 3) and gestational age (GA), birth weight (BW) and BW Fenton percentile (each point represents a single neonate, n=51).

REFERENCES

1. Ancel P-Y, Goffinet F, EPIPAGE-2 Writing Group, Kuhn P, Langer B, Matis J, et al. Survival and morbidity of preterm children born at 22 through 34 weeks' gestation in France in 2011: results of the EPIPAGE-2 cohort study. *JAMA Pediatr*. (2015) 169:230–8. doi: 10.1001/jamapediatrics.2014.3351
2. Ego A. [Definitions: small for gestational age and intrauterine growth retardation]. *J Gynecol Obstet Biol Reprod.* (2013) 42:872–94. doi: 10.1016/j.jgyn.2013.09.012/18
3. Stoll BJ, Hansen NI, Bell EF, Shankaran S, Laptook AR, Walsh MC, et al. Neonatal outcomes of extremely preterm infants from the NICHD neonatal research network. *Pediatrics.* (2010) 126:443–56. doi: 10.1542/peds.2009-2959
4. Barker DJ, Osmond C, Golding J, Kuh D, Wadsworth ME. Growth in utero, blood pressure in childhood and adult life, and mortality from cardiovascular disease. *BMJ.* (1989) 298:564–7. doi: 10.1136/bmj.298.6673.564
5. Barker DJ, Gluckman PD, Godfrey KM, Harding JE, Owens JA, Robinson JS. Fetal nutrition and cardiovascular disease in adult life. *Lancet Lond Engl.* (1993) 341:938–41. doi: 10.1016/0140-6736(93)91224-A
6. Vaag A, Jensen CB, Poulsen P, Brøns C, Pilgaard K, Grunnet L, et al. Metabolic aspects of insulin resistance in individuals born small for gestational age. *Horm Res.* (2006) 65(Suppl. 3):137–43. doi: 10.1159/000091519
7. Beardsall K, Vanhaesebrouck S, Ogilvy-Stuart AL, Vanhole C, Palmer CR, Ong K, et al. Prevalence and determinants of hyperglycemia in very low birth weight infants: cohort analyses of the NIRTURE study. *J Pediatr.* (2010) 157:715–9.e1-3. doi: 10.1016/j.jpeds.2010.04.032
8. Zamir I, Tornevi A, Abrahamsson T, Ahlsson F, Engström E, Hallberg B, et al. Hyperglycemia in extremely preterm infants-insulin treatment, mortality and nutrient intakes. *J Pediatr.* (2018) 200:104–10.e1. doi: 10.1016/j.jpeds.2018.03.049
9. Mitanchez-Mokhtari D, Lahlou N, Kieffer F, Magny J-F, Roger M, Voyer M. Both relative insulin resistance and defective islet beta-cell processing of proinsulin are responsible for transient hyperglycemia in extremely preterm infants. *Pediatrics.* (2004) 113(3 Pt 1):537–41. doi: 10.1542/peds.113.3.537
10. Salis ER, Reith DM, Wheeler BJ, Broadbent RS, Medlicott NJ. Hyperglycaemic preterm neonates exhibit insulin resistance and low insulin production. *BMJ Paediatr Open.* (2017) 1:e000160. doi: 10.1136/bmjpo-2017-000160
11. Gluckman PD, Pinal CS. Regulation of fetal growth by the somatotrophic axis. *J Nutr.* (2003) 133(5 Suppl 2):1741S–6S. doi: 10.1093/jn/133.5.1741S
12. Benyi E, Sävendahl L. The physiology of childhood growth: hormonal regulation. *Horm Res Paediatr.* (2017) 88:6–14. doi: 10.1159/000471876
13. Alsat E, Guibourdenche J, Couturier A, Evain-Brion D. Physiological role of human placental growth hormone. *Mol Cell Endocrinol.* (1998) 140:121–7. doi: 10.1016/S0303-7207(98)00040-9
14. Huang Y, Du M, Zhuang S, Shen Z, Li Y. Impaired growth hormone receptor signaling during non-catch-up growth in rats born small for gestational age. *Horm Res Paediatr.* (2010) 74:106–13. doi: 10.1159/000313374
15. Deng H-Z, Deng H, Cen C-Q, Chen K-Y, Du M-L. Post-receptor crosstalk between growth hormone and insulin signal in rats born small for gestational age with catch-up growth. *PLoS ONE.* (2014) 9:e100459. doi: 10.1371/journal.pone.0100459
16. Monier I, Ancel P-Y, Ego A, Jarreau P-H, Lebeaux C, Kaminski M, et al. Fetal and neonatal outcomes of preterm infants born before 32 weeks of gestation according to antenatal vs. postnatal assessments of restricted growth. *Am J Obstet Gynecol.* (2017) 216:516.e1–516.e10. doi: 10.1016/j.ajog.2017.02.001
17. Hales CN, Barker DJ. The thrifty phenotype hypothesis. *Br Med Bull.* (2001) 60:5–20. doi: 10.1093/bmb/60.1.5

18. Koletzko B, Goulet O, Hunt J, Krohn K, Shamir R, Parenteral Nutrition Guidelines Working Group, et al. 1. Guidelines on paediatric parenteral nutrition of the European society of paediatric gastroenterology, hepatology and nutrition (ESPGHAN) and the European society for clinical nutrition and metabolism (ESPEN), supported by the European society of paediatric research (ESPR). *J Pediatr Gastroenterol Nutr*. (2005) 41(Suppl. 2):S1–87. doi: 10.1097/01.mpg.0000181841.07090.f4
19. Fenton TR. A new growth chart for preterm babies: Babson and Benda's chart updated with recent data and a new format. *BMC Pediatr.* (2003) 3:13. doi: 10.1186/1471-2431-3-13
20. Clark RH, Thomas P, Peabody J. Extrauterine growth restriction remains a serious problem in prematurely born neonates. *Pediatrics.* (2003) 111:986–90. doi: 10.1542/peds.111.5.986
21. Picon L. Effect of insulin on growth and biochemical composition of the rat fetus. *Endocrinology.* (1967) 81:1419–21. doi: 10.1210/endo-81-6-1419
22. Baker J, Liu JP, Robertson EJ, Efstratiadis A. Role of insulin-like growth factors in embryonic and postnatal growth. *Cell.* (1993) 75:73–82. doi: 10.1016/S0092-8674(05)80085-6
23. De Jong M, Cranendonk A, van Weissenbruch MM. Components of the metabolic syndrome in early childhood in very-low-birth-weight infants and term small and appropriate for gestational age infants. *Pediatr Res.* (2015) 78:457–61. doi: 10.1038/pr.2015.118
24. Kistner A, Rakow A, Legnevall L, Marchini G, Brismar K, Hall K, et al. Differences in insulin resistance markers between children born small for gestational age or born preterm appropriate for gestational age. *Acta Paediatr Oslo Nor 1992.* (2012) 101:1217–24. doi: 10.1111/apa.12005
25. Milovanovic I, Njuieyon F, Deghmoun S, Chevenne D, Levy-Marchal C, Beltrand J. SGA children with moderate catch-up growth are showing the impaired insulin secretion at the age of 4. *PLoS ONE.* (2014) 9:e100337. doi: 10.1371/journal.pone.0100337
26. Scheurer JM, Gray HL, Demerath EW, Rao R, Ramel SE. Diminished growth and lower adiposity in hyperglycemic very low birth weight neonates at 4 months corrected age. *J Perinatol Off J Calif Perinat Assoc.* (2016) 36:145–50. doi: 10.1038/jp.2015.154
27. Ramel SE, Long JD, Gray H, Durrwachter-Erno K, Demerath EW, Rao R. Neonatal hyperglycemia and diminished long-term growth in very low birth weight preterm infants. *J Perinatol Off J Calif Perinat Assoc.* (2013) 33:882-6. doi: 10.1038/jp.2013.77
28. Hawkes CP, Grimberg A. Measuring growth hormone and insulin-like growth factor-i in infants: what is normal? *Pediatr Endocrinol Rev PER.* (2013) 11:126-46.
29. Blum WF, Albertsson-Wikland K, Rosberg S, Ranke MB. Serum levels of insulin-like growth factor I (IGF-I) and IGF binding protein 3 reflect spontaneous growth hormone secretion. *J Clin Endocrinol Metab.* (1993) 76:1610–6. doi: 10.1210/jcem.76.6.7684744
30. Ohkawa N, Shoji H, Kitamura T, Suganuma H, Yoshikawa N, Suzuki M, et al. IGF-I, leptin and active ghrelin levels in very low birth weight infants during the first 8 weeks of life. *Acta Paediatr Oslo Nor 1992.* (2010) 99:37–41. doi: 10.1111/j.1651-2227.2009.01516.x
31. Martos-Moreno GA, Barrios V, Sáenz de Pipaón M, Pozo J, Dorronsoro I, Martínez-Biarge M, et al. Influence of prematurity and growth restriction on the adipokine profile, IGF1, and ghrelin levels in cord blood: relationship with glucose metabolism. *Eur J Endocrinol.* (2009) 161:381–9. doi: 10.1530/EJE-09-0193
32. Nawathe AR, Christian M, Kim SH, Johnson M, Savvidou MD, Terzidou V. Insulin-like growth factor axis in pregnancies affected by fetal growth disorders. *Clin Epigenetics.* (2016) 8:11. doi: 10.1186/s13148-016-0178-5
33. Iñiguez G, Ong K, Bazaes R, Avila A, Salazar T, Dunger D, et al. Longitudinal changes in insulin-like growth factor-I, insulin sensitivity, and secretion from birth to age three years in small-for-gestational-age children. *J Clin Endocrinol Metab.* (2006) 91:4645–9. doi: 10.1210/jc.2006-0844
34. Ohkawa N, Shoji H, Ikeda N, Suganuma H, Shimizu T. Relationship between insulin-like growth factor 1, leptin and ghrelin levels and catch-up growth in small for gestational age infants of 27-31 weeks during neonatal intensive care unit admission. *J Paediatr Child Health.* (2017) 53:62–7. doi: 10.1111/jpc.13307
35. Castinetti F, Reynaud R, Quentien M-H, Jullien N, Marquant E, Rochette C, et al. Combined pituitary hormone deficiency: current and future status. *J Endocrinol Invest.* (2015) 38:1–12. doi: 10.1007/s40618-014-0141-2
36. Klammt J, Pfäffle R, Werner H, Kiess W. IGF signaling defects as causes of growth failure and IUGR. *Trends Endocrinol Metab TEM.* (2008) 19:197–205. doi: 10.1016/j.tem.2008.03.003
37. Ester WA, Hokken-Koelega ACS. Polymorphisms in the IGF1 and IGF1R genes and children born small for gestational age: results of large population studies. *Best Pract Res Clin Endocrinol Metab.* (2008) 22:415–31. doi: 10.1016/j.beem.2008.03.001
38. Landmann E, Kollerits B, Kreuder JG, Blum WF, Kronenberg F, Rudloff S. Influence of polymorphisms in genes encoding for insulin-like growth factor (IGF)-I, insulin, and IGF-binding protein (IGFBP)-3 on IGF-I, IGF-II, and IGFBP-3 levels in umbilical cord plasma. *Horm Res Paediatr.* (2012) 77:341–50. doi: 10.1159/000338783
39. Billionnet C, Mitanchez D, Weill A, Nizard J, Alla F, Hartemann A, et al. Gestational diabetes and adverse perinatal outcomes from 716,152 births in France in 2012. *Diabetologia.* (2017) 60:636–44. doi: 10.1007/s00125-017-4206-6
40. Wakeling EL, Brioude F, Lokulo-Sodipe O, O'Connell SM, Salem J, Bliek J, et al. Diagnosis and management of silver-russell syndrome: first international consensus statement. *Nat Rev Endocrinol.* (2017) 13:105–24. doi: 10.1038/nrendo.2016.138
41. Rotwein P. Large-scale analysis of variation in the insulin-like growth factor family in humans reveals rare disease links and common polymorphisms. *J Biol Chem.* (2017) 292:19608. doi: 10.1074/jbc.M117.783639
42. Salis ER, Reith DM, Wheeler BJ, Broadbent RS, Medlicott NJ. Insulin resistance, glucagon-like peptide-1 and factors influencing glucose homeostasis in neonates. *Arch Dis Child Fetal Neonatal Ed.* (2017) 102:F162–6. doi: 10.1136/archdischild-2015-309174
43. Evagelidou EN, Giapros VI, Challa AS, Kiortsis DN, Tsatsoulis AA, Andronikou SK. Serum adiponectin levels, insulin resistance, and lipid profile in children born small for gestational age are affected by the severity of growth retardation at birth. *Eur J Endocrinol. févr.* (2007) 156:271–7. doi: 10.1530/eje.1.02337
44. Hernandez MI, Rossel K, Peña V, Garcia M, Cavada G, Avila A, et al. Patterns of infancy growth and metabolic hormonal profile are different in very-low-birth-weight preterm infants born small for gestational age compared to those born appropriate for gestational age. *Horm Res Paediatr.* (2018) 89:233–45. doi: 10.1159/000487994
45. Tenhola S, Halonen P, Jääskeläinen J, Voutilainen R. Serum markers of GH and insulin action in 12-year-old children born small for gestational age. *Eur J Endocrinol.* (2005) 152:335–40. doi: 10.1530/eje.1.01869
46. Soto N, Bazaes RA, Peña V, Salazar T, Avila A, Iñiguez G, et al. Insulin sensitivity and secretion are related to catch-up growth in small-for-gestational-age infants at age 1 year: results from a prospective cohort. *J Clin Endocrinol Metab.* (2003) 88:3645–50. doi: 10.1210/jc.2002-030031
47. Sato R, Maekawa M, Genma R, Shirai K, Ohki S, Morita H, et al. Final height and cardiometabolic outcomes in young adults with very low birth weight (<1500 g). *PLoS ONE.* (2014) 9:e112286. doi: 10.1371/journal.pone.0112286

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Pediatrics

ORIGINAL RESEARCH
published: 13 September 2021
doi: 10.3389/fped.2021.697100

Neuroprem 2: An Italian Study of Neurodevelopmental Outcomes of Very Low Birth Weight Infants

Licia Lugli[1], Luca Bedetti[1,2,3], Isotta Guidotti[1], Marisa Pugliese[1,4], Odoardo Picciolini[5], Maria Federica Roversi[1], Elisa DellaCasa Muttini[1], Laura Lucaccioni[3], Natascia Bertoncelli[1], Gina Ancora[6], Giancarlo Gargano[7], Fabio Mosca[8], Fabrizio Sandri[9], Luigi Tommaso Corvaglia[10], Agostina Solinas[11], Serafina Perrone[12], Marcello Stella[13], The Neuroprem Working Group, Lorenzo Iughetti[3,14], Alberto Berardi[1] and Fabrizio Ferrari[1]*

[1] Neonatal Intensive Care Unit, University Hospital of Modena, Modena, Italy, [2] PhD Program in Clinical and Experimental Medicine, University of Modena and Reggio Emilia, Modena, Italy, [3] Pediatric Unit, Department of Medical and Surgical Sciences of Mothers, Children and Adults, University of Modena and Reggio Emilia, Modena, Italy, [4] Psychology Unit, University Hospital of Modena and Reggio Emilia, Modena, Italy, [5] Physical and Rehabilitation Medicine, Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, University of Milan, Milan, Italy, [6] Neonatal Intensive Care Unit, Infermi Hospital of Rimini, Rimini, Italy, [7] Neonatal Intensive Care Unit, Azienda Unità Sanitaria Locale-IRCCS, Reggio Emilia, Italy, [8] Neonatal Intensive Care Unit, Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, University of Milan, Milan, Italy, [9] Neonatal Intensive Care Unit, Maggiore Hospital of Bologna, Bologna, Italy, [10] Neonatal Intensive Care Unit, Sant'Orsola Malpighi University Hospital of Bologna, Bologna, Italy, [11] Neonatal Intensive Care Unit, Sant'Anna Hospital of Ferrara, Ferrara, Italy, [12] Neonatal Intensive Care Unit, University Hospital of Parma, Parma, Italy, [13] Neonatal Intensive Care Unit, Bufalini Hospital of Cesena, Cesena, Italy, [14] Department of Medical and Surgical Sciences of Mothers, Children and Adults, Pediatric Postgraduate School, University of Modena and Reggio Emilia, Modena, Italy*

OPEN ACCESS

Edited by:
Krithika Lingappan, Baylor College of Medicine, United States

Reviewed by:
Deepak Jain, Rutgers, The State University of New Jersey, United States
Mirjam Maria Van Weissenbruch, VU University Medical Center, Netherlands
Daynia Ballot, University of the Witwatersrand, South Africa
Tanusha Ramdin, Department of Health, South Africa

***Correspondence:**
Licia Lugli
lugli.licia@policlinico.mo.it;
licia.lugli@virgilio.it

Specialty section:
This article was submitted to Neonatology, a section of the journal Frontiers in Pediatrics

Received: *18 April 2021*
Accepted: *16 August 2021*
Published: *13 September 2021*

Citation:
Lugli L, Bedetti L, Guidotti I, Pugliese M, Picciolini O, Roversi MF, DellaCasa Muttini E, Lucaccioni L, Bertoncelli N, Ancora G, Gargano G, Mosca F, Sandri F, Corvaglia LT, Solinas A, Perrone S, Stella M, The Neuroprem Working Group, Iughetti L, Berardi A and Ferrari F (2021) Neuroprem 2: An Italian Study of Neurodevelopmental Outcomes of Very Low Birth Weight Infants. Front. Pediatr. 9:697100. doi: 10.3389/fped.2021.697100

Background: Despite the increased survival of preterm newborns worldwide, the risk of neurodevelopmental disabilities remains high. Analyzing the outcomes of the preterm population can identify risk factors and enable specific early interventions.

Aims: Neuroprem is a prospective cohort study of very low birth weight (VLBW) infants that aims to evaluate the neurodevelopmental outcomes and risk factors for severe functional disability at 2 years of corrected age.

Methods: Nine Italian neonatal intensive care units participated in the network. The Griffiths Mental Developmental Scales (GMDS-R) or the Bayley Scales of Infant and Toddler Development (BSDI III) and a neuro-functional evaluation (according to the International Classification of Disability and Health and Neuro-Functional Assessment, or NFA ICF-CY) were administered to VLBW infants at 24 months of corrected age. The primary outcome measure was severe functional disability, defined as cerebral palsy, bilateral blindness, deafness, an NFA ICF-CY of >2, a BSDI III cognitive composite score of <2 SD, or a GMDS-R global quotient score of <2 SD. Perinatal risk factors for severe functional disability were assessed through multivariate logistic regression analysis.

Results: Among 502 VLBW survivors who completed the 24-month follow-up, 48 (9.6%) presented severe functional disability, of whom 27 had cerebral palsy (5.4%). Rates of severe functional disability and cerebral palsy were higher in neonates with a lower gestational age ($p < 0.001$). Overall, 147 infants (29.3%) were referred to

neuromotor intervention. In the multivariate regression model, gestational age at birth OR 0.79; 95% CI 0.67–0.90; $p = 0.001$) and periventricular-intraventricular hemorrhage (OR 2.51; 95% CI 1.19–5.26; $p = 0.015$) were significantly associated with severe functional disability.

Conclusion: Neuroprem 2 provides updated information on the neurodevelopmental outcomes of VLBW infants in a large Italian cohort. The overall rate of neurodevelopmental disabilities was quite lower than reported in the previous literature. These data indicate the need for structured follow-up programs from a national neonatal network perspective.

Keywords: very low birth weight, preterm, neuro-developmental outcome, follow-up, network

BACKGROUND

Prematurity is a major global health problem and the leading cause of death in children under 5 years old. Over the past few decades, advances in the clinical care of preterm infants have led to improved survival in the neonatal age group and beyond. Thus, more premature infants survive with neurodevelopmental morbidities of variable severity, often resulting in lifelong disability. The high rates of neurological and developmental problems reported in survivors are concerning to both professionals and the public. Among extremely preterm neonates (22–26 weeks gestation), survival without neurodevelopmental impairment at 2 years of age ranges from 20 to 42% (1–3). Indeed, in absolute numbers, infants born very or moderately preterm represent a large proportion of preterm births, accounting for most children with motor, cognitive, or behavioral deficits and learning disabilities (4–6). Gestational age (GA), birth weight, sex, multiple birth, antenatal corticosteroid administration, neonatal infection, necrotizing enterocolitis (NEC), and major brain lesions, such as periventricular leukomalacia (PVL) and intraventricular hemorrhage (IVH), have been shown to influence both short- and long-term outcomes (7–9). Analyzing outcomes in the preterm population may identify risk factors that could potentially be targeted by specific early intervention (10–12). In addition, decisions on the provision of intensive vs. palliative care and on the counseling of parents of extremely preterm infants are based on the expected incidence of mortality and poor long-term outcome. Therefore, it is of paramount importance that recent, representative outcome data be available. Up-to-date data are scant regarding the neurodevelopment of very low birth weight (VLBW) infants, and national networks on preterm neurological outcomes are still lacking in Italy, but the Neuroprem study has recently produced preliminary data on the neurodevelopmental outcomes of a VLBW cohort in an Italian region during a 1-year period (13). Neuroprem is now expanding to a larger Italian area including nine neonatal intensive care units NICUs and to a longer study period (Neuroprem 2). Herein, we report on a multicenter prospective cohort study of VLBW preterm infants, which evaluated the neurodevelopmental outcomes and risk factors for severe functional disability at 2 years of corrected age.

METHODS

Study Design

Neuroprem is an Italian network that assesses VLBW neurodevelopmental outcomes. Nine tertiary level NICUs in two Italian regions, all participating in the Vermont Oxford Network Database (VON) (14), joined this prospective cohort study. Before starting patient enrollment, the NICUs participated in seminars and meetings to define and share the study protocol. Anonymized data, including perinatal and neurodevelopmental follow-up data, were collected in a common data collection format through a web platform. The study enrolled VLBW infants born from 1 January 2016 through 31 December 2018 (and included in VON) and collected their neurodevelopmental data at 24 months (corrected for prematurity). Genetic abnormalities or major malformations were excluded. Several perinatal factors in the VON were evaluated, including birth weight, GA, site and mode of delivery, ethnicity, gender, multiple gestation, prenatal steroid exposure, Apgar score at first and fifth minute, chorioamnionitis, sepsis, mechanical ventilation, periventricular-intraventricular hemorrhage (PIH), PVL, patent ductus arteriosus (PDA) treatment, NEC, retinopathy of prematurity (ROP) surgery, and breastfeeding at discharge. Chorioamnionitis was defined in the presence of at least two of the following signs: maternal increased C reactive protein, leukocytosis, fever above 38°C, malodorous amniotic fluid, and maternal or fetal tachycardia. Sepsis was considered present in the case of a positive blood culture (14).

Three groups of different GAs were identified: group 1 ($\leq$28 weeks' gestation), group 2 (28 weeks + 1 day to 31 weeks + 6 days of gestation), and group 3 ($\geq$32 weeks' gestation). The study was approved by the Ethics Committee of Univerity Hospital of Modena and Reggio Emilia (protocol 205/2015, no 4818). Written consent was obtained from the parents of each neonate enrolled in the study.

Neurodevelopmental Assessment

In each center, neurodevelopmental follow-up was performed by a multidisciplinary team comprising a neonatologist, a psychologist, a physiotherapist, and a pediatric neurologist. To ensure compliance, parents received appointment reminders by telephone. The survivors were examined neurologically

according to the Amiel-Tison neurological assessment (15) with either the Griffiths Mental Developmental Scales (GMDS-R, 1996) (16) or the Bayley Scales of Infant and Toddler Development (BSDI III, 2006) (17), depending on local protocols. The GMDS-R (0–2 years) provides a general development quotient (GQ) of infants' abilities with a mean of 100, a standard deviation (SD) of 12, and five subscale quotients (locomotor; eye and hand coordination; personal and social; hearing and language; cognitive performance), each with a mean of 100 and an SD of 16 (16). The BSDI III provides standardized composite scores for each of the assessed domains (cognitive, fine, and gross motor; receptive and expressive language; adaptive), with a mean of 100 and an SD of 15 (17). For both the GMDS-R and the BSID-III, the cutoff abnormality was two SDs below the normative mean (13, 18). The BSDI III or GMDS-R results were compared among three groups of different GAs.

The infants were also assessed by neuro-functional evaluation according to the International Classification of Disability and Health (ICF-CY) and Neuro-Functional Assessment (NFA ICF-CY) (19, 20). The NFA ICF-CY–based approach has been implemented successfully in routine follow-up programs for preterm infants, providing early identification of neurodevelopmental delay (19–22). Neuro-functional clinical evaluation was performed for cognitive, linguistic, motor, and adaptive function, and then a global NFA ICF-CY score was assigned (**Appendix A**). All the enrolled patients were also screened for vision (an oculist examination including the fundus oculi) and hearing (brain stem evoked potential). The primary outcome measure was severe functional disability at 2 years of age, corrected for prematurity. Severe functional disability was defined as the presence of at least one among the following outcomes: cerebral palsy (CP), a BSDI III cognitive composite score of <2 SD or a GMDS-R GQ of <2 SD, bilateral blindness (visual acuity < 6/60 in the better eye), bilateral deafness (requiring bilateral hearing aids or unilateral/bilateral cochlear implants), and an NFA ICF-CY > 2. The risk factors for severe functional disability were assessed, and the rate and type of CP were evaluated. CP was defined as a permanent disorder of movement and posture causing activity limitations attributed to non-progressive disturbances that occurred in the developing brain. The classification included spastic CP (monoparesis, hemiparesis, triparesis, tetraparesis, diplegia) and extrapyramidal (dyskinetic) syndromes (23).

Statistical Analysis

Statistical analyses were performed using Stata Direct Statistical Software version 13 (StataCorp LP, USA). Continuous variables were reported by means and SD or by median and interquartile range (IQR), while categorical variables were reported using frequencies. The groups were compared by χ^2 analyses for categorical variables and by Kruskal-Wallis tests as non-parametric tests for continuous variables. Several variables (birth weight, GA, site and mode of delivery, ethnicity, gender, multiple gestation, prenatal steroid exposure, Apgar score at first and fifth minute, chorioamnionitis, sepsis, mechanical ventilation, PIH, PDA treatment, NEC, ROP surgery, and breastfeeding at discharge) were evaluated as possible risk factors for functional disability and were presented in the univariate analysis.

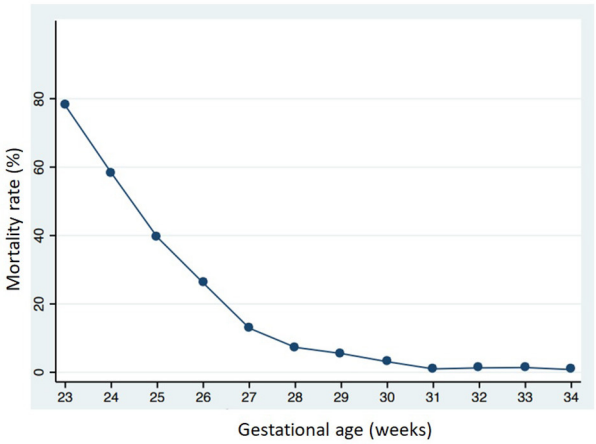


FIGURE 1 | Mortality rate in relation to gestational age at birth.

The multivariate logistic regression model was built on the basis of a stepwise selection, with entry criteria = 0.05 and stay criteria = 0.1. To assess multicollinearity, the correlation coefficient and variance inflation factor (VIF) were checked. A correlation coefficient level of ≤0.9 and a VIF value of >10 were considered critical values. The best subset of predictors in the multivariate models was determined based on the lowest values of Akaike's information criterion and the Bayesian information criterion (BIC). A p-value of <0.05 was considered statistically significant.

RESULTS

Between 1 January 2016 and 31 December 2018, 1,082 VLBW patients were included in the VON database by participating units. Among them, 133 died (12.3%) within the term-corrected age, before discharge from the hospital: 114/278 (41%) in group 1, 16/249 (6.4%) in group 2, and 3/92 (3.3%) in group 3. **Figure 1** shows the mortality rate in relation to GA. Seventeen patients (1.6%) were excluded from the study because of major malformations or genetic anomalies. Among the remaining 932 patients, 502 completed the 24-month neurodevelopmental follow-up (53.9%). **Figure 2** shows the enrollment flow diagram. **Table 1** compares the characteristics of infants with or without the 24-month neurodevelopmental follow-up. Infants with the 24-month neurodevelopmental follow-up had significantly lower GA at birth; they were more likely to be inborn, delivered after a single pregnancy, and delivered after chorioamnionitis. They were also more likely to be treated for PDA and to be breastfed at discharge from hospital **Table 1**. **Table 2** compares the perinatal characteristics of the three groups, divided according to GA. Patients with a lower GA presented higher rates of chorioamnionitis, sepsis, mechanical ventilation, need for PDA treatment, PIH, NEC, and ROP surgery. In contrast, higher GA infants showed a higher rate of breastfeeding at discharge.

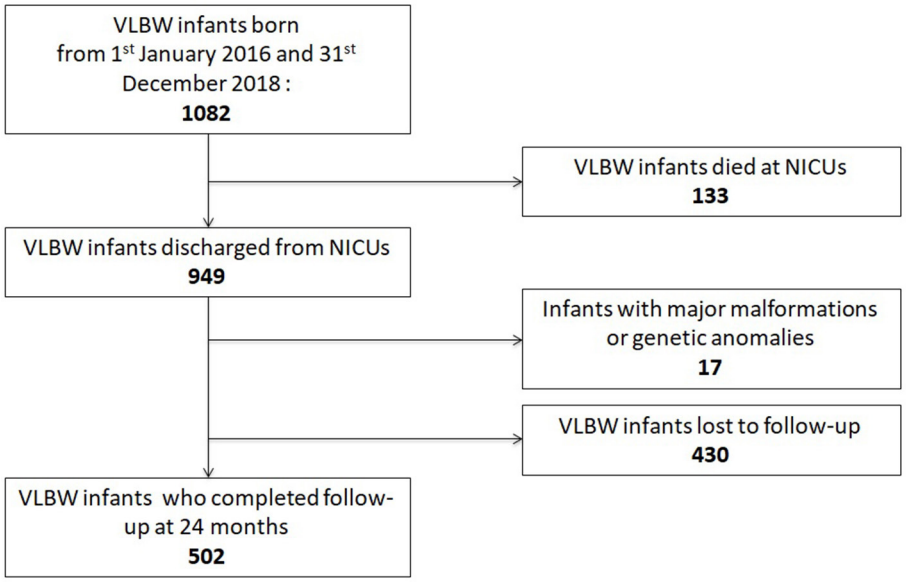


FIGURE 2 | Enrollment flow diagram.

TABLE 1 | Characteristics of infants undergoing or not a 24 months neuro-developmental follow-up.

	Patients with 24 months follow-up *N* = 502	Patients without 24 months follow-up *N* = 430	Missing cases	*p*
Birth weight, median (IQR), g	1,074.5 (870–1,341)	1,270 (1,050–1,415)	–	**<0.001**
Gestational age, median (IQR), wk	29.3 (27.4–31)	30.4 (28.9–32.3)	–	**<0.001**
Outborn, *n* (%)	19 (3.8)	32 (7.4)	–	**0.014**
Ethnicity (Not-Hispanic), *n* (%)	482 (53.2)	424 (46.8)	4	**0.008**
Multiple gestation, *n* (%)	172 (34.3)	194 (45.1)	–	**0.001**
Apgar 1st min, median (IQR)	7 (5–8)	7 (5–8)	–	0.656
Apgar 5th min, median (IQR)	9 (8–9)	9 (8–9)	–	0.115
Cesarean delivery, *n* (%)	94 (18.7)	60 (13.9)	–	0.051
Male gender, *n* (%)	251 (50)	204 (47.4)	–	0.436
Prenatal steroids exposure, *n* (%)	458 (91.9)	387 (90.4)	6	0.406
Chorioamnionitis, *n* (%)	99 (17.8)	58 (13.7)	9	**0.015**
Sepsis, *n* (%)	54 (10.8)	37 (8.6)	–	0.270
Mechanical ventilation, *n* (%)	190 (37.9)	143 (33.3)	–	0.145
Periventricular-intraventricular Hemorrhage, *n* (%)	73 (14.6)	50 (11.7)	4	0.191
Periventricular leukomalacia, *n*(%)	12 (2.4)	7 (1.6)	4	0.412
PDA pharmacological treatment, *n* (%)	130 (25.9)	69 (16.1)	–	**<0.001**
PDA surgical treatment for, *n* (%)	177 (35.3)	98 (22.8)	28	**<0.001**
Necrotizing enterocolitis, *n* (%)	18 (3.6)	23 (5.4)	–	0.191
ROP surgical treatment, *n* (%)	14 (2.8)	11 (2.6)	1	0.833
Human milk feeding at discharge, *n* (%)	342 (68.1)	257 (59.8)	–	**0.008**

PDA, patent ductus arteriosus; ROP, retinopathy of the prematurity.
The values in bold are statistically significant ($p < 0.05$).

Neurodevelopmental Outcome at 24-Month Corrected Age

Severe functional disability occurred in 48/502 infants (9.6%). Seventeen of those 48 patients (35.4%) presented a BSDI III cognitive composite score of <2 SD (two cases) or a GMDS-R GQ of <2 SD (15 cases); eight patients presented CP and a BSDI III cognitive composite score of <2 SD (three cases) or a GMDS-R GQ of <2 SD (five cases); 15 patients had CP; one had blindness; three had deafness; one had CP and blindness; and three patients showed CP and deafness (**Figure 3**). Overall,

TABLE 2 | Comparison of neonatal characteristics among different gestational ages groups in patients completing 24 months follow-up*.

	Group 1 ≤28 wks *N* = 164	Group 2 28 wks + 1 day to 31 wks + 6 days *N* = 249	Group 3 >32 wks *N* = 89	Missing cases	*p*
Birth weight, median (IQR), g	825.5 (705–950)	1,175 (980–1,345)	1,405 (1,303–1,480)	–	**<0.001**
Gestational age, median (IQR), wk	26.5 (25.4–27.4)	29.7 (29–30.6)	33.3 (32.3–33.9)	–	**<0.001**
Outborn, *n* (%)	7 (4.3)	10 (4)	2 (2.3)	–	0.698
Ethnicity (Not-Hispanic), *n* (%)	7 (4.3)	9 (3.6)	2 (2.2)	–	0.720
Multiple gestation, *n* (%)	56 (34.2)	85 (34.1)	31 (34.8)		0.992
Apgar 5th min, median (IQR)	8 (7–9)	9 (8–9)	9 (9–10)	–	**<0.001**
Male gender, *n* (%)	89 (35.5)	120 (47.8)	42 (16.7)	–	0.406
Prenatal steroids exposure, *n* (%)	152 (93.8)	226 (91.5)	80 (89.9)	4	0.508
Chorioamnionitis, *n* (%)	64 (39)	32 (12.9)	3 (3.4)	1	**<0.001**
Sepsis, *n* (%)	41 (25)	12 (4.8)	1 (1.1)	–	**<0.001**
Mechanical ventilation, *n* (%)	127 (77.4)	60 (24.1)	3 (3.4)	–	**<0.001**
Periventricular-intraventricular hemorrhage, *n* (%)	45 (27.6)	20 (8.1)	8 (9)	2	**<0.001**
Periventricular leukomalacia, *n* (%)	4 (2.5)	6 (2.4)	2 (2.3)	2	0.994
PDA pharmacological treatment, *n* (%)	87 (53.1)	42 (16.9)	1 (1)	–	**<0.001**
PDA surgical treatment, *n* (%)	28 (17.8)	2 (0.8)	0	15	**<0.001**
Necrotizing enterocolitis, *n* (%)	13 (7.9)	2 (0.8)	0	–	**<0.001**
ROP surgical treatment, *n* (%)	12 (7.3)	2 (0.8)	0	–	**<0.001**
Human milk feeding at discharge, *n* (%)	87 (53.1)	177 (71.1)	78 (87.6)	–	**<0.001**

*Wks, weeks.*Kruskal-Wallis test was used to compare groups.*
The values in bold are statistically significant ($p < 0.05$).

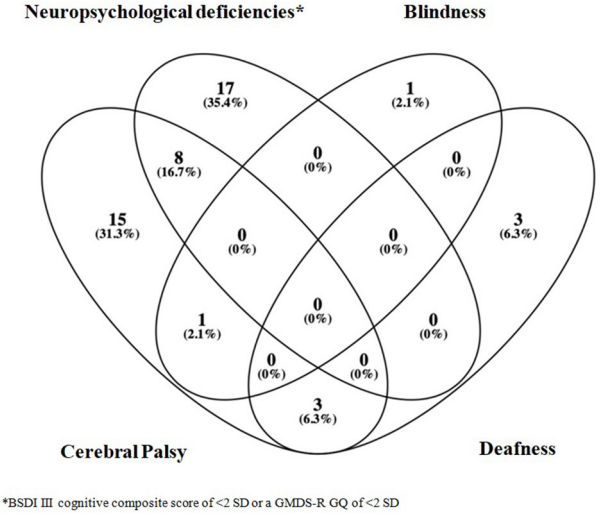


FIGURE 3 | Severe functional disability Venn diagram.

among the 502 followed-up infants, CP was diagnosed in 27 (5.4%) (monoparesis $n = 2$; hemiparesis $n = 7$; diplegia $n = 10$; tetraparesis $n = 8$), whereas deafness occurred in 6 (1.2%) and blindness in two (0.4%) infants.

Table 3 compares the characteristics of infants with or without severe functional disability at age 24 months. Patients with severe functional disability had lower birth weight and GA, lower fifth-minute Apgar scores, and higher rates of mechanical ventilation, NEC, sepsis, treatment for PDA, and cerebral lesions (assessed by cerebral ultrasound) at discharge from hospital.

Severe functional disability was more common in infants with a lower GA: 31 of 164 (18.9%) in group 1; 15 of 249 (6.0%) in group 2; and two of 89 (2.3%) in group 3 ($p < 0.001$). **Figure 4** shows severe functional disability in relation to GA. CP was also more common in infants with a lower GA: 19 of 164 (11.6%) in group 1; six of 249 (2.4%) in group 2; and two of 89 (2.2%) in group 3 ($p < 0.001$). Overall 147 infants (29.3%) were sent to neuromotor rehabilitation during the first 24 months of life, more frequently if they had a lower GA: 83 of 164 (50.6%) in group 1; 58 of 249 (23.3.%) in group 2; and six of 89 (6.7%) in group 3 ($p < 0.001$).

In the univariate regression analysis, several variables were associated with severe functional disability (**Table 4**). The final multivariate regression model included four variables: GA at birth, male gender, sepsis, and PIH (area under ROC curve: 0.75). In this multivariate regression model, only GA at birth and PIH were associated with severe functional disability (**Table 4**).

Cognitive and Neuropsychological Outcome

Among the 502 infants, 177 were evaluated with the BSDI III and 208 with the GMDS-R (data were missing or incomplete for the remaining 117 patients). The GMDS-R subscales, GMDS-R GQ, and BSDI III composite scores were evaluated and compared among the three GA groups. The GMDS-R GQ, GMDS-R locomotor, and GMDS-R personal and social quotient as well

TABLE 3 | Neonatal characteristics of infants with or without severe functional disability at 24 months.

	Patients with severe functional disability *N* = 48	Patients without severe functional disability *N* = 454	Missing cases	*p*
Birth weight, median (IQR), g	861.5 (697.5–1,094.5)	1,104 (895–1,360)	–	**<0.001**
Gestational age, median (IQR), wk	26.9 (25.3–29)	29.5 (27.7–31.3)	–	**<0.001**
Outborn, *n* (%)	4 (8.3)	15 (3.3)	–	0.082
Ethnicity (Not-Hispanic), *n* (%)	3 (6.3)	15 (3.3)	–	0.300
Multiple gestation, *n* (%)	13 (27.1)	159 (35.0)	–	0.270
Apgar 5th min, median (IQR)	9 (7–9)	9 (8–9)	–	**0.038**
Male gender, *n* (%)	31 (64.6)	220 (48.5)	–	**0.034**
Prenatal steroids exposure, *n* (%)	44 (91.7)	414 (92)	4	0.936
Chorioamnionitis, *n* (%)	16 (33.3)	83 (18.3)	1	**0.013**
Sepsis, *n* (%)	14 (29.2)	40 (8.8)	–	**<0.001**
Mechanical ventilation, *n* (%)	33 (68.8)	157 (34.6)	–	**<0.001**
Periventricular-intraventricular hemorrhage, *n* (%)	18 (39.1)	55 (12.1)	2	**<0.001**
Periventricular leukomalacia, *n* (%)	7 (15.2)	5 (1.1)	2	**<0.001**
PDA pharmacological treatment, *n* (%)	20 (41.7)	110 (24.2)	–	**0.009**
PDA surgical treatment, *n* (%)	9 (19.2)	21 (4.8)	15	**<0.001**
Necrotizing enterocolitis, *n* (%)	5 (10.4)	13 (2.9)	–	**0.007**
ROP surgical treatment, *n* (%)	8 (16.7)	6 (1.3)	–	**<0.001**
Human milk feeding at discharge, *n* (%)	22 (45.8)	320 (70.8)	–	**<0.001**

PDA, patent ductus arteriosus; ROP, retinopathy of the prematurity.
Missing cases were excluded from the statistical analyses.
The values in bold are statistically significant ($p < 0.05$).

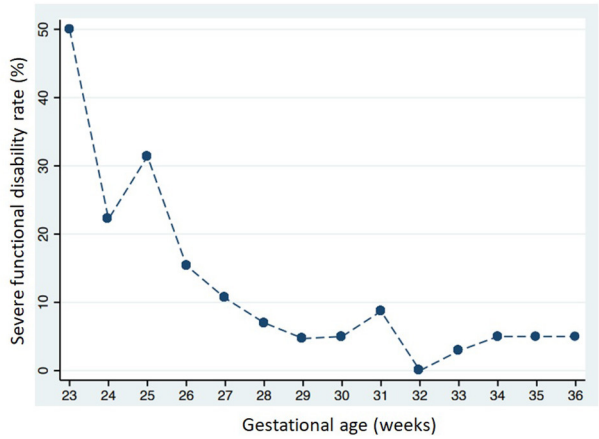


FIGURE 4 | Severe functional disability in relation to gestational age at birth.

as the BSDI III cognitive composite score differed significantly (**Table 5**).

Neuro-Functional Clinical Evaluation (NFA ICF-CY–Based Approach)

Table 6 shows the neuro-functional clinical evaluation. The NFA ICF-CY scores differed significantly between the different GA groups. The GMDS-R GQ and BSDI III cognitive composite scores were lower ($p < 0.001$) in patients with higher NFA ICF-CY scores (**Figures 5**, **6**), distinguishing between patients with major neurodevelopmental anomalies or CP (NFA ICF-CY score > 2) and those with minor anomalies (NFA ICF-CY score ≤ 2).

DISCUSSION

In the current study, severe functional disability and CP affected < 10 and 5.4% of survivors, respectively, whereas the literature on the follow-up of preterm neonates reports a higher prevalence of neurodevelopmental disabilities and CP (ranging from 10 to 15%) (24–28). The lower rate of neurodevelopmental disabilities found in the current study probably reflects differences in the study populations. Most previous studies focused on very or extremely preterm newborns, while the current study addressed all VLBW infants, with a GA ranging from 23 to 33 weeks. In our study, the rate of severe functional disability was significantly higher in infants with a lower GA, as those with a $\leq$28-week GA had eight times higher disability rates than those with a $\geq$32-week GA. Furthermore, infants with lower GAs had higher rates of CP (11.6% in infants of $\leq$28 weeks GA) and were more frequently sent to neuromotor rehabilitation. The lower prevalence of severe functional disability and CP in the survivors could also be biased by the number of non-survivors in the groups, but the mortality rate in our study is similar to others in the literature. Specifically, we found a 12.3% overall mortality rate (41% in infants with $\leq$28 weeks GA), while other studies have reported a 10% mortality rate for under 34 weeks of gestation, 16–18% for under 32 weeks of gestation, and about 40% for extremely preterm infants (29, 30).

TABLE 4 | Association of perinatal data and severe functional disability at 24 month of corrected age.

	Univariate model			Multivariate model		
	OR	**95% CI**	***p***	**OR**	**95% CI**	***p***
Birth weight	0.99	0.99–0.99	**<0.001**			
Gestational age	0.76	0.67–0.85	**<0.001**	0.79	0.67–0.90	**0.001**
Outborn	2.66	0.84–8.36	0.094			
Maternal age	0.95	0.90–0.99	**0.035**			
Ethnicity	0.97	0.55–1.69	0.910			
Multiple gestation	0.69	0.35–1.34	0.273			
APGAR 1st min	0.83	0.72–0.94	**0.006**			
APGAR 5th min	0.85	0.70–1.02	0.087			
Male gender	1.94	1.04–3.60	**0.036**	1.94	0.99–3.77	0.052
Mode of delivery	1.71	0.86–3.37	0.122			
Prenatal steroids exposure	0.96	0.32–2.81	0.936			
Chorioamnionitis	2.23	1.16–4.25	**0.015**			
Sepsis	4.26	2.11–8.59	**<0.001**	2.23	0.99–4.98	0.050
Mechanical ventilation	4.16	2.19–7.89	**<0.001**			
Periventricular-intraventricular hemorrhage	4.66	2.42–8.98	**<0.001**	2.51	1.19–5.26	**0.015**
Periventricular leukomalacia	16.12	4.88–53.15	**<0.001**			
Pharmacological treatment for PDA	2.23	1.21–4.12	**0.010**			
Surgical treatment for PDA	2.37	1.30–4.32	**0.005**			
Necrotizing enterocolitis	3.94	1.34–11.59	**0.013**			
ROP surgical treatment	14.93	4.93–45.16	**<0.001**			
Human milk feeding at discharge	0.35	0.19–0.64	**0.001**			

PDA, patent ductus arteriosus; ROP, retinopathy of the prematurity.
Missing cases were excluded from the statistical analyses.
The values in bold are statistically significant ($p < 0.05$).

TABLE 5 | Comparison of GMDS-R quotients or BSD-III scores among 3 groups with different gestational age.

		GMDS-R/BSDI-III mean values°				
		Group 1 ≤28 wks Total *N* = 118	**Group 2 28 wks + 1 day to 31 wks + 6 days Total *N* = 200**	**Group 3 >32 wks Total *N* = 67**	**Missing cases**	
	GMDS-R *N* = 208	**GMDS-R *N* = 56**	**GMDS-R *N* = 106**	**GMDS-R *N* = 46**		***p*^**
Patients evaluated	Global quotient	95.6 ± 14.1	100.7 ± 13.6	101.7 ± 8.9	4	**0.020**
with GMDS-R*	Locomotor quotient	96.5 ± 20.3	104.2 ± 20.1	105.3 ± 16.0	2	**0.029**
	Personal and social quotient	98.0 ± 17.2	104.5 ± 16.0	105.3 ± 12.2	1	**0.019**
	Hearing and language quotient	90.4 ± 17.9	96.0 ± 17.0	96.6 ± 13.0	2	0.092
	Eye & Hand Coordination quotient	102.3 ± 14.0	104.6 ± 13.9	104.8 ± 10.2	–	0.424
	Performance quotient	95.2 ± 16.2	99.9 ± 15.7	99.5 ± 14.6	–	0.114
	BSDI-III *N* = 177	**BSDI-III *N* = 62**	**BSDI-III *N* = 94**	**BSDI-III *N* = 21**		
Patients evaluated	Cognitive composite score	90.8 ± 11.7	96.2 ± 7.9	101.2 ± 8.8	–	**<0.001**
with BSDI-III*	Motor composite score	88.3 ± 12.8	93.3 ± 7.4	95.5 ± 10.8	9	0.153
	Language composite score	85.9 ± 12.4	90.1 ± 8.5	92 ± 10.3	–	0.095

*Wks, weeks. °Patients with tetraparesis and blindness were excluded. *The number of patients for the different gestational age categories evaluated with BSDI-III was lower than the number of patients evaluated with GMDS-R ($p = 0.037$). Social-emotional score and adaptive scorewere not reported because of missing data (74/177 and 98/177, respectively). ^Kruskal-Wallis test.*
Missing cases were excluded from the statistical analyses.
The values in bold are statistically significant ($p < 0.05$).

TABLE 6 | Neuro-functional clinical evaluation: results according to different gestational age groups.

NFA ICF-CY	Group 1 ≤28 wks *N* = 164	Group 2 28 wks + 1 day to 31 wks + 6 days *N* = 249	≥32 wks *N* = 89	Missing cases	*p*
0	69 (42.4)	163 (65.5)	68 (76.4)	1	<0.001
1	53 (32.5)	55 (22.1)	16 (18.0)		
2	10 (6.1)	16 (6.4)	3 (3.4)		
3	21 (12.9)	11 (4.4)	1 (1.1)		
4	10 (6.1)	4 (1.6)	1 (1.1)		

Wks, weeks.

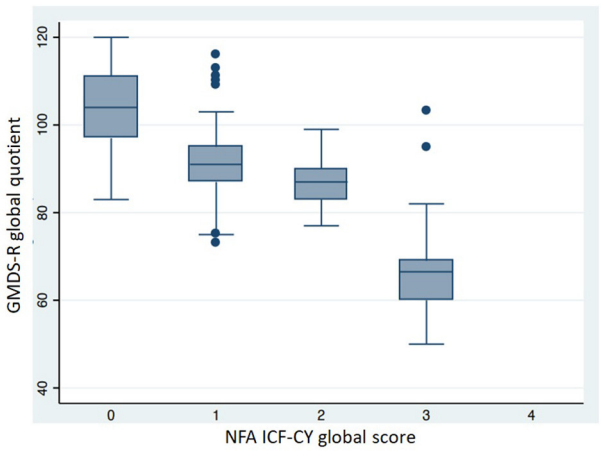


FIGURE 5 | Comparison of GMDS-R global quotient in patients with different NFA ICF-CY score. Patients with tetraparesis and blindness were excluded. Kruskal-Wallis test: $p < 0.001$.

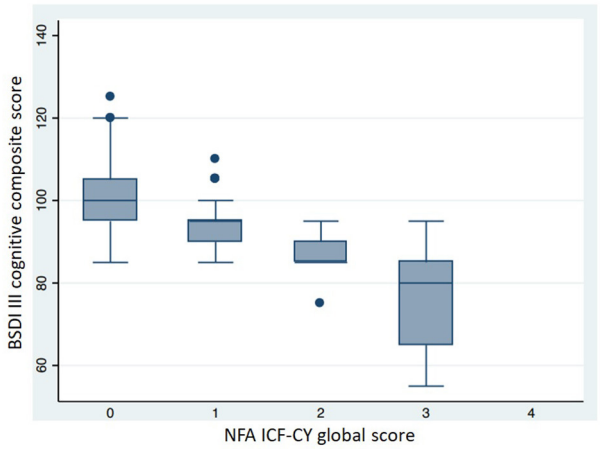


FIGURE 6 | Comparison of BSDI III cognitive composite score in patients with different NFA ICF-CY score**.** Patients with tetraparesis and blindness were excluded. Kruskal-Wallis test: $p < 0.001$.

Extremely preterm infants are at higher risk of neurodevelopmental disabilities, and preterm birth alone is a significant hindrance to the normal neurodevelopmental trajectory from fetus to adult (31). A healthy intrauterine environment for up to 37–40 weeks of gestation benefits neonatal brain development, while early extra-uterine life interferes with normal brain maturation, increasing the risk of neurological impairment even in the absence of documented cerebral lesions. The early identification of children at risk for subsequent developmental problems may inspire interventions, potentially mitigating the course of otherwise persistent disabilities. In the current study, GA at birth and PIH were significantly associated with severe functional disability. These findings confirm that GA is a main risk factor for poor neurodevelopmental outcomes but also point out the role of cerebral lesions.

Patients with lower GAs showed a higher rate of risk factors for poor neurodevelopmental outcomes, such as chorioamnionitis, sepsis, mechanical ventilation, need for PDA treatment, PIH, NEC, and ROP surgery. In patients with a lower GA, the BSDI III cognitive composite score as far as GQ, locomotor, and personal and social quotients (assessed by GMDS-R) were significantly lower. The BSDI III motor composite score was not significantly different, probably because most of the few patients in the study who were assessed with the BSDI III had a higher GA. Interestingly, the NFA ICF-CY was quite consistent with the BSDI III cognitive composite scores and the GMDS-R GQ. Previous studies have shown the relevance of ICF-CY–based data sets in comparing functioning and disability in children of different ages (29–31). The comprehensive neurodevelopmental assessment based on the NFA ICF-CY approach has been implemented successfully in routine follow-up programs for preterm infants, as it is easy to administer and overcomes data set heterogeneity due to the local protocol of evaluation. The NFA ICF-CY is a useful clinical screening tool for evaluating preterm infants' neurodevelopmental profiles, as it integrates neurological, behavioral, and social items. It enables clinicians to focus on children with suspected developmental delay, who consequently need further assessment or intervention (20–22, 32–34), but the BSID III and GMDS-R remain the gold standard of neurodevelopmental testing in preterm infants. In fact, our study's BSDI III cognitive composite score varies widely among patients with serious anomalies (score 3), who are more roughly assessed by the NFA ICF-CY.

Patients with severe functional disabilities showed documented brain lesions in more than 50% of cases (PIH and PVL in 39 and 15% of cases, respectively). Brain injury is a well-known risk factor for poor neurodevelopmental outcomes. In the current study, cerebral damage was assessed by ultrasounds, and brain lesions were roughly classified according to the VON in PIH and PVL. PIH was significantly associated with severe functional disability, while PVL was less frequent and not associated with a poor outcome, probably due to the small number of children included in the study. Recent studies show that neonatal brain injury, assessed by a standardized magnetic resonance imaging (MRI) scoring system, is associated with short-term neurodevelopmental outcomes, but environmental

factors are also important for cognitive development, especially for children with mild neonatal brain injury (35).

The strengths of the Neuroprem study include its prospective design and a very recent enrollment period as well as the inclusion of moderate and very preterm neonates, whose outcomes are reported infrequently. Indeed, the enrollment period (2016–2018) is very close to the present day, reflecting the effect on neonatal outcomes of the most advanced intensive care and support techniques for newborns. By contrast, previous studies included cohorts of preterm infants born before 2015, although the cohorts were larger than ours (1–3, 26, 36).

Our study has some limitations. The first major limitation is that different, although validated, neurodevelopmental scales (the BSDI III, GMDS-R, and NFA ICF-CY) were combined to define disability in our multicenter study because either the BSDI III or the GMDS-R was used depending on the local protocol. The standardization of developmental tests among centers is desirable, but it requires staff training and is costly and time consuming. To overcome data heterogeneity or missing BSDI III/GMDS-R data, the NFA ICF-CY was adopted by all the centers.

A second limitation is incomplete follow-up data for 43% of the neonates, and some might argue that this constitutes selection bias. However, some previous studies report similar dropout rates and suggest an excess of poorly performing children among those not evaluated (37), as children from more disadvantaged families are often lost to follow-up. In the current study, despite the quite high dropout rate, infants who completed follow-up had a lower GA, lower birth weight, and additional risk factors for poor neurodevelopmental outcomes. Therefore, although we lack information regarding the socioeconomic status of the children lost at follow-up, we assume that our results do not underestimate poor outcomes. At the same time, strategies aimed at improving follow-up compliance are desirable in a multicenter context.

A third limitation is that the follow-up did not extend beyond the age of 2 years, and only severe functional disabilities were investigated. Hence, we did not assess mild neurological dysfunction and preschool age performance, which may be impaired in various neuropsychological domains, even in patients without major disabilities. However, the NFA ICF-CY begins to express preliminary data on minor neurodevelopmental anomalies (an NFA ICF-CY score of 2), such as minor motor disorders, minor deficits in cognitive functioning, and regulation difficulties, whose characteristics must be redefined at later ages. Finally, MRI data are lacking, and brain lesions were not described in detail, but these could be areas for future research.

In conclusion, this study provides updated information on the neurodevelopmental outcomes of VLBW infants in a large Italian cohort. The overall rate of neurodevelopmental disabilities was quite lower than in data in the previous literature, and GA remains one of the main risk factors for poor neurodevelopmental outcomes. Interestingly, in this study, CP accounted for just over half of severe functional dysfunction, while the remaining cases presented severe neuropsychological or sensory-neural deficiencies. Neuroprem 2, by providing data on contemporary VLBW outcomes, supports further follow-up programs from a national network perspective. Such networks contribute to promoting access to formal neurodevelopmental evaluation and to timely rehabilitative interventions.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**, further inquiries can be directed to the corresponding author/s.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the Emilia Romagna Ethics Committee (protocol 205/2015, n 4818). Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin.

AUTHOR CONTRIBUTIONS

LLug, FF, and FM contributed to conception and design of the study. MR, NB, ED, MS, AS, and SP organized the database. LB, IG, and LLuc performed the statistical analysis. MP, GA, GG, FS, LC, and OP wrote sections of the manuscript. AB and LI critically revised the manuscript for important intellectual content. Neuroprem Working Group contributed to the follow-up of patients and to data collection. All authors contributed to manuscript revision, read, and approved the submitted version.

FUNDING

This study was funded by Pier Franco and Luisa Mariani foundation of Milan, Italy, awarding a grant to LLug.

ACKNOWLEDGMENTS

The authors acknowledge the Mariani Foundation of Milan, who sustained and sponsored the study, and the personnel of all NICUs participating in the study.

NEUROPREM WORKING GROUP

Antonella Luglio (Pediatric Post-graduate School University Hospital of Modena and Reggio Emilia, Modena, Italy), Sara Grandi (Neonatal Intensive Care Unit, Infermi Hospital of Rimini, Rimini, Italy), Valentina Fiorini (Neonatal Intensive Care Unit, Azienda USL-IRCCS, Reggio Emilia, Italy), Piero Catenazzi (Neonatal Intensive Care Unit, Maggiore Hospital of Bologna, Bologna, Italy), Elisa Ballardini (Neonatal Intensive Care Unit, Sant'Anna Hospital of Ferrara, Ferrara, Italy), Sabrina Moretti (Neonatal Intensive Care Unit, University Hospital

of Parma, Parma, Italy), Daniela Turoli (Italian Neonatal Network, University of Milan, Milan, Italy; Neonatal Intensive Care Unit, Manzoni Hospital of Lecco, Lecco, Italy), Arianna Aceti (Neonatal Intensive Care Unit, Sant'Orsola Malpighi University Hospital of Bologna, Bologna, Italy), Roberto Bellù (Italian Neonatal Network, University of Milan, Milan, Italy; Neonatal Intensive Care Unit, Manzoni Hospital of Lecco, Lecco, Italy), Luca Ori (Neonatal Intensive Care Unit, University Hospital of Modena, Modena, Italy), Silvia Braibanti (Neonatal Intensive Care Unit, Azienda USL-IRCCS, Reggio Emilia, Italy), Alessandra Boncompagni (Neonatal Intensive Care Unit, University Hospital of Modena, Modena, Italy), Caterina Spada (Neonatal Intensive Care Unit, Bufalini Hospital of Cesena, Cesena, Italy), and Lara Valeri (Pediatric Post-graduate School University Hospital of Modena and Reggio Emilia, Modena, Italy).

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fped.2021.697100/full#supplementary-material

REFERENCES

1. Moore T, Hennessy EM, Myles J, Johnson SJ, Draper SE, Costeloe LK, et al. Neurological and developmental outcome in extremely preterm children born in England in 1995 and 2006: the EPICURE studies. *BMJ*. (2012) 345:e7961. doi: 10.1136/bmj.e7961
2. Serenius F, Källén K, Blennow M, Ewald U, Fellman V, Holmström G, et al. Neurodevelopmental outcome in extremely preterm infants at 2.5 years after active perinatal care in Sweden. *JAMA*. (2013) 309: 120–81. doi: 10.1001/jama.2013.3786
3. Younge N, Goldstein RF, Bann CM, Eunice Kennedy Shriver National Institute of Child Health and Human Development Neonatal Research Network. Survival and neurodevelopmental outcomes among Periviable infants. *N Engl J Med*. (2017) 376:617–28. doi: 10.1056/NEJMoa1605566
4. Marlow N. Neurocognitive outcome after very preterm birth. *Arch Dis Child Fetal Neonatal Ed*. (2004) 89:F224–8. doi: 10.1136/adc.2002.019752
5. Hack M, Taylor HG, Drotar D, Schluchter M, Cartar L, Andreias L, et al. Chronic conditions, functional limitations, and special health care needs of school-aged children born with extremely low-birth-weight in the 1990s. *JAMA*. (2005) 294:318–25. doi: 10.1001/jama.294.3.318
6. Marlow N, Wolke D, Bracewell MA, Samara M. EPICure Study Group: Neurologic and developmental disability at six years of age after extremely preterm birth. *N Engl J Med*. (2005) 352:9–19. doi: 10.1056/NEJMoa041367
7. Asztalos EV, Roberts RS, Robertson CM, Sauve RS, Whitield MF. Impact of bronchopulmonary dysplasia, brain injury, and severe retinopathy on the outcome of extremely low-birth-weight infants at 18 months: results from the trial of indomethacin prophylaxis in preterms. *JAMA*. (2003) 289:1124–9. doi: 10.1001/jama.289.9.1124
8. Bassler D, Stoll BJ, Schmidt B, Asztalos EV, Roberts RS, Robertson CM, et al. Using a count of neonatal morbidities to predict poor outcome in extremely low birth weight infants: added role of neonatal infection. *Pediatrics*. (2009) 123:313–8. doi: 10.1542/peds.2008-0377
9. Stoll BJ, Hansen NI, Adams-Chapman I, Fanaroff AA, Hintz SR, Vohr B, et al. Neurodevelopmental and growth impairment among extremely low-birth-weight infants with neonatal infection. *JAMA*. (2004) 292:2357–65. doi: 10.1001/jama.292.19.2357
10. Schlapbach LJ, Adams M, Proietti E, Aebischer M, Grunt S, Borradori-Tolsa C, et al. Outcome at two years of age in a Swiss national cohort of extremely preterm infants born between 2000 and 2008. *BMC Pediatr*. (2012) 12:198. doi: 10.1186/1471-2431-12-198
11. Larroque B, Ancel PY, Marret S, Marchand L, André M, Arnaud C, et al. Neurodevelopmental disabilities and special care of 5-year old children born before 33 weeks of gestation (the EPIPAGE study): a longitudinal cohort study. *Lancet*. (2008) 371:813–20. doi: 10.1016/S0140-6736(08)60380-3
12. Larroque B, Ancel PY, Marchand-Martin L, Cambonie G, Fresson J, Pierrat V, et al. Special care and school difficulties in 8-year-old very preterm children: the Epipage cohort study. *PLoS ONE*. (2001) 6:e21361. doi: 10.1371/journal.pone.0021361
13. Lugli L, Pugliese M, Plessi C, Berardi A, Guidotti I, Ancora G, et al. Neuroprem: the Neuro-developmental outcome of very low birth weight infants in an Italian region. *Ital J Pediatr*. (2020) 46:26. doi: 10.1186/s13052-020-0787-7
14. Vermont Oxford Network. *Vermont Oxford Database Manual of Operations for Infants Born in 2009. Release 13.0*: Burlington, VT: Vermont Oxford Network (2009).
15. Gosselin J, Gahagan S, Amiel-Tison C. The Amiel-Tison neurological assessment at term: conceptual and methodological continuity in the course of follow-up. *Ment Retard Dev Disabil Res Rev*. (2015) 11:34–51. doi: 10.1002/mrdd.20049
16. Griffiths R. *Griffiths Mental Developmental Scale- Revised: Birth to 2 Years (GMDS-R)*. Firenze: Hogrefe (1996).
17. Bayley N. *The Bayley scales of infant and toddler development (BSID-III)*. San Antonio, TX: Psychological Corporation (2006).
18. Picciolini O, Squarza C, Fontana C, Giannì ML, Cortinovis I, Gangi S, et al. Neurodevelopmental outcome of extremely low birth weight infants at 24 months corrected age: a comparison between Griffiths and Bayley Scales. *BMC Pediatr*. (2015) 15:139. doi: 10.1186/s12887-015-0457-x
19. World Health Organization. *The International Classification of Functioning, Disability and Health for Children and Youth: ICF-CY*. Geneva, Switzerland: World Health Organization (2007).
20. Picciolini O, Giannì ML, Vegni C, Fumagalli M, Mosca F. Usefulness of an early neurofunctional assessment in predicting neurodevelopmental outcome in very low birthweight infants. *Arch Dis Child Fetal Neonatal Ed*. (2006) 91:F111–7. doi: 10.1136/adc.2005.073262
21. Giannì ML, Picciolini O, Vegni C, Gardon L, Fumagalli M, Mosca F. Twelve-month neurofunctional assessment and cognitive performance at 36 months of age in extremely low birth weight infants. *Pediatrics*. (2007) 120:1012–9. doi: 10.1542/peds.2006-3364
22. Picciolini O, Montirosso R, Porro M, Gianni ML, Mosca F. Neurofunctional assessment at term equivalent age can predict 3-year neurodevelopmental outcomes in very low birth weight infants. *Acta Paediatr*. (2016) 105:e47–53. doi: 10.1111/apa.13248
23. Bax M, Goldstein M, Rosenbaum P, Leviton A, Paneth N, Dan B, et al. Proposed definition and classification of cerebral palsy. *Dev Med Child Neurol*. (2005) 47:571–6. doi: 10.1017/S001216220500112X
24. Marlow N, Hennessy EM, Bracewell MA, Wolke D, and EPICure study group. Motor and executive function at 6 years of age after extremely preterm birth. *Pediatrics*. (2007) 120:793–804. doi: 10.1542/peds.2007-0440
25. Pugliese M, Rossi C, Guidotti I, Gallo C, Della Casa E, Bertoncelli N. Preterm birth and developmental problems in infancy and preschool age part II: cognitive, neuropsychological and behavioral outcomes. *J Matern Fetal Neonatal Med*. (2013) 26:1653–7. doi: 10.3109/14767058.2013.794205
26. Pierrat V, Marchand-Martin L, Arnaud C, Kaminski M, Resche-Rigon M, Lebeaux C, et al. Neurodevelopmental outcome at 2 years for preterm children born at 22 to 34 weeks' gestation in France in 2011: EPIPAGE-2 cohort study. *BMJ*. (2017) 358:j3448. doi: 10.1136/bmj.j3448
27. Mahoney K, Bajuk B, Oei J, Lui K, Abdel-Latif ME, NICUS Network. Risk of neurodevelopmental impairment for outborn extremely preterm infants in an Australian regional network. *J Matern Fetal Neonatal Med*. (2017) 30:96–102. doi: 10.3109/14767058.2016.1163675
28. Delobel-Ayoubm M, Arnaud C, White-Koning M, Casper C, Pierrat V, Garel M, et al. Behavioral problems and cognitive performance at 5 years of age

after very preterm birth: the EPIPAGE study. *Pediatrics.* (2009) 123:1485–92. doi: 10.1542/peds.2008-1216
29. Matthews TJ, MacDorman MF, Thoma ME. Infant mortality statistics from the 2013 period linked birth/infant death data set. *Natl Vital Stat Rep.* (2015) 64:1–30.
30. Rysavy MA, Horbar JD, Bell EF, Li L, Greenberg LT, Tyson JE, et al. Assessment of an updated neonatal research network extremely preterm birth outcome model in the vermont oxford network. *JAMA Pediatr.* (2020) 174:e196294. doi: 10.1001/jamapediatrics.2019.6294
31. Jois RS. Understanding long-term neurodevelopmental outcomes of very and extremely preterm infants: a clinical review. *Aust J Gen Pract.* (2019) 48:26–32. doi: 10.31128/AJGP-04-18-4545
32. Ibragimova N, Granlund M, Bjorck-Akesson E. Field trial of ICF version for children and youth (ICF-CY) in Sweden: logical coherence, developmental issues and clinical use. *Dev Neurorehabil.* (2009) 12:3–11. doi: 10.1080/17518420902777001
33. Giovannetti AM, Raggi A, Leonardi M, Mosca F, Gangi S, Sabbadini S, et al. Usefulness of ICF-CY to define functioning and disability: a retrospective study on children born with very low birth weight. *Early Hum Dev.* (2013) 89:825–31. doi: 10.1016/j.earlhumdev.2013.07.014
34. Fontana C, Picciolini O, Fumagalli M, Mosca F, Bernardelli G, Leonardi M, et al. A longitudinal ICF-CY-based evaluation of functioning and disability of children born with very low birth weight. *Int J Rehabil Res.* (2016) 39:296–301. doi: 10.1097/MRR.0000000000000183
35. Jansen L, van Steenis A, van den Berg-Huysmans AA, Wiggers-de Bruine ST, Rijken M, de Vries LS, et al. Associations between neonatal MRI and short- and long-term neurodevelopmental outcomes in a longitudinal cohort of very preterm children. *J Pediatr.* (2021) 234:46–53.e2. doi: 10.1016/j.jpeds.2021.02.005
36. Gardon L, Picciolini O, Squarza C, Frigerio A, Giannì ML, Gangi S, et al. Neurodevelopmental outcome and adaptive behaviour in extremely low birth weight infants at 2 years of corrected age. *Early Hum Dev.* (2019) 128:81–5. doi: 10.1016/j.earlhumdev.2018.12.013
37. Wolke D, Söhne B, Ohrt B, Riegel K. Follow-up of preterm children: important to document dropouts. *Lancet.* (1995) 345:447. doi: 10.1016/S0140-6736(95)90425-5

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's Note: All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

frontiers in Pediatrics

ORIGINAL RESEARCH
published: 07 October 2021
doi: 10.3389/fped.2021.731877

Preterm Birth Affects Early Motor Development in Pigs

Charlotte Vanden Hole[1†], Miriam Ayuso[1], Peter Aerts[2], Steven Van Cruchten[1], Thomas Thymann[3], Per Torp Sangild[3] and Chris Van Ginneken[1]*

[1] Laboratory of Comparative Perinatal Development, Department of Veterinary Sciences, Faculty of Biomedical, Pharmaceutical and Veterinary Sciences, University of Antwerp, Antwerp, Belgium, [2] Laboratory of Functional Morphology, Department of Biology, Faculty of Sciences, University of Antwerp, Antwerp, Belgium, [3] Comparative Pediatrics and Nutrition, University of Copenhagen, Copenhagen, Denmark

OPEN ACCESS

Edited by:
Lynette Kay Rogers, The Research Institute at Nationwide Children's Hospital, United States

Reviewed by:
Chantal Lau, Baylor College of Medicine, United States
Oceane Schmitt, University of Veterinary Medicine Vienna, Austria

***Correspondence:**
Miriam Ayuso
miriam.ayusohernando@uantwerpen.be

†Present address:
Charlotte Vanden Hole, Flanders Research Institute for Agriculture, Fisheries and Food (ILVO)—Animal Sciences Unit, Melle, Belgium

Specialty section:
This article was submitted to Neonatology, a section of the journal Frontiers in Pediatrics

Received: *28 June 2021*
Accepted: *26 August 2021*
Published: *07 October 2021*

Citation:
Vanden Hole C, Ayuso M, Aerts P, Van Cruchten S, Thymann T, Sangild PT and Van Ginneken C (2021) Preterm Birth Affects Early Motor Development in Pigs. Front. Pediatr. 9:731877. doi: 10.3389/fped.2021.731877

Background: Preterm infants frequently show neuromotor dysfunctions, but it is not clear how reduced gestational age at birth may induce developmental coordination disorders. Advancing postnatal age, not only post-conceptional age, may determine neuromuscular development, and early interventions in preterm newborns may improve their later motor skills. An animal model of preterm birth that allows early postnatal detection of movement patterns may help to investigate this hypothesis.

Methods: Using pigs as a model for moderately preterm infants, preterm (106-day gestation, equivalent to 90% of normal gestation time; $n = 38$) and term (115-day gestation, equivalent to 99% of normal gestation time; $n = 20$) individuals were delivered by cesarean section and artificially reared until postnatal day 19 (preweaning period). The neuromotor skills of piglets were documented using spatiotemporal gait analyses on video recordings of locomotion at self-selected speed at postnatal age 3, 4, 5, 8, and 18 days. Results were controlled for effects of body weight and sex.

Results: Both preterm and term piglets reached mature neuromotor skills and performance between postnatal days 3–5. However, preterm pigs took shorter steps at a higher frequency, than term piglets, irrespective of their body size. Within preterm pigs, males and low birth weight individuals took the shortest steps, and with the highest frequency.

Conclusion: Postnatal development of motor skills and gait characteristics in pigs delivered in late gestation may show similarity to the compromised development of gait pattern in preterm infants. Relative to term pigs, the postnatal delay in gait development in preterm pigs was only few days, that is, much shorter than the 10-day reduction in gestation length. This indicates rapid postnatal adaptation of gait pattern after reduced gestational age at birth. Early-life physical training and medical interventions may support both short- and long-term gait development after preterm birth in both pigs and infants.

Keywords: locomotion, prematurity, pig, birth weight, spatio-temporal gait analysis

INTRODUCTION

Preterm birth (birth before 37 weeks of gestation) is known to interrupt brain growth and maturation *in utero*, potentially affecting postnatal neurodevelopment, especially of cerebellar and periventricular brain structures (1, 2). In addition, clinical complications associated with preterm birth (such as hypoxia, ischemia, inflammation) can further hinder postnatal neurodevelopment (2). Thus, it is not surprising to note high prevalence of neuromotor dysfunctions and poor movement coordination in preterm infants (1, 3–5). Even for infants not diagnosed with specific brain defects (e.g., cerebral palsy) and having normal intelligence, preterm birth may result in motoric challenges, as evidenced by 20–40% incidence of moderate motor impairments, developmental coordination disorders, and neurological dysfunctions (1, 3, 4, 6). Preterm birth is a multifactorial syndrome that requires support at many levels, including the need to facilitate optimal neurodevelopmental outcomes. However, in order to support evidence-based interventions, more basic data on locomotion in preterm newborns are required. In many aspects, preterm pigs born at 90% gestation have proven to be an excellent brain model for preterm infants (7), in addition to aspects of gut and nutritional functions (8). The preterm pig as a model for neuromuscular development in preterm infants is less explored. In contrast to the immediate locomotion after birth of term piglets, 90% gestation preterm pigs show 3–5-day delays in their normal standing and walking (9–11). While this postnatal motor development is much faster than in preterm infants, the preterm–term differences in piglets may be used to investigate basic mechanisms and possible interventions in states of immaturity. Further, the gross anatomy of the brain (12), its perinatal growth and developmental trajectories are similar in pigs and infants (13), suggesting that consequences of preterm birth may be similar, although different in their timing (8, 14). Specifically, for locomotion, it appears that underlying mechanisms of development and locomotor modules in the neuronal networks of the spinal cord are similar among mammals (15–18).

Previous studies on motor skills in preterm pigs showed that more days were required to achieve basic motor skills (time to first stand and walk), with lower overall physical activity level, compared with term pigs (9–11). Similarly, in preterm infants delayed or absent physical movement predict a delay in onset of first walking (6) and walking coordination (1, 19). A previous study on gait development at 1 week of age in preterm and term pigs revealed only minor differences, although shorter strides and step lengths were present in preterm pigs after normalization for their smaller size (9). Interestingly, shorter strides are also observed in preterm infants, although such effects may disappear at later ages (1, 6). No longitudinal postnatal locomotion studies comparing preterm and term counterparts are available in pigs or infants. Studies in preterm pigs show that organ systems respond widely different to reduced gestational age at birth, either with fast adaptation after birth (lungs, gut, immunity) or with more prolonged defects, related to their immature state at birth (e.g., brain, bone, metabolism) (8, 20). Whether postnatal, rather than post-conceptional age, is the main driver of neuromuscular maturation is unclear. Rapid postnatal adaptation of neuromuscular development would encourage specialized neonatal support to reduce later dysfunctions (6, 21, 22).

The present study compares motor skill development in piglets that are born preterm (90% gestational age) (P) and term (T) at different time-points: 3, 4, 5, 8, and 18 days postnatally. We compare different aspects of gait between both groups and along the set of time-points: motor performance (speed, stride length, stride frequency), neuromotor skill/maturation (normalized motor performance and the different components of a stride cycle, for example, normalized step length, duty factor), and gait variability [for more info, see **Materials and Methods** section and previous publications (23, 24).We hypothesize that postnatal age rather than post-conceptual age drives gait development. Specifically, we hypothesize that preterm pigs show no developmental delay in motor performance, an equally long period of neuromotor maturation and a similar gait variability compared to pig born near term. Considering that low birth weight and sex have been reported to affect morbidities and motor development in infants (4, 5, 25–27) and preterm pigs (10, 20), we made subgroup analyses according to weight (0–25 vs. 50–75% birth weight percentiles) and sex (male, female).

MATERIALS AND METHODS

Study Animals

Four litters of pigs (Danish Landrace × Large White × Duroc) (litter sizes 20–23) were used in this study. One litter was born by cesarean section at term (gestation day 115; term = 115–117 days, T piglets, $n = 20$, birth weight (live born piglets) 1,049 ± 222 g). Three other litters were delivered preterm at 90% gestation through a cesarean section (gestation day 106; P piglets, $n = 38$, birth weight (live born piglets) 883 ± 199 g), as outlined previously (14). In brief, the piglets were resuscitated immediately after cesarean section and placed in oxygenated and temperature-controlled incubators. Within 3 h after birth, a catheter was placed *via* the transected umbilical artery to allow parenteral nutrition support, and an orogastric feeding tube was placed to allow enteral bolus feeding. The postnatal handling of these two groups was identical and followed the protocol outlined in (14). Both T and P piglets were nourished enterally with increasing amounts of raw bovine milk with lactose added (Variolac, 6 g/l, Arla Foods Ingredients, Århus, Denmark). During the first 7 days of life, the pigs were fed with parenteral nutrition (modified Kabiven solution, Fresenius Kabi, Bad Homburg, Germany) in addition to their enteral diet. All the piglets received the same amount of nutrition (relative to body weight). The clinical condition of each piglet was evaluated at least twice per day. During the first 5 days of life, these piglets were kept individually in heated incubators

Abbreviations: AIDF, AI duty factor; AIF, AI stride frequency; AIL, AI stride length; AISL, AI step length; AIST, AI stance duration; AISW, AI swing duration; *df*, duty factor; *f*, stride frequency; *g*, gravitational acceleration; h_{swmax}, maximum swing height; HLL, hind limb length; l_{step}, step length; l_{stride}, stride length; t_{st}, stance duration; t_{sw}, swing duration; *u*, self-selected speed.

with supplemental oxygen during the first 12 h after birth. Later, they were transferred to larger open cages. P and T piglets were not mixed.

All piglets were euthanized on postnatal day 19 using initial induction of anesthesia (mixture of zolazepam, tiletamine, ketamine, butorphanol, and xylacin) followed by intracardiac injection of a lethal dose of sodium pentobarbital. All experimental procedures were approved by the Danish Animal Experiments Inspectorate (2014-15-0201-00418).

Video Sequences

Piglets were gently encouraged to walk at voluntary speed through a custom-made corridor (fitted with a reference grid), while lateral view video recordings were made (12.8-megapixel, 50 Hz, JVC GC-PX100, JVC Kenwood Corporation, Kanagawa, Japan) at five time points: 3, 4, 5, 8, and 18 days after birth. At each recording day, three successive movies per piglet were recorded. Only videos that had at least one completed cycle without pausing or falling were retained for further analysis. In each retained sequence, one complete stride cycle was analyzed. More details on the recording and selection of the video sequences are found in previous papers (23, 24).

Gait Analysis

In each of the video sequences, five body landmarks were digitized field-by-field using Matlab (MathWorks, Natick, MA, USA), using a free work package written by Ty Hedrick (University of North Carolina, USA; http://www.unc.edu/~thedrick/software1.html). The first four points were the most distal point of the distal phalanx (claw of the fourth toe) of each leg, and the fifth point was the eye or a dark spot on the skin. The latter body point was included to measure overall forward displacement of the body throughout a stride. An image showing the setup of the recording has been previously published by our group and depicts all the reference points used in the gait analysis (23). From our previous gait studies, we know that the choice of the landmark does not introduce extra variability to the dataset, as long as the landmarks are easily tracked throughout the stride (23, 24).

A linear dimension—related to locomotion—was necessary for the normalization of certain variables [dynamic similarity principle by Alexander and Jayes (28); see **Table 1**]. In previous gait analysis of the piglet, functional hind limb length (HLL), that is, the distance between the most distal part of the distal phalanx (of the fourth toe of the hindlimb) and the tail base, was used (23, 24). HLL was measured once in each sequence, in the frame where the hind limb closest to the camera was on the floor and supporting the piglet's weight. In addition, two points on the reference grid were digitized for scaling purposes (for more information on the setup and an image depicting all these landmarks, we refer the reader to (23).

Fourteen gait variables were calculated using a custom-written Matlab script [made by Goyens, see (23)], based on the digitization of the abovementioned five body landmarks. An overview of all variables, including definitions, formulas, and normalizations (if applicable), is found in **Table 1**. Variables can be subdivided into three main categories: motor performance, neuromotor skill/maturation, and gait variability (23, 24). Motor performance included absolute values of self-selected speed (u) and its components stride frequency (f) and stride length (l_{stride}). Neuromotor skill/maturation included all spatiotemporal gait variables that were normalized to HLL (made dimensionless), in accordance with the dynamical similarity put forth by Alexander and Jayes (28). The evolution of these variables over time indicates neuromotor maturation, whereas differences in these variables between groups indicate a difference in neuromotor skill as such. Gait variability was measured through asymmetry indices (AIs). The smaller the AI, the larger the symmetry. Theoretically, they can range from 200 to 0% (30). Variables were calculated per piglet (u and u'), per leg pair (AIs) or per leg (spatiotemporal gait variables, both absolute and normalized).

Statistics

Generalized regression models were used to test whether condition [T ($n = 20$) or P ($n = 38$)] and age after birth (and their interaction) had a significant effect on the different outcome variables. When constructing the models, it was considered whether a variable was calculated per pig (body weight, HLL, u, u′), leg (all absolute and normalized spatiotemporal gait variables with the exception of u and u′), or legpair (AIs). In case of variables that were leg or legpair specific, leg or legpair was added as a fixed effect, and a random factor for leg or legpair nested in piglet could be added (when proven a significant addition to the model through log-likelihood testing). *Post hoc* testing was approached differently, depending on the investigated effect and variable. When comparing legs, *post hoc* testing with Tukey's correction was applied. In case of an age effect, *post hoc* testing with Dunnett's correction was applied. In this case, day 18 (as the most mature age) was used as a reference, as this reduced the number of between-group comparisons and because we were interested in the maturation pattern and not the day-to-day-variation.

The testing of P-MALE ($n = 21$) vs. P-FEMALE ($n = 17$) pigs was done on the entire preterm dataset. For selecting which piglets were P-LBW and P-NORM, per litter the lowest-quartile (0–25th percentile) and the third-quartile (50–75th percentile) birth weights were calculated. This led to a P-LBW group of nine filmed piglets (birth body weight 660 ± 251 g) and a P-NORM group of nine filmed piglets (birth body weight 990 ± 130 g). The statistical analysis itself was the same as in the T vs. P dataset, with "condition" being replaced by "BW-category" or "sex." Interactions between these two effects could not be included in the model, because the P-LBW/P-NORM dataset was too small to include sex as an effect.

RESULTS

Morphometrics

There was no interaction between condition (P vs. T), sex or birth weight and postnatal age for both body weight, and HLL ($p > 0.05$). Thus, only the overall effects of age and gestational age were statistically assessed.

TABLE 1 | Summary of all used variables (abbreviations (ABB), definitions, and formulas, including normalization procedure [NP, adapted from (23)].

Variable	ABB	Definition	Formula	NP
Gravitational acceleration	g	–	–	–
Self-selected speed	u	The forward movement during one cycle divided by the duration of the cycle. Animals are able to move in an unrestrained, voluntary way.	fl_{stride}	$\frac{u}{\sqrt{HLL\ g}}$
Stride frequency	f	Inverse of the period between two consecutive footfalls of a certain leg.	$\frac{u}{l_{stride}}$	$f\sqrt{HLL\ g}$
Stride length	l_{stride}	The forward movement during one stride or cycle.	$\frac{u}{f}$	$\frac{l_{stride}}{HLL}$
Stance duration	t_{st}	The period of contact between a limb and the ground.	–	$\frac{t_{st}}{\sqrt{HLL/g}}$
Swing duration	t_{sw}	The period of limb flight.	–	$\frac{t_{sw}}{\sqrt{HLL/g}}$
Step length	l_{step}	The forward movement during one step (stance phase only).	–	$\frac{l_{step}}{HLL}$
Duty factor	df	The fraction of the cycle for which the limb is in contact with the ground.	–	NA
Maximum swing height	h_{swmax}	The maximum vertical distance the leg is lifted from the ground during the swing phase.	–	$\frac{h_{swmax}}{HLL}$
AI stride frequency	AIF	Asymmetry index of the stride frequency. Adapted from (29).	$\frac{(f_R - f_L)}{0.5\ (f_R+f_L)}\ 100\%$	–
AI stride length	AIL	Asymmetry index of the stride length. Adapted from (29).	$\frac{(l_{stride,\ R} - l_{stride,L})}{0.5\ (l_{stride,R}+\ l_{stride,L})}\ 100\%$	–
AI stance duration	AIST	Asymmetry index of the stance duration. Adapted from (29).	$\frac{(t_{st,R} - t_{st,L})}{0.5\ (t_{st,R}+t_{st,L})}\ 100\%$	–
AI swing duration	AISW	Asymmetry index of the swing duration. Adapted from (29).	$\frac{(t_{sw,R} - t_{sw,L})}{0.5\ (t_{sw,R}+t_{sw,L})}\ 100\%$	–
AI step length	AISL	Asymmetry index of the step length. Adapted from (29).	$\frac{(l_{step,R} - l_{step,L})}{0.5\ (l_{step,R}+l_{step,L})}\ 100\%$	–
AI duty factor	AIDF	Asymmetry index of the duty factor. Adapted from (29).	$\frac{(df_R - df_L)}{0.5\ (df_R+df_L)}\ 100\%$	–

When normalized, variables are indicated with ′ in the text.

The body weight of the piglets in the age groups 3, 4, 5, and 8 days was significantly different from that at the reference age d18 [$p < 0.001$ for P ($n = 38$), T ($n = 20$), P-LBW ($n = 9$), P-NORM ($n = 9$), P-MALE ($n = 21$), and P-FEMALE ($n = 17$)] (**Table 2**). Additional *post-hoc* analysis—comparing all age groups with each other—revealed that the body weight of P and T piglets (as well as P-LBW, P-NORM, P-MALE, and P-FEMALE) remained constant from d3 up to and including d8 and increased between d8 and d18 ($p < 0.001$ for d3–d8 vs. d18). Overall, body weight was lower in P compared to T piglets ($p = 0.001$). Body weight was overall lower in P-LBW piglets compared to P-NORM piglets ($p < 0.001$) but did not differ between P-MALE and P-FEMALE.

HLL in each age group was significantly lower from that at the reference age d18 [$p < 0.0001$ for P ($n = 38$), T ($n = 20$), P-LBW ($n = 9$), P-NORM ($n = 9$), P-MALE ($n = 21$), and P-FEMALE ($n = 17$)] (**Table 3**). Additional *post-hoc* analysis—comparing all age groups with each other—revealed HLL remained constant from d3 up and including d5 and increased between d5 over d8 to d18. In addition, P piglets had shorter legs, as evidenced by a shorter HLL ($p < 0.001$). HLL was overall lower in P-LBW piglets compared to P-NORM piglets ($p < 0.001$) but did not differ between P-MALE and P-FEMALE.

Motor Performance

u did not show any interaction between condition (P vs. T) and postnatal age and was not different between P ($n = 38$) and T ($n = 20$) piglets. u increased with postnatal age ($p < 0.001$). Irrespective of P or T, piglets at d3–8 had a significantly lower u compared to d18 (d3: $p < 0.001$, d4: $p = 0.010$, d5: $p = 0.002$, d8: $p = 0.029$) (**Figure 1A**) (data points = 215). As for the components of u, we did note an interaction between condition and age (l_{stride}: $p < 0.001$; f: $p = 0.002$) resulting in differences between P and T piglets. l_{stride} in both groups increased up to d18 ($p < 0.001$ for all group comparisons). At every time point, l_{stride} was higher for T piglets, compared to P piglets [$p = 0.010$ (d3), $p < 0.001$ (d4–18)]. T piglets increased their f from d3 to d18 ($p = 0.021$), but other age differences were not observed in both T and P piglets. f was significantly lower in T, compared to P piglets at d3, d5, and d8 ($p < 0.001$, $p = 0.001$, $p = 0.024$, respectively) (**Figure 1B**) (data

TABLE 2 | Body weight (mean ± SD; kg) according to gestational age at birth (condition) (preterm at gestational age 106 days—term at gestation age 115 days) and postnatal age (3, 4, 5, 8, and 18 days), birth weight (low birth weight (LBW): 0–25th percentile—normal birth weight (NORM): 50–75th percentile), and sex (female vs. male).

	Effect					
	Condition		Birth weight (preterms)		Sex (preterms)	
Age	Preterm	Near term	LBW	NORM	Female	Male
d3	0.90 ± 0.22^{a}	1.03 ± 0.23^{a}	0.65 ± 0.18^{a}	0.93 ± 0.77^{a}	0.99 ± 0.20^{a}	0.84 ± 0.22^{a}
d4	0.87 ± 0.14^{a}	1.08 ± 0.24^{a}	0.68 ± 0.19^{a}	0.91 ± 0.77^{a}	0.89 ± 0.19^{a}	0.88 ± 0.12^{a}
d5	0.98 ± 0.23^{a}	1.08 ± 0.24^{a}	0.73 ± 0.21^{a}	0.96 ± 0.11^{a}	0.99 ± 0.20^{a}	0.95 ± 0.25^{a}
d8	1.05 ± 0.26^{a}	1.13 ± 0.24^{a}	0.77 ± 0.21^{a}	1.11 ± 0.22^{a}	1.11 ± 0.27^{a}	1.02 ± 0.26^{a}
d18	1.73 ± 0.28^{b}	1.57 ± 0.30^{b}	1.54 ± 0.11^{b}	1.82 ± 0.24^{b}	1.69 ± 0.30^{b}	1.77 ± 0.27^{b}

There was no interaction between condition, sex, or birth weight and postnatal age. ^{a,b}*Different superscripts indicate significant differences with postnatal age within condition, birth weight, or sex.*

TABLE 3 | Hindlimb length (HLL) (mean ± SD; m) according to gestational age when cesarean-section derived (condition) (preterm at gestational age 106 days—term at gestation age 115 days) and postnatal age (3, 4, 5, 8, and 18 days), birth weight (low birth weight (LBW): 0–25th percentile—normal birth weight (NORM): 50–75th percentile), and sex (female vs. male).

	Effect					
	Condition		Birth weight (preterms)		Sex (preterms)	
Age	Preterm	Near term	LBW	NORM	Female	Male
d3	0.14 ± 0.02^{a}	0.15 ± 0.01^{a}	0.13 ± 0.02^{a}	0.14 ± 0.01^{a}	0.15 ± 0.01^{a}	0.14 ± 0.02^{a}
d4	0.14 ± 0.01^{a}	0.16 ± 0.01^{a}	0.13 ± 0.01^{a}	0.14 ± 0.01^{a}	0.14 ± 0.01^{a}	0.14 ± 0.01^{a}
d5	0.14 ± 0.01^{a}	0.15 ± 0.01^{a}	0.13 ± 0.01^{a}	0.15 ± 0.01^{a}	0.15 ± 0.01^{a}	0.14 ± 0.02^{a}
d8	0.16 ± 0.02^{b}	0.17 ± 0.01^{b}	0.14 ± 0.02^{b}	0.16 ± 0.01^{b}	0.16 ± 0.02^{b}	0.15 ± 0.02^{b}
d18	0.18 ± 0.02^{c}	0.17 ± 0.01^{c}	0.16 ± 0.01^{c}	0.18 ± 0.01^{c}	0.17 ± 0.02^{c}	0.18 ± 0.01^{c}

There was no interaction between condition, sex, or birth weight and postnatal age. HLL was lower in P as compared to T piglets and in P-LBW piglets compared to P-NORM piglets. $^{a-c}$*Different superscripts indicate significant differences with postnatal age within condition, birth weight, or sex.*

points = 860). This means that T piglets took bigger steps at a lower frequency than P piglets, resulting in a similar speed.

u was not different for P-LBW ($n = 9$) and P-NORM ($n = 9$) piglets (**Figure 1C**) (data points = 60). Similar to T vs. P piglets, this u was achieved differently: P-NORM piglets had a higher l_{stride} and a lower f, compared to P-LBW ($p < 0.001$ and $p = 0.016$, respectively). l_{stride} was increased (all comparisons with d18 $p < 0.001$), whereas f was dropped with age (only d3 differed from d18, $p < 0.001$) (**Figure 1D**) (data points = 240).

P-MALE ($n = 21$) and P-FEMALE ($n = 17$) piglets did not differ in u (**Figure 1E**) (data points = 134), although l_{stride} was higher for P-FEMALE at d3 ($p < 0.001$) and f was overall higher in P-MALE ($p = 0.001$). Both groups increased their l_{stride} (all comparisons with d18 $p < 0.001$) and overall dropped their f (only d3 differed from d18, $p < 0.001$) (**Figure 1F**) (data points = 536).

Neuromotor Control—Normalized Motor Performance

u' was not different between P ($n = 38$) and T ($n = 20$) piglets and increased in a similar fashion. Irrespective of P or T, u' was lower at d3 and d5, compared to d18 ($p = 0.001$, 0.050, respectively), indicating that the piglets were able to obtain a mature normalized speed between d5 and d8 (**Figure 2A**) (data points = 215). For l_{stride}', the maturation period was longer in P piglets, which showed lower values until d8, compared to d18 ($p < 0.001$). However, in T piglets, d8 and d18 were not significantly different anymore (d3–d5 vs. d18 $p < 0.001$). l_{stride}' was consistently shorter in P piglets ($p < 0.001$ at all ages). f' showed a fairly inconsistent maturation pattern in T piglets with d3 and d8 (but not d4 and d5) being significantly different from the reference age at d18 ($p < 0.001$ and $p = 0.014$, respectively). This gait characteristic did not show maturational changes in P piglets. At d3, d5, and d8, f' was significantly higher in P piglets, compared to T piglets ($p < 0.001$, $p = 0.018$, 0.004, respectively) (**Figure 2B**) (data points = 860).

While u' did not differ between P-LBW ($n = 9$) and P-NORM ($n = 9$) (**Figure 2C**) (data points = 60), f' was overall higher for P-LBW ($p = 0.003$), while l_{stride}' was higher for P-NORM ($p < 0.001$) (**Figure 2D**) (data points = 240). u' did not show any age-related changes in P-LBW and P-NORM. However, l_{stride}' increased (all comparisons $p < 0.001$) whereas f' discretely dropped (d3 vs. d18: $p = 0.003$) (**Figure 2D**).

Similarly, u' did not differ between P-MALE ($n = 21$) and P-FEMALE ($n = 17$) piglets and did not differ with postnatal age (**Figure 2E**) (data points = 134). f' was higher ($p = 0.001$) whereas l_{stride}' (at d3, 8, and 18; $p < 0.001$, 0.018, 0.037, respectively) was lower for P-MALE, compared to P-FEMALE.

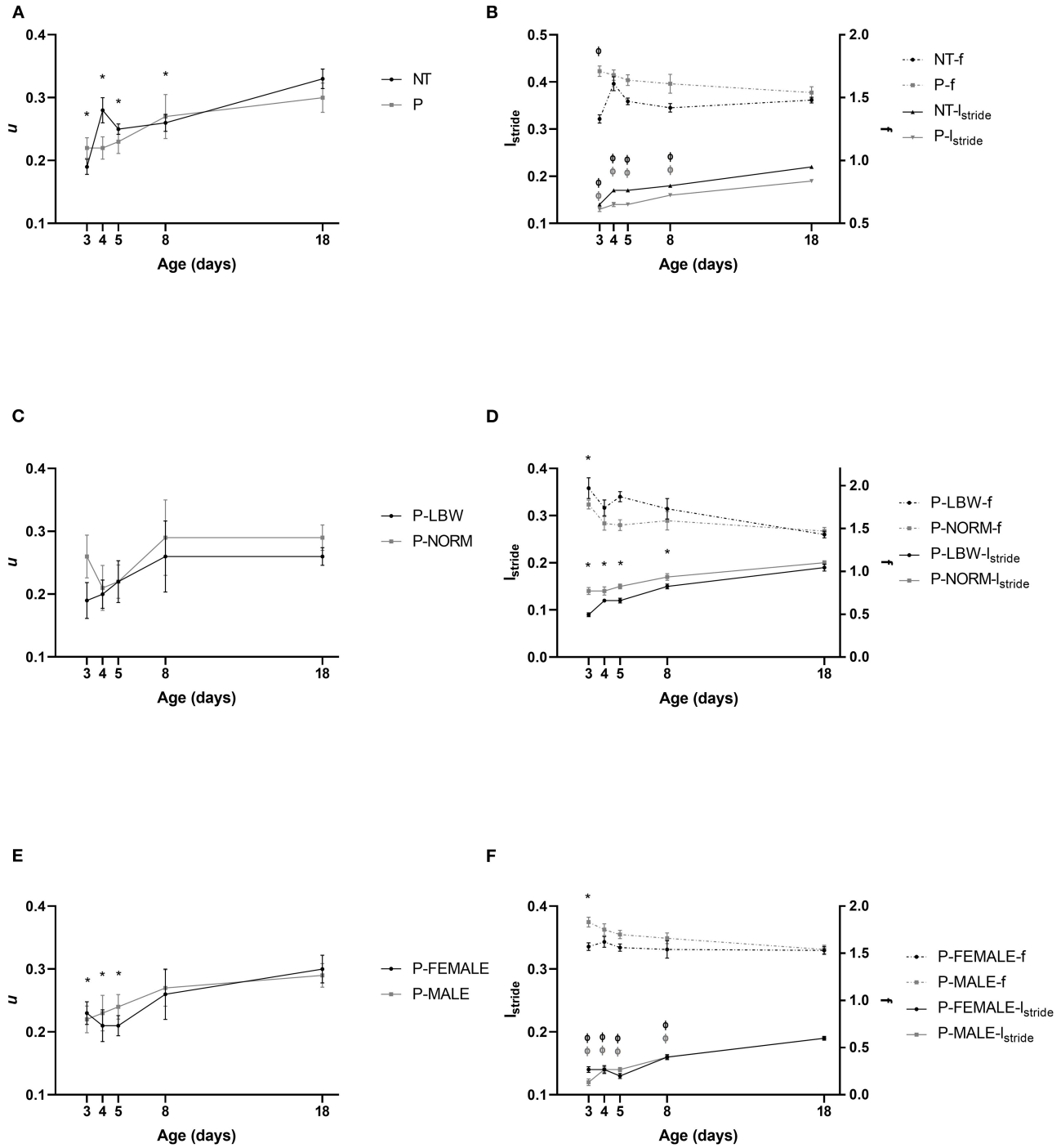


FIGURE 1 | Motor performance. All values are mean ± SE. **(A)** Speed (u, ms^{-1}) according to condition (P: preterm, gray; T: term, black) and age. u of P and T piglets is similar. Mean values indicated with * differ from d18. **(B)** For stride length (l_{stride}, m, full line) and stride frequency (f, s^{-1}, dotted line), an interaction was observed between condition (P vs. T) and postnatal age. l_{stride} was higher in T than in P pigs. f was lower in T, compared to P pigs at d3, d5, and d8). Mean values of l_{stride} indicated with "Φ" differ from d18 for P and T. Except for T piglets increasing their f from d3 to d18, T and P pigs did not change their f with age. **(C)** Speed (u, ms^{-1}) of preterm pigs did not differ with birth weight (normal birth weight (P-NORM); low birth weight (P-LBW)) and postnatal age. **(D)** Stride length (l_{stride}, m, full line) and stride frequency (f, s^{-1}, dotted line) according to birth weight (P-NORM vs. P-LBW) and postnatal age. P-NORM piglets had a higher l_{stride} and a lower f, compared to P-LBW. Mean values indicated with * differ from d18 for l_{stride} and for f. **(E)** Speed (u, ms^{-1}) according to sex of preterm piglets (female: P-FEMALE; male: P-MALE) and age. No differences are noted with sex. Mean values indicated with * differ from d18. **(F)** Stride length (l_{stride}, m, full line) and stride frequency (f, s^{-1}, dotted line) according to sex (P-MALE vs. P-FEMALE) and postnatal age. There was an interaction of postnatal age and sex for l_{stride}, indicating that l_{stride} was higher for P-FEMALE at d3 whereas the effect for f was not depending on postnatal age and f was overall higher in P-MALE. Mean values of indicated with "Φ" and "*" differ from d18 for l_{stride} and f, respectively. Black colors indicate differences in T while gray colors indicate differences in P piglets.

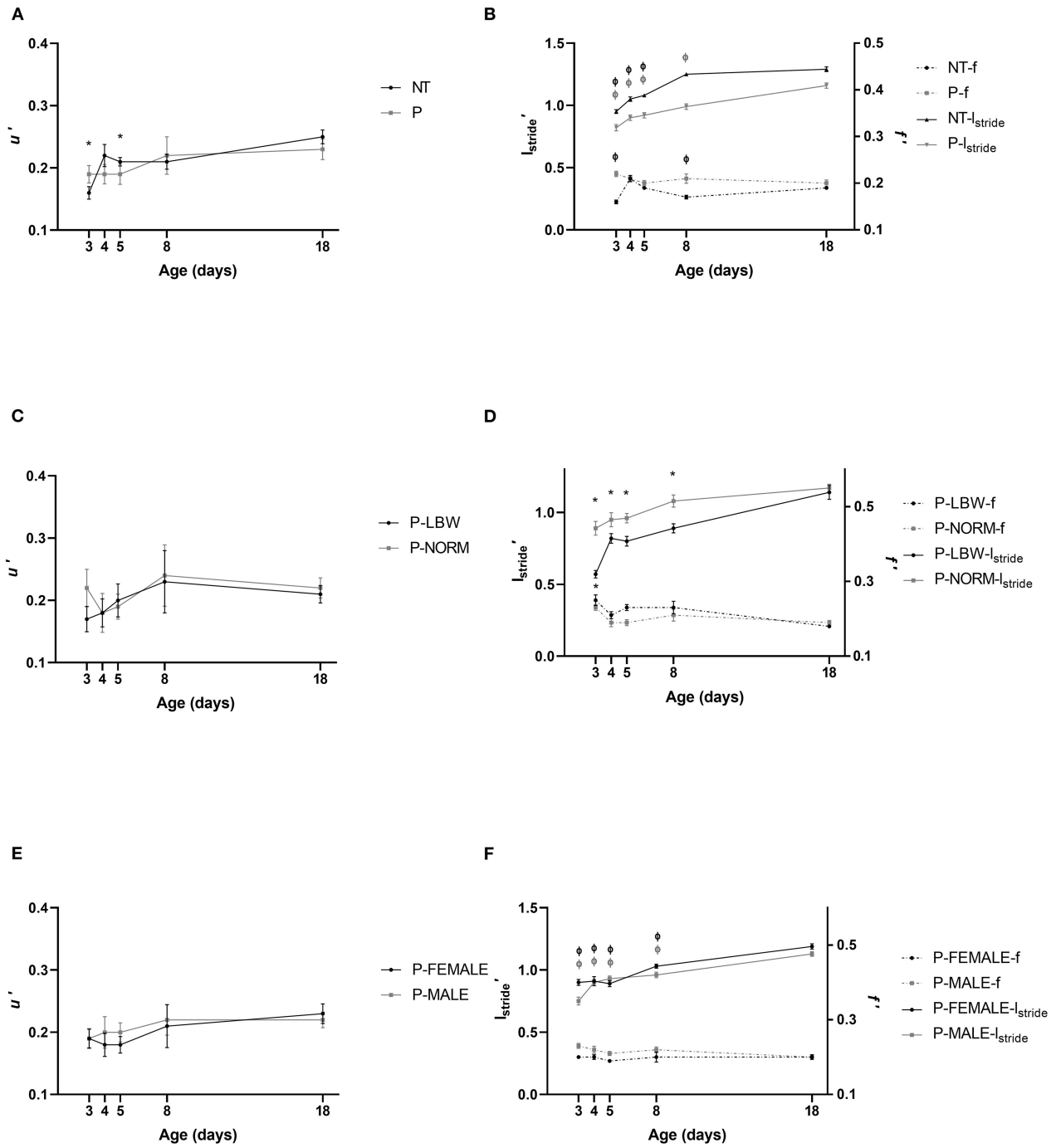


FIGURE 2 | Neuromotor control. All values are mean ± SE. **(A)** Normalized speed (u') according to condition (P: preterm, gray; T: term, black) and age. u' of P and T piglets is similar. Mean values indicated with * differ from d18. **(B)** For normalized stride length (l'_{stride}, full line) and stride frequency (f', dotted line), an interaction was observed between condition (P vs. T) and postnatal age. $l_{stride}{}'$ was shorter in P piglets. At d3, d5, and d8, f' was higher in P piglets, compared to T piglets. Mean values of $l_{stride}{}'$ and f' indicated with "Φ" were different from d18. **(C)** Normalized speed (u') of preterm pigs did not differ with birth weight (normal birth weight (P-NORM); low birth weight (P-LBW) and postnatal age. **(D)** Normalized stride length (l'_{stride}, full line) and normalized stride frequency (f', dotted line) according to birth weight (P-NORM vs. P-LBW) and postnatal age. $l_{stride}{}'$ was higher for P-NORM, and f' was overall higher for P-LBW. Mean values of $l_{stride}{}'$ and f' indicated with "*" were different from d18. **(E)** Normalized speed (u') did not differ according to sex of preterm piglets (female: P-FEMALE; male: P-MALE) and postnatal age. **(F)** Normalized stride length (l'_{stride}, full line) and normalized stride frequency (f', dotted line) according to sex (P-MALE vs. P-FEMALE) and postnatal age. There was an interaction of postnatal age and sex for l'_{stride} indicating that l_{stride} was higher for P-FEMALE at d3, 8, and 18, while f' was overall higher in P-MALE. Mean values of indicated with "Φ" differ significantly from d18 for l'_{stride}. Black colors indicate differences in T while gray colors indicate differences in P piglets.

TABLE 4 | Duty factor (*Df*) (mean ± SD; m) according to gestational age when cesarean-section derived (condition) (preterm at gestational age 106 days—term at gestation age 115 days) and postnatal age (3, 4, 5, 8, and 18 days), birth weight (low birth weight (LBW): 0–25th percentile—normal birth weight (NORM): 50–75th percentile), and sex (female vs. male).

	Effect					
	Condition		Birth weight (preterms)		Sex (preterms)	
Age	Preterm	Near term	LBW	NORM	Female	Male
d3	0.64 ± 0.08^{a}	0.68 ± 0.09^{a}	0.60 ± 0.08	0.61 ± 0.09	0.65 ± 0.08	0.63 ± 0.09
d4	$0.63 \pm 0.12^{a,b}$	$0.65 \pm 0.10^{a,b}$	0.64 ± 0.07	0.64 ± 0.12	0.65 ± 0.11	0.62 ± 0.12
d5	$0.63 \pm 0.09^{a,b}$	$0.64 \pm 0.07^{a,b}$	0.62 ± 0.09	0.66 ± 0.09	0.64 ± 0.08	0.63 ± 0.09
d8	$0.63 \pm 0.11^{a,b}$	$0.61 \pm 0.01^{a,b}$	0.61 ± 0.11	0.62 ± 0.12	0.66 ± 0.12	0.61 ± 0.11
d18	0.64 ± 0.07^{b}	0.63 ± 0.05^{b}	0.64 ± 0.04	0.66 ± 0.07	0.65 ± 0.07	0.63 ± 0.06

There was no interaction between condition, sex, or birth weight and postnatal age. Df was higher in P-FEMALE when compared with P-MALE. $^{a-b}$Different superscripts indicate significant differences with postnatal age within condition, birth weight, or sex.

l_{stride}' increased with age in both P-MALE and P-FEMALE (all comparisons with d18, $p < 0.001$) (**Figure 2F**) (data points = 536).

Neuromotor Control—Normalized Gait Characteristics

For *df*, we noted a short window of maturation, evidenced by the larger value at d3, compared to d18 ($p < 0.001$) (**Table 4**). *Df* was lower for P-MALE ($n = 21$), compared to P-FEMALE ($n = 17$) ($p = 0.019$).

The mean value of t_{st}' was similar in both groups except at d3, when it was higher for T piglets ($n = 20$) compared to P piglets ($n = 38$) ($p < 0.001$). Mean t_{sw}' was lower for P than for T piglets at most time points ($p < 0.001$ at d3 and d8, $p = 0.017$, 0.033 at d5 and d18) (**Figure 3A**) (data points = 860). In T piglets, t_{st}' was significantly higher at d3 compared to d18 ($p = 0.002$), while there was no maturation visible in P piglets. t_{sw}' had a longer window of maturation in T piglets, with both d4 and d8 exhibiting significantly higher values compared to d18 ($p < 0.001$, for both). In P piglets, t_{sw}' for d3 was lower when compared with d18 ($p = 0.010$). t_{st}' was higher in P-NORM ($n = 9$) compared to P-LBW ($n = 9$) ($p = 0.034$) (**Figure 3B**) (data points = 240) and for P-FEMALE ($n = 17$) compared to P-MALE ($n = 21$) ($p = 0.001$) (**Figure 3C**) (data points = 536) while t_{sw}' did not differ. As observed in the comparison between T and P piglets, t_{st}' did not show any differences with postnatal age, while the effect of postnatal age on t_{sw}' was confirmed when comparing P-FEMALE with P-MALE ($p = 0.004$).

l_{step}' was higher in T pigs ($n = 20$) compared with P ($n = 38$) pigs ($p < 0.001$) while h_{swmax}' was similar. l_{step}' increased (all comparisons: $p < 0.001$) whereas h_{swmax}' dropped with postnatal age (all comparisons: $p < 0.001$). The latter occurred in a leg-dependent manner: h_{swmax}' was higher at d3, d4, d5, and d8, compared to d18 for LF, RF, and LF. For RF, d8 did not differ from d18 anymore. l_{step}' was overall higher for P-NORM ($n = 9$) ($p < 0.001$) while h_{swmax}' did not differ between P-LBW ($n = 9$) and P-NORM. l_{step}' (d3, d8, d18; $p < 0.001$, $p < 0.001$, $p = 0.001$, respectively) and h_{swmax}' ($p = 0.001$) were lower for P-MALE ($n = 21$), compared to P-FEMALE ($n = 17$) (**Table 5**).

Gait Symmetry

P piglets ($n = 38$) showed a higher AIL ($p < 0.001$) and AISL (only the front legs, $p < 0.001$), compared to T piglets ($n = 20$). AIF, AIST, AISW, and AIDF did not differ between P and T piglets. There was some maturation visible for several of the asymmetry variables (similar in T and P piglets). AIL was significantly higher at d3, d4, and d5 compared to d18 ($p = 0.008$, 0.019, 0.028, respectively) (**Figure 4A**) (data points = 430). A similar observation was seen when looking at P-MALE ($n = 21$) and P-FEMALE ($n = 17$) ($p = 0.031$) (**Figure 4**) (data points = 268) but not in the preterm pigs belonging to selected birth weight categories (**Figure 4**) ($n = 18$, data points = 120). Higher values for AISL were noted at d3, d4, and d8, compared to d18 ($p = 0.008$, 0.005, 0.011, respectively) (**Figure 4D**). For AISW and AIDF, only d8 was significantly higher than d18 ($p = 0.002$, 0.002, respectively). Only one of the asymmetry indices differed between P-LBW ($n = 9$) and P-NORM ($n = 9$) piglets. AIF was lower for P-LBW at d4 and d18 ($p = 0.028$, 0.041, respectively). None of the asymmetry indices were different between P-MALE (n = 21) and P-FEMALE ($n = 17$) piglets (**Figure 4**).

DISCUSSION

The Effect of Premature Birth

As expected, both body weight and HLL were lower in P compared to T piglets during the first week of postnatal life. This indicates that when the preterms are born, they are indeed smaller due to a shortened period of growing *in utero*. However, by d18 this difference disappeared, showing that P piglets catch up with T piglets after a week.

When looking at motor performance (measured by *u*), P and T piglets performed equally well. This was somewhat surprising since, given their lower muscle mass and lesser muscle anabolic response (31), we expected that preterm pigs would be overall slower. However, it is possible, that, relative to total body weight, their muscle mass is equally (or more) developed. We have found this to be true for low birth weight piglets in our previous studies (24, 32), and this is also the case in preterm piglets vs. their term counterparts at 26 days of age (14). Thus, their muscle mass might allow them to produce enough force to keep up

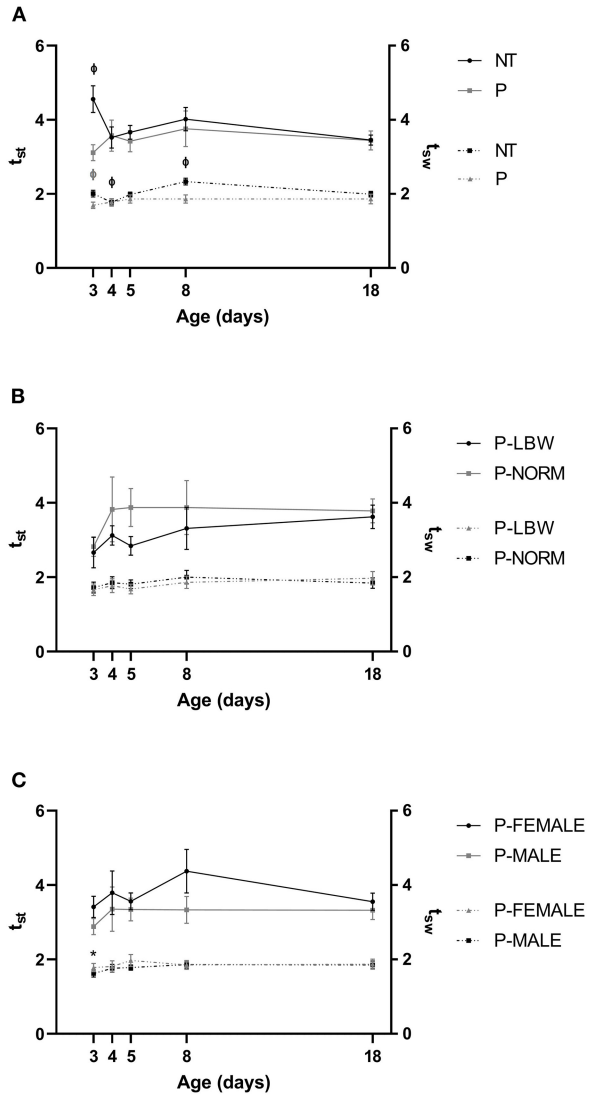


FIGURE 3 | Normalized stance and swing duration. All values are mean ± SE. **(A)** Normalized stance duration (t'_{st}; full line) and swing duration (t'_{sw}; dotted line) according to condition (P: preterm, gray; T: term, black) and age. Mean values for t'_{st} at d3 and t_{sw}' at d3, d5, d8, and d18 were higher in T than in P. Mean values of t'_{st} and t_{sw}' indicated with "Φ" were different from d18. **(B)** Normalized stance duration (t'_{st}; full line) and swing duration (t'_{sw}; dotted line) according to birth weight (normal birth weight (P-NORM); low birth weight (P-LBW) and postnatal age. t'_{st} was higher in P-NORM compared to P-SGA. **(C)** Normalized stance duration (t'_{st}; full line) and swing duration (t'_{sw}; dotted line) according to sex (female: P-FEMALE; male: P-MALE) and postnatal age. t'_{st} was higher in P-FEMALE compared to P-MALE. Mean values of t_{sw}' indicated with "*" were different from d18. Black colors indicate differences in T while gray colors indicate differences in P piglets.

their motor performance (voluntary self-selected speed), which would translate into a higher frequency that counterbalances the smaller strides of P piglets. Additionally, the lack of a difference in motor performance might indicate that gait performance has little to do with post-conceptional age in late gestation, but more with postnatal age and environmental factors. Not only did both groups remain in a heated incubator for 5 days before being transferred to an open cage, but also they received the same combination of enteral/parenteral food (relative to body weight), taking a possible difference in feed intake or route of administration (enteral vs. parenteral) that may affect gait development (10, 32) out of the equation.

Overall neuromotor maturation (u') was not delayed in P piglets and reached maturity between d5 and d8 in both P and T piglets. This supports our hypothesis that postnatal age and environment, rather than post-conceptional age, affect neuromuscular development. At first sight, our results are surprising, because other studies have demonstrated that preterm pigs show motor coordination delays during the first weeks after birth (10, 14, 33). According to Andersen et al. (14), these delays are generally shorter than the reduction in gestation length, which indicates that preterm pigs do show some developmental plasticity. In their study, piglets were born 12 days preterm, but basic motor function (first time standing up and first time walking), locomotion, and balance/coordination scores suggested delays of 2, 5, and 11 days, respectively. A delay of 2–3 days in basic motor function in preterm vs. term piglets was confirmed by Obelitz-Ryom et al. (11). In this study, the majority of both preterm and term piglets were standing up and walking by the third postnatal day. A delay in the onset of walking (1.43–2.16 months) is seen in preterm infants (6) and rabbits (34). In rabbits, the lower motor score observed in preterm (28 days of post-conceptional age) vs. term (31 days post-conceptional age) newborns corresponded with lower neuron densities in the former (34). As such, it is likely that we "missed" these delays in our motor performance and neuromotor development data, because recordings were only made from day 3 onward and precocial animals show a relative mature brain and neuromuscular functions by the end of gestation (10, 35).

When looking into the neuromotor skills (normalized gait characteristics), P and T piglets have different strategies to reach a mature neuromotor performance if we consider normalized speed as the proxy for the latter. This differing repertoire is in line with several studies in preterm infants, for example, (1, 6). Different patterns of neuromotor skills' development were observed in our study: 1) no maturation in P piglets (f', t'_{st}), 2) slightly longer maturation (l'_{stride}, t'_{st}), or 3) similar pattern of maturation (df, l'_{step}) when compared to T piglets. Based on these results, one might consider preterm pigs to suffer from a "stunted" maturation for certain aspects of the gait that is compensated for by other neuromotor skills. This is reflected in a differently looking gait at the age of 18 days in P piglets irrespective of their body dimensions: P piglets take shorter steps (indicated by shorter l'_{step}, shorter l'_{stride}, shorter time between footfalls shown by shorter t'_{sw}) at a higher frequency (indicated by an overall higher f') than T piglets. In preterm infants during childhood, a shorter stride length was also reported (6). As neuromotor maturation is completed by d18 (23, 24), it is likely that this difference in neuromotor skills (gait characteristics) between P and T piglets remains during the rest of their life. A follow-up study in adult pigs is needed

TABLE 5 | ormalized step length (l_{step}') and maximal swing height (h_{swmax}') (mean ± SD; m) according to gestational age when cesarean-section derived (preterm at gestational age 106 days—term at gestation age 115 days) and postnatal age (3, 4, 5, 8, and 18 days), birth weight (low birth weight (LBW): 0–25th percentile—normal birth weight (NORM): 50–75th percentile), and sex (female vs. male).

	Effect					
	Condition		Birth weight (preterms)		Sex (preterms)	
Age	Preterm	Near term	LBW	NORM	Female	Male
l_{step}'						
d3	0.53 ± 0.14[a]	0.65 ± 0.12[a]	0.36 ± 0.09[a]	0.55 ± 0.14[a]	0.59 ± 0.11[a]	0.48 ± 0.14[a]
d4	0.55 ± 0.12[a]	0.69 ± 0.15[a]	0.51 ± 0.12[a]	0.58 ± 0.14[a]	0.57 ± 0.13[a]	0.53 ± 0.11[a]
d5	0.58 ± 0.13[a]	0.71 ± 0.12[a]	0.49 ± 0.13[a]	0.62 ± 0.13[a]	0.59 ± 0.11[a]	0.58 ± 0.14[a]
d8	0.62 ± 0.15[a]	0.78 ± 0.15[a]	0.53 ± 0.13[a]	0.67 ± 0.18[a]	0.68 ± 0.12[a]	0.59 ± 0.15[a]
d18	0.75 ± 0.09[b]	0.84 ± 0.12[b]	0.74 ± 0.10[b]	0.76 ± 0.08[b]	0.78 ± 0.11[b]	0.72 ± 0.07[b]
h_{swmax}'						
d3	0.14 ± 0.05[a]	0.14 ± 0.04[a]	0.15 ± 0.06[a]	0.14 ± 0.05[a]	0.15 ± 0.05[a]	0.13 ± 0.05[a]
d4	0.14 ± 0.05[a]	0.13 ± 0.04[a]	0.13 ± 0.05[a]	0.13 ± 0.04[a]	0.14 ± 0.05[a]	0.14 ± 0.05[a]
d5	0.14 ± 0.05[a]	0.14 ± 0.04[a]	0.13 ± 0.05[a]	0.14 ± 0.06[a]	0.16 ± 0.06[a]	0.13 ± 0.05[a]
d8	0.12 ± 0.05[a]	0.14 ± 0.04[a]	0.13 ± 0.05[a]	0.13 ± 0.05[a]	0.13 ± 0.06[a]	0.12 ± 0.04[a]
d18	0.09 ± 0.04[b]	0.09 ± 0.03[b]	0.08 ± 0.03[b]	0.08 ± 0.03[b]	0.10 ± 0.05[b]	0.09 ± 0.04[b]

l_{step}' was higher in T pigs compared with P pigs, in P-NORM compared to P-SGA and in P-FEMALE compared with P-MALE. l_{step}' differed with postnatal age. h_{swmax}' was higher in P-FEMALE when compared with P-MALE and dropped with postnatal age. [a–b]Different superscripts within a column indicate significant differences with postnatal age within condition, birth weight, or sex.

to confirm this as in a more detailed study focusing on 3–5 postnatal age. Such a study—including challenges such as hurdles and treadmills—can provide more insight in the "gait repertoire" of preterm vs. term pigs of which this study hints that this is differing.

Taking a closer look at gait variability, we compare the balance/coordination results of Andersen et al. (14) with our results, which are strikingly different. Where they suggest a delay of 11 days for preterm pigs, compared to term pigs, we see no difference between groups in achieving a symmetrical gait pattern. The most likely explanation is the different way of studying balance/coordination between Andersen et al. (14) and our study. Where we digitized and calculated AIs, they relied on scoring by the experimenters. We observed that both T and P pigs took a fairly long time to achieve a symmetrical gait, with some variables only reaching a stable value between d8 and 18. This was expected for P piglets. However, we expected T piglets to have an already mature state at d3, as we know from a previous study that term piglets achieve gait symmetry within the first day after birth (23, 24). Why this is not the case in this study might be explained by the different rearing conditions and "exercise" in these studies, where in Vanden Hole et al. (23, 24) the piglets were vaginally delivered, did not receive artificial feeding, and remained with the sow in a farm environment. The postnatal environment also proved its importance in rat pups, which suffer from abnormal locomotion that lasted even until adulthood when rats were subjected to both prenatal (i.e., intrauterine hypoperfusion) and postnatal (i.e., sensorimotor restriction) insults (36). Studies on gait symmetry (and stability) in human preterms are inconclusive on whether or not a difference exists and whether this is maintained throughout development (6).

The Effect of LBW

The body weight of both groups of preterm piglets increased over time, but the difference in body weight at birth between P-LBW and P-NORM piglets was maintained, indicating that P-LBW piglets do not show catch-up growth, relative to their normal-size preterm littermates, within the first 18 days. However, this difference in body weight resulting from fetal growth restriction did not affect motor performance, indicating that, relative to total body weight, muscle mass in P-LBW and P-NORM piglets is similar. It could be expected that in P-LBW piglets the energy reserve at birth is lower, as we also found in term LBW pigs (32). In term LBW pigs, this is probably the main reason for the lower performance in growth-restricted animals (24). However, in our current study, the lesser energy reserves are replenished rapidly by the artificial rearing and pigs are assessed at a later age.

P-LBW and P-NORM piglets also show the same pattern of neuromotor maturation in all of the investigated variables. With regard to f', l_{stride}', and l_{step}', a difference in neuromotor skill was detected. However, it must be noted that by d18 differences are reduced to a minimum, implying that the gait looks the same in P-LBW and P-NORM. The lack of visible differences in neuromotor maturation is possibly due to the lack of video recordings during the first 3 days, a period where other studies have reported delays in basic neuromotor skill (11, 14). Most of the pigs in these studies were capable of standing up and walking within the first 3 days after birth. In addition, the delays in first-time standing up and first-time walking were only 15 and 17 h, respectively. In order to register these fairly short delays, a future study should keep in mind that time points for gait measurements should be closer together and start at the time of first-time walking. In a previous study, detailing neuromotor development in the first 4 days in low birth weight vs. normal birth weight term

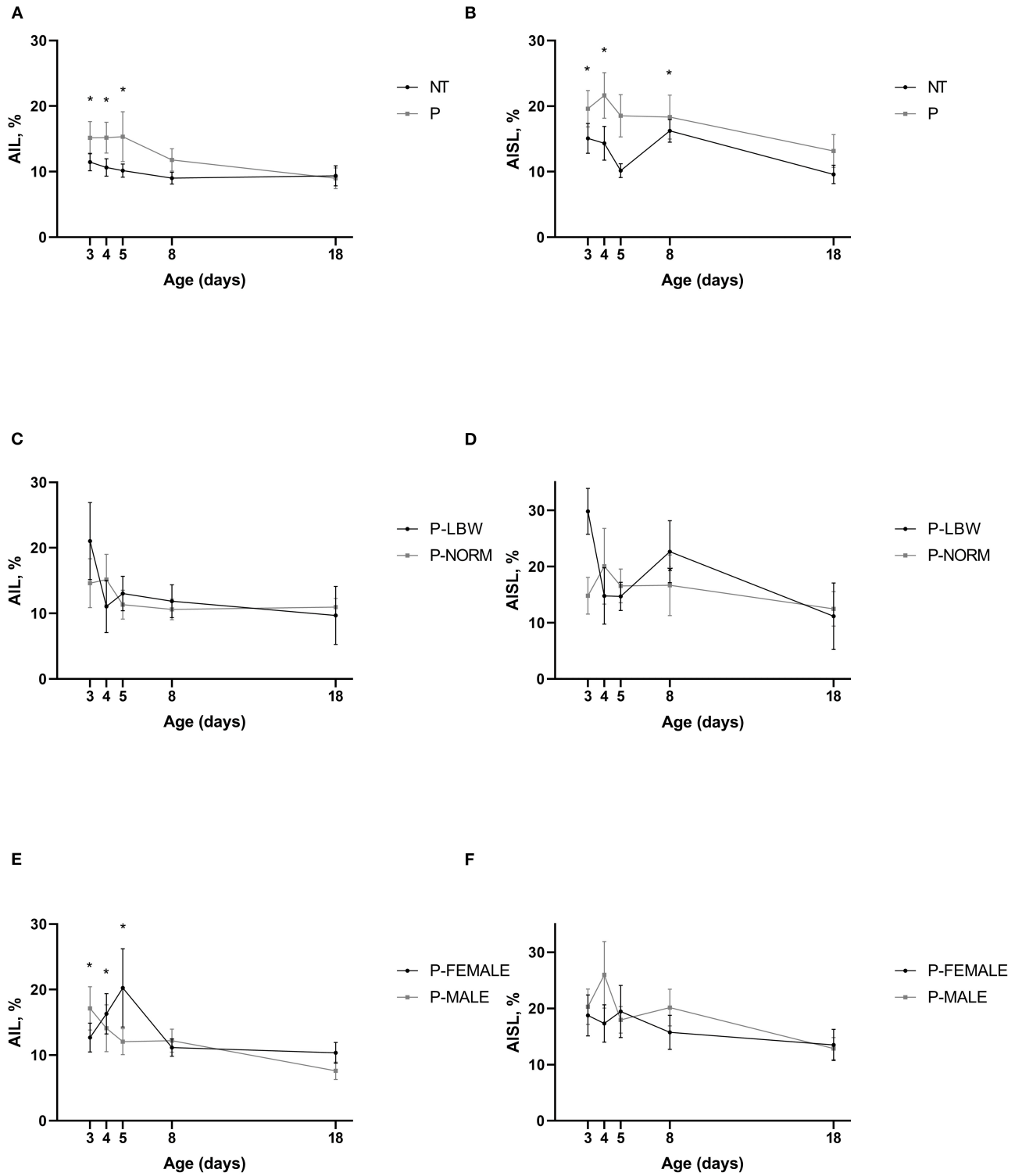


FIGURE 4 | Gait symmetry. All values are mean ± SE. Asymmetry index stride length (AIL) according to postnatal age and **(A)** condition (P: preterm, gray; T: term, black), **(B)** birth weight (normal birth weight (P-NORM); low birth weight (P-LBW), and **(C)** sex (female: P-FEMALE; male: P-MALE). AIL is higher in P than in T piglets. Mean values indicated with * differ significantly from d18. Asymmetry index step length (AISL) according to postnatal age and **(D)** condition (P: preterm, gray; T: term, black), **(E)** birth weight (normal birth weight (P-NORM); low birth weight (P-LBW), and **(F)** sex (female: P-FEMALE; male: P-MALE). AISL is higher in P than in T piglets (front leg). Mean values indicated with * differ significantly from d18.

piglets, we found no differences in time of maturation, but we did find a difference in neuromotor skill (more specifically, f', t_{sw}', and t_{st}') (24). With the exception of AIF, gait variability was the same in P-LBW and P-NORM piglets. These results are in line with previous results on gait variability in low birth weight and normal birth weight term piglets, where also no difference in gait variability was detected (24).

Overall, our results suggest that growth restriction hardly affects gait development in preterm pigs between 3 and 18 days of life when artificially reared.

The Effect of Sex

Body weight and HLL were not different in P-MALE and P-FEMALE piglets across the entire studied period. In agreement with their body dimensions (similar leg length, muscle mass) and the same artificial rearing scheme, motor performance was the same in both groups. Neuromotor maturation shows the same pattern in P-MALE and P-FEMALE piglets, but neuromotor skill often differed between sexes (with the exception of u′). Most variables showed a larger mean value in P-FEMALE, except for f′ which was higher in P-MALES. It is important to keep in mind though that these differences in neuromotor skill do not imply a better or worse gait pattern in either sex. This just means that their gait, relative to their body dimensions, is slightly different, an observation we also saw when comparing P with T piglets. Simply put, whereas P-MALES take more, but shorter, steps to cover a certain distance, P-FEMALES will take fewer, but larger steps. Gait variability was the same in both groups across the entire studied period. These fairly limited differences in gait between sexes are consistent with Bæk et al. (20) and with our previous studies on term piglets (23) where no sex-related differences regarding overall motor performance were reported.

Relevance

Piglets born at 90% gestation are often considered to have an overall survival capacity and gut function of 28–30-week-old infants [see review by (8)], while the developing brain may be more similar to 34–37 week old infants, the so-called "late preterm infants" (14, 33). In agreement with this, the development of the skeletal and nervous systems is considered to be slightly faster in newborn piglets than in babies, as reviewed elsewhere (37, 38). As such, our results will be most relevant for the latter category of preterm infants. These late preterm infants have only recently become a topic of interest. Although they comprise the bulk part of preterm births [considering the increasing number of elective cesarean sections (39), they remained fairly unrepresented in studies because, compared to early preterm infants, their deficiencies (after birth and later in life) seemed less severe (1, 2, 4, 6, 40). However, it has become clear that late preterm infants experience a substantial mortality and neonatal morbidity [see review by (41)]. Compared to term infants, they exhibit higher rates of temperature instability, respiratory distress, hypoglycemia, jaundice, etc. (42–44). Also, later in life, these infants experience difficulties in speaking, writing, mathematics, behavior, and physical education and are at higher risk for motor impairment (1, 2, 4, 6, 45). In our study, we put the preterm piglet forward to study the effect of late prematurity on neuromotor skill development. Our results show that the precocious nature of the pig with regard to neuromotor development is preserved in case of preterm birth since most of the effects of prematurity resolved by days 3–5. Thus, when studying effects of gestational age, birth weight, sex, and postpartum environment (nutritional interventions, exercise) on neuromotor development using the preterm piglet, the focus should lay on the first days after delivery. It must be considered that pigs delivered prematurely were not able to stand and walk within the first hours after life. It was only on day 3 when most of the piglets included in this study were able to walk and therefore be recorded. This may be a limitation of this model, since the first postnatal hours are critical in the development of neuromotor skills. In addition, the results show that neuromuscular development—as seen for other organs systems—is highly plastic and capable to catch up, showing that postnatal rather than post-conceptional age is the main driver. This indicates that there is a window of opportunity to optimize the neuromotor performance in the case of prematurity. In addition, the gait of the preterm pig shares many characteristics (shorter stride length, delayed-onset first walking, reduced repertoire) with that of the human infant, putting the preterm pig at the forefront as a translational model.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author/s.

ETHICS STATEMENT

The animal study was reviewed and approved by National Ethics Committee on Animal Experimentation (protocol no. 2012-15-2934-00193).

AUTHOR CONTRIBUTIONS

TT and PS conceived and designed the piglet experiment. PA, CVand, and CVan conceived and designed the gait analysis experiment. CVand collected the data and performed the analysis. CVan, SV, PA, TT, and PS contributed the data and/or analysis tools. All authors discussed the results and contributed to the final manuscript. CVand, CVan, and MA provided the graphs and translated these discussions into the paper.

FUNDING

This work was supported by the University of Antwerp (grant number GOA-33927). The experimental work was funded by Arla Foods Ingredients and the Innovation Fund Denmark (NEOMUNE) grant. The authors declare that this study received funding from Arla Foods Ingredients. The funder was not

involved in the study design, collection, analysis, interpretation of data, the writing of this article or the decision to submit it for publication.

ACKNOWLEDGMENTS

Authors are grateful to the funding bodies.

REFERENCES

1. Hadders-Algra M. Two distinct forms of minor neurological dysfunction: perspectives emerging from a review of data of the Groningen Perinatal Project. *Dev Med Child Neurol.* (2002) 44:561–71. doi: 10.1017/S0012162201002560
2. Kinney HC. The near-term (late preterm) human brain and risk for periventricular leukomalacia: a review. *Semin Perinatol.* (2006) 30:81–8. doi: 10.1053/j.semperi.2006.02.006
3. Fallang B, Hadders-Algra M. Postural behavior in children born preterm. *Neural Plast.* (2005) 12:175–82; discussion 263–72. doi: 10.1155/NP.2005.175
4. van Hoorn JF, Schoemaker MM, Stuive I, Dijkstra PU, Rodrigues Trigo Pereira F, van der Sluis CK, et al. Risk factors in early life for developmental coordination disorder: a scoping review. *Dev Med Child Neurol.* (2021) 63:511–9. doi: 10.1111/dmcn.14781
5. Oudgenoeg-Paz O, Mulder H, Jongmans MJ, van der Ham IJM, Van der Stigchel S. The link between motor and cognitive development in children born preterm and/or with low birth weight: a review of current evidence. *Neurosci Biobehav Rev.* (2017) 80:382–93. doi: 10.1016/j.neubiorev.2017.06.009
6. Albesher RA, Spittle AJ, McGinley JL, Dobson FL. Gait characteristics of children born preterm. *Neoreviews.* (2019) 20 e397–e408. doi: 10.1542/neo.20-7-e397
7. Mudd AT, Dilger RN. Early-life nutrition and neurodevelopment: use of the piglet as a translational model. *Adv Nutr.* (2017) 8:92–104. doi: 10.3945/an.116.013243
8. Sangild PT, Thymann T, Schmidt M, Stoll B, Burrin DG, Buddington RK. Invited review: the preterm pig as a model in pediatric gastroenterology. *J Anim Sci.* (2013) 91:4713–29. doi: 10.2527/jas.2013-6359
9. Holme Nielsen C, Bladt Brandt A, Thymann T, Obelitz-Ryom K, Jiang P, Vanden Hole C, et al. Rapid postnatal adaptation of neurodevelopment in pigs born late preterm. *Dev Neurosci.* (2018) 40:586–600. doi: 10.1159/000499127
10. Cao M, Andersen AD, Van Ginneken C, Shen RL, Petersen SO, Thymann T, et al. Physical activity level is impaired and diet dependent in preterm newborn pigs. *Pediatr Res.* (2015) 78:137–44. doi: 10.1038/pr.2015.73
11. Obelitz-Ryom K, Bering SB, Overgaard SH, Eskildsen SF, Ringgaard S, Olesen JL, et al. Bovine milk oligosaccharides with sialyllactose improves cognition in preterm pigs. *Nutrients.* (2019) 11:1335. doi: 10.3390/nu11061335
12. Lind NM, Moustgaard A, Jelsing J, Vajta G, Cumming P, Hansen AK. The use of pigs in neuroscience: modeling of brain disorders. *Neurosci Behav Rev.* (2007) 31:728–51. doi: 10.1016/j.neubiorev.2007.02.003
13. Dobbing J, Sands J. Comparative aspects of the brain growth spurt. *Early Hum Dev.* (1979) 3:79–83. doi: 10.1016/0378-3782(79)90022-7
14. Andersen AD, Sangild PT, Munch SL, van der Beek EM, Renes IB, Van Ginneken C, et al. Delayed growth, motor function and learning in preterm pigs during early postnatal life. *Am J PhysiolRegul Integr Comp Physiol.* (2016) 310:R481–92. doi: 10.1152/ajpregu.00349.2015
15. Dominici N, Ivanenko YP, Cappellini G, D'Avella A, Mondi V, Cicchese M, et al. Locomotor primitives in newborn babies and their development. *Science.* (2011) 334:997–9. doi: 10.1126/science.1210617
16. Lacquaniti F, Ivanenko YP, Zago M. Development of human locomotion. *Curr Opin Neurobiol.* (2012) 22:822–8. doi: 10.1016/j.conb.2012.03.012
17. Ivanenko YP, Poppele RE, Lacquaniti F. Motor control programs and walking. *Neuroscientist.* (2006) 12:339–48. doi: 10.1177/1073858406287987
18. Ivanenko YP, Poppele RE, Lacquaniti F. Five basic muscle activation paterns account for muscle activity during human locomotion. *J Physiol.* (2004) 556:267–82. doi: 10.1113/jphysiol.2003.057174
19. Hamer EG, Bos AF, Hadders-Algra M. Specific characteristics of abnormal general movements are associated with functional outcome at school age. *Early Hum Dev.* (2016) 95:9–13. doi: 10.1016/j.earlhumdev.2016.01.019
20. Bæk O, Cilieborg MS, Nguyen DN, Bering SB, Thymann T, Sangild PT. Sex-specific survival, growth, immunity and organ development in preterm pigs as models for immature newborns. *Front Pediatr.* (2021) 9:626101. doi: 10.3389/fped.2021.626101
21. Blauw-Hospers CH, Hadders-Algra M. A systematic review of the effects of early intervention on motor development. *Dev Med Child Neurol.* (2005) 47:421–32. doi: 10.1017/S0012162205000824
22. Yu JJ, Burnett AF, Sit CH. Motor skill interventions in children with developmental coordination disorder: a systematic review and meta-analysis. *Arch Phys Med Rehabil.* (2018) 99:2076–99. doi: 10.1016/j.apmr.2017.12.009
23. Vanden Hole C, Goyens J, Prims S, Fransen E, Ayuso Hernando M, Van Cruchten S, et al. How innate is locomotion in precocial animals? A study on the early development of spatio-temporal gait variables and gait symmetry in piglets. *J Exp Biol.* (2017) 220:2706–16. doi: 10.1242/jeb.157693
24. Vanden Hole C, Cleuren S, Van Ginneken C, Prims S, Ayuso M, Van Cruchten S, et al. How does intrauterine crowding affect locomotor performance in newborn pigs? A study of force generating capacity and muscle composition of the hind limb. *PLoS ONE.* (2018) 13:e0209233. doi: 10.1371/journal.pone.0209233
25. Kristensen S, Salihu HM, Keith LG, Kirby RS, Fowler KB, Pass MA. SGA subtypes and mortality risk among singleton births. *Early Hum Dev.* (2007) 83:99–105. doi: 10.1016/j.earlhumdev.2006.05.008
26. Davis NM, Ford GW, Anderson PJ, Doyle LW. Developmental coordination disorder at 8 years of age in a regional cohort of extremely-low-birthweight or very preterm infants. *Dev Med Child Neurol.* (2007) 49:325–30. doi: 10.1111/j.1469-8749.2007.00325.x
27. Aylward GP. Neurodevelopmental outcomes of infants born prematurely. *J Dev Behav Pediatr.* (2014) 35:394–407. doi: 10.1097/01.DBP.0000452240.39511.d4
28. Alexander R, Jayes A. A dynamic similarity hypothesis for the gaits of quadrupedal mammals. *J Zool.* (1983) 201:135–52. doi: 10.1111/j.1469-7998.1983.tb04266.x
29. Robinson RO, Herzog W, Nigg BM. Use of force platform variables to quantify the effects of chiropractic manipulation on gait symmetry. *J Manipulative Physiol Ther.* (1987) 10:172–6.
30. Meijer E, van Nes A, Back W, van der Staay FJ. Clinical effects of buprenorphine on open field behaviour and gait symmetry in healthy and lame weaned piglets. *Vet J.* (2015) 206:298–303. doi: 10.1016/j.tvjl.2015.10.016
31. Naberhuis J, Suryawan A, Nguyen H, Hernandez-Garcia A, Fiorotto M, Stoll B, et al. Prematurity blunts feeding-induced stimulation of translation initiation signaling and protein synthesis in muscle of neonatal piglets. *Faseb J.* (2018) 31:141. doi: 10.1096/fasebj.31.1_supplement.141.8
32. Vanden Hole C, Ayuso M, Aerts P, Prims S, Van Cruchten S, Van Ginneken C. Glucose and glycogen levels in piglets that differ in birth weight and vitality. *Heliyon.* (2019) 5:e02510. doi: 10.1016/j.heliyon.2019.e02510
33. Bergström A, Kaalund SS, Skovgaard K, Andersen AD, Pakkenberg B, Rosenørn A, et al. Limited effects of preterm birth and the first enteral nutrition on cerebellum morphology and gene expression in piglets. *Physiol Rep.* (2016) 4:e12871. doi: 10.14814/phy2.12871
34. van der Merwe J, van der Veeken L, Ferraris S, Gsell W, Himmelreich U, Toelen J, et al. Early neuropathological and neurobehavioral consequences of preterm birth in a rabbit model. *Sci Rep.* (2019) 9:3506. doi: 10.1038/s41598-019-53779-x
35. Pond WG, Mersmann HJ. *Biology of the Domestic Pig*. London: Comstock Pub Associates; Cornell University Press (2001).
36. Coq JO, Kochmann M, Lacerda DC, Khalki H, Delcour M, Toscano AE, et al. From cerebral palsy to developmental coordination disorder: development of preclinical rat models corresponding to recent epidemiological changes.

Ann Phys Rehabil Med. (2019) 63:422–30. doi: 10.1016/j.rehab.2019.10.002
37. M3(R2); ICH EIMR. Guidance on non-clinical safety studies for the conduct of human clinical trials and marketing authorization for pharmaceuticals. In: *International Conference on Harmonisation*. Japan (2009).
38. Ayuso M, Buyssens L, Stroe M, Valenzuela A, Allegaert K, Smits A, et al. The neonatal and juvenile pig in pediatric drug discovery and development. *Pharmaceutics.* (2020) 13:44. doi: 10.3390/pharmaceutics13010044
39. Davidoff MJ, Dias T, Damus K, Russell R, Bettegowda VR, Dolan S, et al. Changes in the gestational age distribution among U.S. singleton births: impact on rates of late preterm birth, 1992 to 2002. *Semin Perinatol.* (2006) 30:8–15. doi: 10.1053/j.semperi.2006.01.009
40. Kramer MS, Demissie K, Yang H, Platt RW, Sauvé R, Liston R. The contribution of mild and moderate preterm birth to infant mortality. Fetal and Infant Health Study Group of the Canadian Perinatal Surveillance System. *JAMA.* (2000) 284:843–9. doi: 10.1001/jama.284.7.843
41. Saigal S, Doyle LW. An overview of mortality and sequelae of preterm birth from infancy to adulthood. *Lancet.* (2008) 371:261–9. doi: 10.1016/S0140-6736(08)60136-1
42. Raju TN. The problem of late-preterm (near-term) births: a workshop summary. *Pediatr Res.* (2006) 60:775–6. doi: 10.1203/01.pdr.0000246074.73342.1e
43. Wang ML, Dorer DJ, Fleming MP, Catlin EA. Clinical outcomes of near-term infants. *Pediatrics.* (2004) 114:372–6. doi: 10.1542/peds.114.2.372
44. Escobar GJ, McCormick MC, Zupancic JA, Coleman-Phox K, Armstrong MA, Greene JD, et al. Unstudied infants: outcomes of moderately premature infants in the neonatal intensive care unit. *Arch Dis Child Fetal Neonatal Ed.* (2006) 91:F238–44. doi: 10.1136/adc.2005.087031
45. Huddy CL, Johnson A, Hope PL. Educational and behavioural problems in babies of 32–35 weeks gestation. *Arch Dis Child Fetal Neonatal Ed.* (2001) 85:F23–8. doi: 10.1136/fn.85.1.F23

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's Note: All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

frontiers in Pediatrics

ORIGINAL RESEARCH
published: 30 November 2021
doi: 10.3389/fped.2021.754989

Adrenal Steroid Metabolism and Blood Pressure in 5- to 7-Year-Old Children Born Preterm as Compared to Peers Born at Term

Eva Landmann[1], Markus Brugger[2,3,4,5], Verena Blank[6], Stefan A. Wudy[7,8], Michaela Hartmann[7,8], Konstantin Strauch[2,3,4,5] and Silvia Rudloff[7,9]*

[1] Department of Pediatric Hematology and Oncology, Center of Child and Adolescent Medicine, Justus Liebig University Giessen, Giessen, Germany, [2] Institute of Medical Biometry and Epidemiology, Philipps-University Marburg, Marburg, Germany, [3] Institute of Genetic Epidemiology, Helmholtz Zentrum München - German Research Center for Environmental Health, Neuherberg, Germany, [4] Institute of Medical Information Processing, Biometry and Epidemiology - IBE, Chair of Genetic Epidemiology, LMU Munich, Munich, Germany, [5] Institute of Medical Biostatistics, Epidemiology and Informatics (IMBEI), University Medical Center, Johannes Gutenberg University, Mainz, Germany, [6] Department of Neuropediatrics, Center of Child and Adolescent Medicine, Justus Liebig University Giessen, Giessen, Germany, [7] Department of Pediatrics and Neonatology, Justus Liebig University Giessen, Giessen, Germany, [8] Steroid Research and Mass Spectrometry Unit, Division of Pediatric Endocrinology and Diabetology, Center of Child and Adolescent Medicine, Justus-Liebig-University Giessen, Giessen, Germany, [9] Institute of Nutritional Science, Justus Liebig University Giessen, Giessen, Germany

OPEN ACCESS

Edited by:
Lynette Kay Rogers, The Research Institute at Nationwide Children's Hospital, United States

Reviewed by:
Viral Jain, University of Alabama at Birmingham, United States
Herbert Kurz, Clinic Donaustadt, Austria

****Correspondence:***
Eva Landmann
eva.landmann@paediat.med.uni-giessen.de

Specialty section:
This article was submitted to Neonatology, a section of the journal Frontiers in Pediatrics

Received: *07 August 2021*
Accepted: *27 October 2021*
Published: *30 November 2021*

Citation:
Landmann E, Brugger M, Blank V, Wudy SA, Hartmann M, Strauch K and Rudloff S (2021) Adrenal Steroid Metabolism and Blood Pressure in 5- to 7-Year-Old Children Born Preterm as Compared to Peers Born at Term. Front. Pediatr. 9:754989. doi: 10.3389/fped.2021.754989

Background: Previous studies indicated preterm birth to be a risk factor for hypertension in adolescence and adulthood. However, studies in children investigating the underlying mechanisms are scarce.

Objective: We hypothesized children born preterm to have higher excretion of cortisol and/or androgen metabolites per day concomitantly with higher blood pressure as compared to peers born at term. We thus aimed to compare urinary steroid profiles and blood pressure between 5- to 7-year-old children born preterm and peers born at term. Furthermore, aldosterone precursor excretion per day was compared between both groups.

Methods: Blood pressure was measured in 236 children (preterms $n = 116$; gestational age 29.8 ± 2.6 (30; 24–33) weeks [mean ± standard deviation (median; range)]) using an automatic oscillometric device. Urinary steroid profiles were determined in 24-h urine samples (preterms $n = 109$; terms $n = 113$) using gas chromatographic-mass spectrometric analysis. To assess excretion of cortisol and androgen metabolites per day, major cortisol and androgen metabolites were summed, respectively. To assess aldosterone excretion per day tetrahydrocorticosterone, 5α-tetrahydrocorticosterone, and tetrahydro-11-deydrocorticosterone were summed.

Results: Multiple regression analyses showed prematurity to be associated with systolic but not with diastolic blood pressure. When adjusted for potential confounders (prematurity, gender, age at day of examination, being born small for gestational age, breastfeeding, accelerated weight gain during infancy, family history of cardiovascular disease, parental hypertension, and body mass index) prematurity was shown to be associated with an increase in systolic blood pressure by 2.87 mmHg (95% confidence

interval 0.48–5.27; $p = 0.02$). Cortisol, androgen metabolite, and aldosterone precursor excretion per day were not higher in individuals born preterm. In contrast to our hypothesis, multiple regression analysis showed prematurity to independently decrease cortisol and aldosterone precursor excretion per day ($p < 0.001$ and 0.04, respectively).

Conclusion: This study provides further evidence for systolic blood pressure to be higher after preterm birth as early as at the age of 5 to 7 years. However, this seems not to be explained by elevated excretion of cortisol and/or androgen metabolites.

Keywords: blood pressure, preterm, prepubertal, steroid metabolism, preterm birth, cortisol, adrenal steroid, developmental origin of adult disease

INTRODUCTION

Several studies indicate higher blood pressure (BP) values in adolescents and in adults born preterm (1–9). Data on blood pressure before adolescence in children born preterm as compared to children born at term are comparatively scarce. Two groups (10, 11) described higher BP in toddlers born preterm as compared to toddlers born at term. Some reports on BP in school-age children born preterm had been restricted to specific groups, such as individuals born small for gestational age (SGA) (12) or born at an extremely low gestational age (13). Only recently, data on blood pressure in 5- to 7-year-old children born preterm as compared to peers born at term were published (14–17). Further data are needed to elucidate whether, already at early school-age, individuals born preterm exhibit differences in BP as compared to peers born at term as reported in most—but not all—of these recent studies.

The underlying mechanisms that could lead to higher blood pressure as a sequela of preterm birth are not fully understood.

Increased BP after preterm birth might derive from altered hypothalamus-pituitary-adrenal (HPA) axis function. A stressful perinatal period—as it is experienced by children born preterm—might modify HPA axis functions (18, 19) with subsequent alterations in cortisol metabolism.

The aim of the present study was to compare (a) adrenal steroid metabolism and (b) BP between 5 and 7-year-old children born at a gestational age of 33 weeks or below (preterm group) and a group of children born at a gestational age between 37 and 41 weeks (term group). We hypothesized children born preterm to have higher excretion of cortisol and/or androgen than their peers born at term. We also hypothesized children born preterm to have higher BP as compared to children born at term.

To comprehensively describe adrenal steroid metabolism, we further measured the excretion of dihydroepiandrosterone (DHEA) and of aldosterone precursors per day [as urinary excretion of mineralocorticoids per day (ME/d)] in children born preterm and in children born at term by performing urinary steroid profiles in 24-h urine samples using gas chromatographic-mass spectrometric analysis.

Abbreviations: AE/d, urinary excretion of androgen metabolites per day; BMI, body mass index; BP, blood pressure; BSA, body surface area; CE/d, urinary excretion of cortisol per day; DBP, diastolic blood pressure; DHEA, dehydroepiandrosterone; HPA, hypothalamic-pituitary-adrenal axis; ME/d, urinary excretion of mineralocorticoids per day; OR, odds ratio; SBP, systolic blood pressure; SGA, small for gestational age.

SUBJECTS AND METHODS

Study Population and Ethics

All children who participated in the study were between 5 and 7 years old at the day of examination. Preterm children of a gestational age of 33 weeks or below were included in the study (preterm group). The control group comprised children of a gestational age between 37 and 41 weeks (term group). Children were recruited from the region of Hesse, Germany. We aimed to motivate as many parents of children born preterm as possible to participate in the study. Therefore, we chose several ways to recruit children born preterm. In Germany, one way to comprehensively circulate information to parents is the obligatory health examination before school entry, which is performed by the local public health services. The health examination is obligatory for all children, including mentally and/or physically handicapped children. All public health services within the radius of 120 km distributed information on the study to parents of children born preterm when they showed up for their obligatory health examination. In addition, pediatric offices within the same circumference were asked to distribute information on the study to the parents of children born preterm. Children born at term were also recruited through the obligatory health examinations before school entry as well as via newspaper advertisements. Recruitment and characteristics of both groups have been previously described in detail (20).

Exclusion criteria for all children were: type-1 diabetes, chromosomal abnormalities, major disability (gross motor function classification system > II) (21), chronic illness, and systemic corticosteroid therapy. All children had to be prepubertal, i.e., each child had to be Tanner stage I as ascertained by the study physician. Gestational age was taken from the hospital birth records. Detailed data concerning pregnancy, perinatal history, previous medical history, nutrition, growth, and weight gain were taken from the documents of the obligatory well-child visits and were complemented by information obtained from medical records. Additional information on the child's history as well as on family history was obtained by a structured interview at the day of examination.

Being born SGA was defined as a birth weight below the 10th percentile according to the percentiles of Voigt (22) for German newborns. Accelerated weight gain during infancy was defined as an increase in the percentile values of more than 25 percentiles between birth and the first birthday. A history of parental hypertension was ascertained if at least one parent reported to receive antihypertensive medication. A family history of cardiovascular disease was ascertained if at least one parent or grandparent had suffered coronary heart disease and/or myocardial infarction and/or stroke.

The study was approved by the local research ethics committee and written parental consent was obtained.

Anthropometry

Body weight and length were measured as recommended by Stolzenberg et al. (23) and body mass index (BMI) was calculated (24).

BP Measurements

After a 10-min rest, with the subject in a sitting position, BP was measured from the right upper arm three times with an interval of at least two min using an automatic device (Welch Allyn®, 52000 Series, Akanetateles Falls, New York, NY, USA). The measurements were taken by a trained study nurse. The cuff was chosen depending on the size of the child's upper arm as recommended by the American Academy of Pediatrics (25). The arithmetic means of three measurements for systolic and diastolic BP (SBP and DBP) were included in the analysis.

Urinary Adrenal Steroid Profiles in 24-H Urine Samples by Gas Chromatography-Mass Spectrometry

The study participants collected a 24-h urine sample for quantification of adrenal steroid hormone metabolites. The 24-h urine sample was collected within two weeks after the study visit.

Urinary steroid profiles were analyzed using quantitative data that were generated by gas chromatography-mass spectrometry (Agilent Technologies 6890) analysis as described previously (26, 27).

To assess urinary cortisol excretion per day (CE/d), the seven major urinary glucocorticoid metabolites, i.e., tetrahydrocortisol, 5α-tetrahydrocortisol, α-cortol, β-cortol, tetrahydrocortisone, α-cortolone, and β-cortolone were summed (27).

To assess urinary overall androgen metabolite excretion of the adrenals per day (AE/d), the sum of androsterone, etiocholanolone, 5-androstene-3β, 17α-diol, 5-androstene-3β,17β-diol, DHEA, 16α-hydroxy-DHEA, and androstenetriol-16α was calculated (26).

To assess aldosterone precursor excretion per day (ME/d), tetrahydrocorticosterone, 5α-tetrahydrocorticosterone, and tetrahydro-11-deydrocorticosterone were summed.

Absolute amounts of metabolites, sums, and ratios calculated were divided by the absolute amount of 24-h urinary creatinine excretion. For comparisons between term and preterm born children, these values were additionally divided by BMI or body surface area (BSA). Furthermore, these comparisons were done separately for boys and girls, because reference values for urinary steroid metabolites differ between both genders (26, 27).

Statistical Methods

For continuous data, two sample *t*-tests were applied in case of a normal distribution, Mann-Whitney U tests when the values were not distributed normally. Because data on urinary steroid profiles can contain an appreciable number of zero values, we applied a two-part permutation test (28). Dichotomous variables were compared using Fisher's exact test.

Since being born SGA is known to be associated with the development of metabolic and cardiovascular disease later in life, all analyses were also performed excluding children with SGA.

Further, multiple regression analyses were calculated to assess the influence of prematurity on blood pressure and urinary steroid profiles. The regressions were performed as follows.

(1) Multiple regression analyses were performed to describe the influence of prematurity on SBP and DBP. Initially, the following variables were included in the full model: prematurity, gender, age at day of examination, SGA, breastfeeding, accelerated weight gain during infancy, family history of cardiovascular disease, parental hypertension, and BMI.

(2) Multiple regression analyses were calculated to assess the influence of prematurity on CE/d, on AE/d, on ME/d, and on DHEA/d. Log-transformation was performed for the outcomes CE/d, AE/d, and ME/d, but not for DHEA/d, for which regression diagnostics showed that the residuals of the full model with DHEA/d as log-transformed outcome variable were not normally distributed. Therefore, we applied a Box-Cox transformation (29) to DHEA/d in order to obtain normally distributed residuals. In addition to the variable prematurity, the variables gender, age at examination, and being born SGA were included as potential confounders in the full model. In a secondary analysis, either BSA or BMI was added to the full model since body fat is known to be an endocrine organ.

(3) Backward elimination based on Akaike's information criterion (AIC) was chosen as the model selection algorithm to identify the best-fitting models based on the full models of (1) and (2). For the model selections regarding urinary steroid profiles in (2), both BSA and BMI were added to the full model.

Prematurity, gender, SGA, breastfeeding, accelerated weight gain during infancy, family history of cardiovascular disease, and parental hypertension were included as dichotomous variables in the regression models. Age at day of examination, BMI, and BSA were included as continuous variables in the regression models.

A p-value < 0.05 was considered statistically significant. Statistical analyses were performed with statistical software package R, version 4.0.3 (30).

RESULTS

Baseline Characteristics and Anthropometric Data

A total of 236 children were included in the study. Out of these, 116 children were born preterm, i.e., of a gestational age of 24 to 33 weeks, and 120 children were born at term (37 to 41 weeks of gestational age). Baseline characteristics and anthropometric data of both groups are summarized in **Table 1**.

TABLE 1 | Baseline characteristics of children born preterm and children born at term.

	Preterm group ($n = 116$)	Term group ($n = 120$)	p-value	OR/MD[b]	95% CI
Boys/Girls	75/41	67/53	0.18	1.44	0.85; 2.50
Age (years)[a]	6.6 ± 0.8	6.6 ± 0.9	0.98	0.003	−0.21; 0.22
Gestational age (weeks)[a]	29.8 ± 2.6	39.4 ± 1.2	< 0.001	−9.67	−10.20; −9.16
Birth weight (grams)[a]	1,434 ± 470	3,486 ± 484	< 0.001	−2,052	−2,174; −1,930
SGA	9/116	8/120	0.80	1.18	0.39; 3.65
Body weight (kg)[a]	21.8 ± 4.7	24.3 ± 5.2	< 0.001	−2.58	−3.85; −1.30
Body height (cm)[a]	120 ± 8	123 ± 8	< 0.001	−3.69	−5.66; −1.73
BMI (kg/m^2)[a]	15.1 ± 1.9	15.9 ± 2.0	0.003	−0.79	−1.29; −0.28
Family history of cardiovascular disease	19/116	18/120	0.86	1.11	0.54; 2.31
Parental hypertension	23/116	10/120	0.01	0.37	0.15; 0.86
BMI father (kg/m^2)[a]	26.9 ± 3.9	26.5 ± 3.4	0.48	0.34	−0.61; 1.29
BMI mother (kg/m^2)[a]	26.3 ± 6.7	25.3 ± 4.7	0.20	0.99	−0.52; 2.49

[a] *Presented as means and standard deviation.*
[b] *For dichotomous variables, the odds ratio (OR) is given as effect estimate, whereas the mean difference (MD) is provided for quantitative variables.*

Blood Pressure

SBP and DBP were on average higher in preterm-born children than in term-born children, although the differences were not statistically significant. However, after excluding SGA, a statistically significant difference was found for DBP between the two groups (**Table 2**).

Multiple regression analysis ($n = 221$; with $n = 109$ and $n = 112$ for preterm and term born children, respectively) showed prematurity and BMI to be associated with higher SBP (effect estimates: prematurity 2.87, 95% confidence interval (CI) 0.48–5.27; $p = 0.02$; BMI 1.31, CI 0.77–1.85; $p < 0.001$, adjusted r-squared 0.095). Thus, the variable "being born prematurely" increased SBP by 2.87 mmHg when corrected for potential confounders. After backward elimination, only the variables prematurity and BMI remained in the model (effect estimate for prematurity 3.01, CI 0.97–5.05; $p = 0.004$, and 1.32, CI 0.81–1.83; $p < 0.001$ for BMI; adjusted r-squared 0.11).

For DBP as the dependent variable, no significant association with any predictor was found when performing multiple regression analyses.

Adrenal Steroid Profiling in 24-H Urine Samples

24-h urine samples were available from a total of 222 children. **Table 3** summarizes data characterizing the adrenal steroid metabolism.

CE/d

In boys, no significant differences in CE/d were found between those born preterm and those born at term, regardless of whether the values were divided by BMI or BSA. In girls, higher CE/d was shown in those born at term as compared to girls born preterm, regardless of whether the values were divided by BMI or BSA. For all children (data not shown), higher values were shown for children born at term as compared to those born preterm. The differences described remained significant after exclusion of children born SGA. Multiple regression analysis ($n = 221$) showed the variable prematurity to independently decrease urinary CE/d ($p < 0.001$). Moreover, when BMI or BSA were additionally included in the model, the variable prematurity still significantly decreased urinary CE/d ($p < 0.001$ and $p = 0.006$, respectively). The optimal model according to the AIC included both prematurity and BSA ($p = 0.004$ for prematurity and $p = 0.01$ for BSA; adjusted r-squared 0.08).

ME/d

In boys, in girls, and in all children (data not shown), no significant differences with respect to ME/d were found between children born preterm and children born at term, regardless of whether the values were divided by BMI or BSA or children born SGA were excluded from the analyses.

Multiple regression analyses showed the variable prematurity to significantly decrease ME/d ($p = 0.04$). This effect was slightly lower when either BMI ($p = 0.06$) or BSA ($p = 0.09$) were added to the model. After backward elimination, only prematurity remained in the model ($p = 0.03$ and adjusted r-squared 0.016).

AE/d and DHEA/d

In all children, whether analyzed separately as boys and girls or independent of their gender (data not shown), no significant differences with respect to AE/d or DHEA/d were found between children born preterm and children born at term, regardless of whether the values were divided by BMI or BSA or children born SGA were excluded from the analyses. Multiple regression analyses also showed no influence of the variable prematurity on AE/d or DHEA/d.

DISCUSSION

We compared BP and urinary steroid profiles between 5 and 7-year-old children born preterm and peers born at term.

Study Population

We defined children of a gestational age of <34 completed weeks as our group of children born preterm since we aimed to include

TABLE 2 | Raw blood pressure values in children born preterm and children born at term.

		Preterm group ($n = 116$)	Term group ($n = 120$)	Difference [95% CI]	p value
Systolic blood pressure [mmHg][a]	All children	107.6 ± 8.2	105.8 ± 7.7	1.82 [−0.23; 3.87]	0.08
	SGA excluded	107.6 ± 8.0	105.8 ± 7.8	1.86 [−0.25; 3.98]	0.08
Diastolic blood pressure [mmHg][a]	All children	66.5 ± 6.2	65.0 ± 5.3	1.47 [0; 2.94]	0.05
	SGA excluded	66.6 ± 6.2	65.1 ± 5.2	1.58 [0.06; 3.11]	0.04

[a] *Presented as means and standard deviation.*

TABLE 3 | Adrenal steroid metabolites in children born preterm and children born at term.

		Boys			Girls		
		Preterm group ($n = 70$)	Term group ($n = 64$)	P^b	Preterm group ($n = 39$)	Term group ($n = 49$)	P^b
CE/d[a]	SGA included	3.71 (0.96–9.80)	3.75 (1.23–18.89)	0.21	3.28 (0.98–14.46)	4.81 (1.17–14.44)	0.02
	SGA excluded[†]	3.79 (0.96–9.80)	3.75 (1.23–18.89)	0.20	3.28 (0.98–14.46)	4.84 (1.17–14.44)	0.03
ME/d[a]	SGA included	0.44 (0.09–1.24)	0.46 (0.10–2.66)	0.59	0.42 (0.12–2.09)	0.51 (0.13–1.69)	0.19
	SGA excluded[†]	0.45 (0.09–1.24)	0.46 (0.10–2.66)	0.55	0.42 (0.12–2.09)	0.52 (0.12–1.69)	0.15
AE/d[a]	SGA included	0.24 (0.02–1.76)	0.24 (0.06–1.51)	0.85	0.25 (0.05–1.59)	0.38 (0.04–1.41)	0.14
	SGA excluded[†]	0.25 (0.02–1.76)	0.25 (0.06–1.51)	0.99	0.25 (0.05–1.59)	0.38 (0.04–1.41)	0.15
DHEA/d[a]	SGA included	0.01 (0.00–0.50)	0.01 (0.00–0.11)	0.71	0.01 (0.00–0.47)	0.02 (0.00–0.11)	0.70
	SGA excluded[†]	0.01 (0.00–0.50)	0.01 (0.00–0.11)	0.71	0.01 (0.00–0.47)	0.02 (0.00–0.09)	0.65

[a] *Values are divided by body mass index and are given in μg/mg urinary creatinine secretion per day. Data are presented as median and range.*
[b] *P value of the two-part permutation test.*
[†] *When SGA excluded in boys: preterm group n = 65 and term group n = 60; when SGA excluded in girls: preterm group n = 35 and term group n = 45.*

an extended spectrum of children born preterm, but also to have a group of preterm children that clearly differed from children born at term. We therefore included children born extremely and moderately preterm. Kramer et al. (31) defined moderate preterm birth as being born at a gestational age of 32 and 33 completed gestational weeks. A gestational age of 34 weeks and more has been suggested to define the cutoff for "late-preterm" (32, 33). The same cutoff has been used by Sipola-Leppänen et al. (5) who reported increased BP in adolescent girls born preterm.

We chose the narrow age span of 5–7 years to investigate BP and steroid metabolism between individuals born preterm and individuals born at term in order to minimize age-related variability and to thus enable better comparability of the values obtained. Further, we examined children younger than 8 years in order to study children that were unambiguously prepubertal and in whom adrenarche, i.e., adrenal androgen secretion, had not started yet.

Blood Pressure

In our group of 5-to 7-year-old children, prematurity was shown to be associated with an increase in SBP by 2.87 mmHg when adjusted for potential confounders. Differences in DBP, however, can mostly be explained by confounders and hence are not directly related to prematurity.

Our findings are in line with recent studies in late preschool and early school age children born preterm that report higher blood pressure readings as compared to age-matched children born at term (14, 17) and/or an increased prevalence of hypertension in preterm born children (15).

In conjunction with studies reporting higher BP in toddlers born preterm (10, 11), it may be assumed that in children born preterm, BP starts to deviate from normal BP at a young age and continues to deviate throughout childhood and adolescence. This is in line with a report on an inverse relationship between gestational age and BP in 9–12-year-old children (34).

The underlying mechanisms of increased BP values in individuals born preterm, however, have not yet been clarified. Several associations have been described: Singhal et al. (35) reported breast milk consumption to be associated with lower BP in adolescents born prematurely. Vohr et al. (36) reported higher weight gain within the first three years of life to be associated with higher BP in adolescents born preterm, while Bonamy et al. (10) described an inverse association between post-neonatal weight gain and BP in children born preterm at 2.5 years of age.

In our group of children born preterm, multiple regression analyses showed neither breastfeeding nor accelerated weight gain during infancy to be associated with SBP at the age of 5 to 7 years. Furthermore, multiple regression analyses did not show associations between SBP and the variables being born SGA, gender, cardiovascular disease in the family, and parental hypertension, thus supporting the hypothesis that high SBP in adolescents and adults born preterm has a perinatal origin, primarily linked to low gestational age.

Adrenal Steroid Profiling in 24-H Urine Samples

We hypothesized differences in BP between individuals born preterm and individuals born at term to be explained by

differences in cortisol metabolism: A stressful perinatal period as it is experienced by children born preterm might result in programming of the HPA axis (18, 19) resulting in increased secretion of cortisol and its metabolites. Subclinical hypercortisolism is associated with increased BP (37, 38) and might thus mediate the link between prematurity and higher BP values in later life. Positive correlations between BP and serum cortisol levels measured at the age of 2 years were reported in a subgroup of boys with very low birthweight (39).

Urinary Cortisol Excretion in 24-H Urine Samples

In contrast to our hypothesis, prematurity was not associated with higher CE/d, but with significantly lower CE/d in preterms as compared to their peers born at term.

Recently, other groups also studied cortisol in former preterm children compared to children born at term of a similar age group as in our study, i.e., 6–7 years: Watterberg et al. (40) also hypothesized preterm-born children to have increased cortisol compared to term-born children. They measured salivary cortisol levels four times during a study visit and in addition three specimen were obtained at home to analyze diurnal patterns. In contrast to their hypothesis, they reported similar cortisol levels in preterm-born children compared to term-born children but with a blunted morning cortisol.

Brummelte et al. (41) measured cortisol levels in three saliva samples collected during a study visit in which children underwent cognitive testing. In addition, cortisol was measured in eight saliva samples collected at home over two consecutive days. They described similar cortisol profiles in preterm and full-term children except for cortisol levels measured at bedtime. Bedtime cortisol levels were higher in the preterm group.

Cortisol levels were also studied in preterm-born children that were younger or older than 6–7 years. Studies performed in younger children had indicated elevated cortisol secretion (42) in individuals born preterm. At the age of 18 months corrected for gestational age, Grunau et al. (42) measured higher cortisol concentrations in a cohort of children born at low gestational age as compared to peers born at term. Studies in preterm-born children studied at an older age than the children examined in our study also hint at higher cortisol secretion: Buske-Kirschbaum et al. (43) showed higher cortisol concentrations after awakening in 18 preterm children aged 8–14 years as compared to 18 control children. In morning urine samples obtained from 36 children aged 8–11 years who were born preterm, Gohlke et al. (44) reported higher cortisol, cortisol metabolites and adrenal androgens as compared to 36 peers born at term.

Taken together, data on cortisol secretion in children born preterm as compared to peers born at term are inconsistent. At least, data obtained from 6–7-year-old children, do not point to higher cortisol secretion in preterm-born children as compared to term-born children. However, studies performed in older children rather indicate increased cortisol secretion in former preterms.

Differences with respect to the age groups studied, the specific study populations, the study designs as well as the methods applied for cortisol measurements do not allow unconfined comparisons between all those studies. For example, the age ranges of 8–11 years (44) or even 8–14 years (43) encompass adrenarche and puberty which might influence cortisol metabolism. Moreover, different methods for measuring cortisol can be used. Measurements in saliva reflect cortisol production at selected points in time (40–43). Urinary steroid profiling as applied by Gohlke et al. (44) in morning urine samples and in our study in 24-h urinary specimens, however, represent integrated measurements, i.e., excretion rates of cortisol and thus allow for the assessment of hormonal production rates.

Data obtained from adults are inconsistent, i.e., in a prospective follow-up study including 147 30-year-olds born at term and 311 subjects of the same age group born preterm, Dalziel et al. (1) could not show an association between prematurity and early morning serum cortisol levels. In addition—and in accordance with our results—preterm birth was shown to be associated with increased SBP. In a comparatively small group, Walker et al. (45) showed lower total urinary cortisol metabolite excretion in 22- to 25-year-old women who were born preterm and appropriate for gestational age as compared to peers born at term.

Urinary Androgen Metabolite, DHEA and Aldosterone Precursor Secretion

Adrenal hyperandrogenism and higher ME/d might also explain higher BP values in children born preterm. We also studied AE/d, DHEA/d separately, and ME/d in our group.

In our study, prematurity was not associated with higher AE/d or DHEA/d. In contrast to our hypothesis, prematurity was even associated with lower ME/d.

A few studies also compared adrenal androgens between individuals born preterm and born at term. In contrast to our study, their results rather indicate higher adrenal androgen secretion. Meuwese et al. (46) measured higher DHEA concentrations in serum from young adults born preterm. Watterberg et al. (40) measured salivary DHEA in 5–7-year-old children born preterm as compared to children born at term. In morning urine samples obtained from 36 children aged 8–11 years who were born preterm, Gohlke et al. (44) reported higher cortisol and cortisol metabolites and adrenal androgens as compared to 36 peers born at term. Again, the study population as well as the method applied, do not allow an unconfined comparison with our results. Firstly, the chosen age range of 8–11 years is an age that encompasses adrenarche and enhanced adrenarche in the group of children born preterm may influence the results. Secondly, although urinary steroid metabolites were quantified using gas chromatography-mass spectrometry, morning urine samples reflect adrenal steroid metabolism less precisely than 24-h urine samples.

We did not expect CE/d and ME/d to be even lower in children born preterm than in children born at term. The underlying biological mechanism remains unclear. One might speculate that the adrenal cortex remains smaller after preterm birth just as it had been shown for kidney size (47). A reduced adrenal cortex area might affect adrenal steroid metabolism.

Also, one might speculate that prolonged exposure to stress as it is often experienced by the preterm neonate during the perinatal period might lead to a hypoactive rather than a hyperactive HPA axis function. This hypothesis is supported by the finding of lower HPA axis responses in an adult preterm cohort during stress-test (48). Consistent with the results of that study, our results argue against a major role of HPA axis and adrenal steroid metabolism in explaining increased BP values found in individuals born preterm. We speculate that other mechanisms, e.g., reduced nephron endowment [reviewed in (49) and (50)] or variations in capillary density as recently described by Lewandowski et al. (51) underlie higher BP values measured in individuals born preterm. Exposure to hyperoxia and oxidative stress in the neonatal period might also contribute to the increased risk of increased BP in neonates born preterm as reviewed in (52).

Strengths and Limitations of the Study

Strengths of the current study include the completeness of data on BP measurements and comprehensiveness of data on urinary adrenal steroid profiles. Furthermore, urinary adrenal steroid profiling in 24-h urinary specimens represent integrated measurements, i.e., excretion rates, and thus allow for the assessment of hormonal production rates. This is in contrast to most studies revealing determinations of steroid concentration in blood or saliva at selected time points only. A further strength of the study is the comparably large group of children born preterm. To the best of our knowledge, urinary steroid profiles have not been studied in larger groups of preterm-born children of that age group. Moreover, we consider the choice of a narrow age range in prepubertal children as an advantage of the study. Limitations of the study are the cross-sectional study design and the fact that neonatal data have not been collected prospectively.

Summary and Perspective

Our data indicate that—as early as at the age of 5 to 7 years—individuals born preterm seem to be at increased risk for exhibiting higher SBP as compared to peers born at term. This might have implications for their cardiovascular health later in life.

However, our results do not support the hypothesis that elevations in adrenal steroid metabolites explain higher SBP values found in the group of children born preterm. We speculate that other mechanisms or a combination thereof contribute to the increased hypertension risk in individuals born preterm. The identification of possible contributors remains crucial since it might enable the implementation of pre- and postnatal strategies to prevent long-term cardiovascular morbidity.

DATA AVAILABILITY STATEMENT

The dataset presented in this article is not readily available for reasons of data protection. Requests to access the dataset should be directed to the corresponding author.

ETHICS STATEMENT

This studies involving human participants were reviewed and approved by Ethic Committee of the Faculty of Medicine, Justus-Liebig-University Giessen, Giessen, Germany. Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin.

AUTHOR CONTRIBUTIONS

EL conceptualized and designed the study and drafted the initial manuscript. MB and KS carried out the statistical analyses and contributed to the draft of the manuscript. VB carried out the initial data collection with regard to medical examinations and initial analyses. As part of her doctoral thesis she contributed to the initial draft of the manuscript. SW and MH signed responsible for the analyses of steroid metabolites in urine including the interpretation of the data. SR was a co-author of the funding proposal, she thus substantially contributed to the study design, coordination of the project, and critically revised and reviewed the manuscript. All authors contributed to the article and approved the submitted version.

FUNDING

This study was supported by the German Research Foundation (DFG grant no. LA 2428/1-1).

ACKNOWLEDGMENTS

We thank the study nurses, Sandra Stephan and Susanne Stingl, for their thoughtful handling. We also thank the public health services, the pediatricians, and physiotherapists for their support in recruiting the children. We especially wish to thank the children and their families for participating in this study.

REFERENCES

1. Dalziel SR, Parag V, Rodgers A, Harding JE. Cardiovascular risk factors at age 30 following pre-term birth. *Int J Epidemiol.* (2007) 36:907–15. doi: 10.1093/ije/dym067
2. Keijzer-Veen MG, Dulger A, Dekker FW, Nauta J, van der Heijden BJ. Very preterm birth is a risk factor for increased systolic blood pressure at a young adult age. *Pediatr Nephrol.* (2010) 25:509–16. doi: 10.1007/s00467-009-1373-9
3. de Jong F, Monuteaux MC, van Elburg RM, Gillman MW, Belfort MB. Systematic review and meta-analysis of preterm birth and later systolic blood pressure. *Hypertension.* (2012) 59:226–34. doi: 10.1161/HYPERTENSIONAHA.111.181784
4. Parkinson JR, Hyde MJ, Gale C, Santhakumaran S, Modi N. Preterm birth and the metabolic syndrome in adult life: a systematic review and meta-analysis. *Pediatrics.* (2013) 131:e1240–63. doi: 10.1542/peds.2012-2177
5. Sipola-Leppänen M, Vääräsmäki M, Tikanmäki M, Hovi P, Miettola S, Ruokonen A, et al. Cardiovascular risk factors in adolescents born preterm. *Pediatrics.* (2014) 134:e1072–e81. doi: 10.1542/peds.2013-4186
6. Sipola-Leppänen M, Karvonen R, Tikanmäki M, Matinolli HM, Martikainen S, Pesonen AK, et al. Ambulatory blood pressure and

its variability in adults born preterm. *Hypertension.* (2015) 65:615–21. doi: 10.1161/HYPERTENSIONAHA.114.04717
7. Skudder-Hill L, Ahlsson F, Lundgren M, Cutfield WS, Derraik JGB. Preterm birth is associated with increased blood pressure in young adult women. *J Am Heart Assoc.* (2019) 8:e012274. doi: 10.1161/JAHA.119.012274
8. Cheong JLY, Haikerwal A, Wark JD, Irving L, Garland SM, Patton GC, et al. Cardiovascular health profile at age 25 years in adults born extremely preterm or extremely low birthweight. *Hypertension.* (2020) 76:1838–46. doi: 10.1161/HYPERTENSIONAHA.120.15786
9. Crump C, Sundquist J, Sundquist K. Risk of hypertension into adulthood in persons born prematurely: a national cohort study. *Eur Heart J.* (2020) 41:1542–50. doi: 10.1093/eurheartj/ehz904
10. Bonamy AK, Kallen K. Norman M. High blood pressure in 25-year-old children born extremely preterm. *Pediatrics.* (2012) 129:e1199–204. doi: 10.1542/peds.2011-3177
11. Duncan AF, Heyne RJ, Morgan JS, Ahmad N, Rosenfeld CR. Elevated systolic blood pressure in preterm very-low-birth-weight infants </=3 years of life. *Pediatr Nephrol.* (2011) 26:1115–21. doi: 10.1007/s00467-011-1833-x
12. Willemsen RH, de Kort SW, van der Kaay DC, Hokken-Koelega AC. Independent effects of prematurity on metabolic and cardiovascular risk factors in short small-for-gestational-age children. *J Clin Endocrinol Metab.* (2008) 93:452–8. doi: 10.1210/jc.2007-1913
13. Bolton CE, Stocks J, Hennessy E, Cockcroft JR, Fawke J, Lum S, et al. The EPICure study: association between hemodynamics and lung function at 11 years after extremely preterm birth. *J Pediatr.* (2012) 161:595–601.e2. doi: 10.1016/j.jpeds.2012.03.052
14. Posod A, Odri Komazec I, Kager K, Pupp Peglow U, Griesmaier E, Schermer E, et al. Former very preterm infants show an unfavorable cardiovascular risk profile at a preschool age. *PLoS ONE.* (2016) 11:e0168162. doi: 10.1371/journal.pone.0168162
15. Vohr BR, Heyne R, Bann C, Das A, Higgins RD, Hintz SR, et al. High blood pressure at early school age among extreme preterms. *Pediatrics.* (2018) 142:e20180269. doi: 10.1542/peds.2018-0269
16. Vollsaeter M, Halvorsen T, Markestad T, Oymar K, Ueland PM, Meyer K, et al. Renal function and blood pressure in 11 year old children born extremely preterm or small for gestational age. *PLoS ONE.* (2018) 13:e0205558. doi: 10.1371/journal.pone.0205558
17. Edstedt Bonamy AK, Mohlkert LA, Hallberg J, Liuba P, Fellman V, Domellof M, et al. Blood pressure in 6-year-old children born extremely preterm. *J Am Heart Assoc.* (2017) 6:e005858. doi: 10.1161/JAHA.117.005858
18. Seckl JR, Meaney MJ. Glucocorticoid Programming. *Ann N Y Acad Sci.* (2004) 1032:63–84. doi: 10.1196/annals.1314.006
19. Finken MJ, van der Voorn B, Hollanders JJ, Ruys CA, de Waard M, van Goudoever JB, et al. Programming of the hypothalamus-pituitary-adrenal axis by very preterm birth. *Ann Nutr Metab.* (2017) 70:170–4. doi: 10.1159/000456040
20. Huke V, Rudloff S, Brugger M, Strauch K, Berthold LD, Landmann E. Prematurity is not associated with intra-abdominal adiposity in 5- to 7-year-old children. *J Pediatr.* (2013) 163:1301–6. doi: 10.1016/j.jpeds.2013.06.035
21. Palisano R, Rosenbaum P, Walter S, Russell D, Wood E, Galuppi B. Development and reliability of a system to classify gross motor function in children with cerebral palsy. *Dev Med Child Neurol.* (1997) 39:214–23. doi: 10.1111/j.1469-8749.1997.tb07414.x
22. Voigt M, Schneider KT, Jährig K. Analysis of a 1992 birth sample in Germany. 1: new percentile values of the body weight of newborn infants. *Geburtshilfe Frauenheilkd.* (1996) 56:550–8. doi: 10.1055/s-2007-1023283
23. Stolzenberg H, Kahl H, Bergmann KE. Body measurements of children and adolescents in Germany. Results of the German health interview and examination survey for children and adolescents (KiGGS.) *Bundesgesundheitsblatt Gesundheitsforschung Gesundheitsschutz.* (2007) 50:659–569. doi: 10.1007/s00103-007-0227-5
24. Quetelet LA. A treatise on man and the development of his faculties. 1842. *Obes Res.* (1994) 2:72–85. doi: 10.1002/j.1550-8528.1994.tb00047.x
25. National High Blood Pressure Education Program Working Group on High Blood Pressure in Children and Adolescents. The fourth report on the diagnosis, evaluation, and treatment of high blood pressure in children and adolescents. *Pediatrics.* (2004) 114(Suppl. 2):555–76. doi: 10.1542/peds.114.2.S2.555
26. Remer T, Boye KR, Hartmann MF, Wudy SA. Urinary markers of adrenarche: reference values in healthy subjects, aged 3-18 years. *J Clin Endocrinol Metab.* (2005) 90:2015–21. doi: 10.1210/jc.2004-1571
27. Wudy SA, Hartmann MF, Remer T. Sexual dimorphism in cortisol secretion starts after age 10 in healthy children: urinary cortisol metabolite excretion rates during growth. *Am J Physiol Endocrinol Metab.* (2007) 293:E970–6. doi: 10.1152/ajpendo.00495.2006
28. Neuhäuser M, Boes T, Jöckel KH. Two-part permutation tests for DNA methylation and microarray data. *BMC Bioinform.* (2005) 6:35. doi: 10.1186/1471-2105-6-35
29. Box GEP, Cox DR. An analysis of transformations. *J R Stat Soc Series B Stat Methodol.* (1964) 26:211–52. doi: 10.1111/j.2517-6161.1964.tb00553.x
30. R Core Team. *R: A Language and Environment for Statistical* Computing. Vienna: R Foundation for Statistical Computing (2020).
31. Kramer MS, Demissie K, Yang H, Platt RW, Sauve R, Liston R. The contribution of mild and moderate preterm birth to infant mortality. Fetal and infant health study group of the canadian perinatal surveillance system. *JAMA.* (2000) 284:843–9. doi: 10.1001/jama.284.7.843
32. Engle WA. A recommendation for the definition of "late preterm" (near-term) and the birth weight-gestational age classification system. *Semin Perinatol.* (2006) 30:2–7. doi: 10.1053/j.semperi.2006.01.007
33. Raju TN, Higgins RD, Stark AR, Leveno KJ. Optimizing care and outcome for late-preterm (near-term) infants: a summary of the workshop sponsored by the National Institute of Child Health and Human Development. *Pediatrics.* (2006) 118:1207–14. doi: 10.1542/peds.2006-0018
34. Inomata S, Yoshida T, Koura U, Tamura K, Hatasaki K, Imamura H, et al. Effect of preterm birth on growth and cardiovascular disease risk at school age. *Pediatr Int.* (2015) 57:1126–30. doi: 10.1111/ped.12732
35. Singhal A, Cole TJ, Lucas A. Early nutrition in preterm infants and later blood pressure: two cohorts after randomised trials. *Lancet.* (2001) 357:413–9. doi: 10.1016/S0140-6736(00)04004-6
36. Vohr BR, Allan W, Katz KH, Schneider KC, Ment LR. Early predictors of hypertension in prematurely born adolescents. *Acta Paediatr.* (2010) 99:1812–8. doi: 10.1111/j.1651-2227.2010.01926.x
37. Baudrand R, Campino C, Carvajal CA, Olivieri O, Guidi G, Faccini G, et al. Increased urinary glucocorticoid metabolites are associated with metabolic syndrome, hypoadiponectinemia, insulin resistance and ß cell dysfunction. *Steroids.* (2011) 76:1575–81. doi: 10.1016/j.steroids.2011.09.010
38. Martins LC, Conceicao FL, Muxfeldt ES, Salles GF. Prevalence and associated factors of subclinical hypercortisolism in patients with resistant hypertension. *J Hypertens.* (2012) 30:967–73. doi: 10.1097/HJH.0b013e3283521484
39. de Jong M, Cranendonk A, van Weissenbruch MM. Salivary and serum cortisol and relation to blood pressure in infancy and early childhood in very-low-birth-weight infants. *Pediatr Res.* (2015) 78:476–9. doi: 10.1038/pr.2015.128
40. Watterberg KL, Hintz SR, Do B, Vohr BR, Lowe J, Newman JE, et al. Adrenal function links to early postnatal growth and blood pressure at age 6 in children born extremely preterm. *Pediatr Res.* (2019) 86:339–47. doi: 10.1038/s41390-018-0243-1
41. Brummelte S, Chau CM, Cepeda IL, Degenhardt A, Weinberg J, Synnes AR, et al. Cortisol levels in former preterm children at school age are predicted by neonatal procedural pain-related stress. *Psychoneuroendocrinology.* (2015) 51:151–63. doi: 10.1016/j.psyneuen.2014.09.018
42. Grunau RE, Haley DW, Whitfield MF, Weinberg J, Yu W, Thiessen P. Altered basal cortisol levels at 3, 6, 8 and 18 months in infants born at extremely low gestational age. *J Pediatr.* (2007) 150:151–6. doi: 10.1016/j.jpeds.2006.10.053
43. Buske-Kirschbaum A, Krieger S, Wilkes C, Rauh W, Weiss S, Hellhammer DH. Hypothalamic-pituitary-adrenal axis function and the cellular immune response in former preterm children. *J Clin Endocrinol Metab.* (2007) 92:3429–35. doi: 10.1210/jc.2006-2223
44. Gohlke B, Wudy SA, Stutte S, Bartmann P, Hartmann MF, Woelfle J. Increased steroid excretion in children with extremely low birth weight at a median age of 98 years. *Horm Res Paediatr.* (2015) 84:331–7. doi: 10.1159/000441031
45. Walker BR, Irving RJ, Andrew R, Belton NR. Contrasting effects of intrauterine growth retardation and premature delivery on adult cortisol secretion and metabolism in man. *Clin Endocrinol (Oxf).* (2002) 57:351–5. doi: 10.1046/j.1365-2265.2002.01606.x

46. Meuwese CL, Euser AM, Ballieux BE, van Vliet HA, Finken MJJ, Walther FJ, et al. Growth-restricted preterm newborns are predisposed to functional adrenal hyperandrogenism in adult life. *Eur J Endocrinol.* (2010) 163:681–9. doi: 10.1530/EJE-10-0471
47. Keijzer-Veen MG, Devos AS, Meradji M, Dekker FW, Nauta J, van der Heijden BJ. Reduced renal length and volume 20 years after very preterm birth. *Pediatr Nephrol.* (2010) 25:499–507. doi: 10.1007/s00467-009-1371-y
48. Kaseva N, Wehkalampi K, Pyhala R, Moltchanova E, Feldt K, Pesonen AK, et al. Blunted hypothalamic-pituitary-adrenal axis and insulin response to psychosocial stress in young adults born preterm at very low birth weight. *Clin Endocrinol (Oxf).* (2014) 80:101–6. doi: 10.1111/cen.12251
49. Chong E, Yosypiv IV. Developmental programming of hypertension and kidney disease. *Int J Nephrol.* (2012) 2012:760580. doi: 10.1155/2012/760580
50. Carmody JB, Charlton JR. Short-term gestation, long-term risk: prematurity and chronic kidney disease. *Pediatrics.* (2013) 131:1168–79. doi: 10.1542/peds.2013-0009
51. Lewandowski AJ, Davis EF, Yu G, Digby JE, Boardman H, Whitworth P, et al. Elevated blood pressure in preterm-born offspring associates with a distinct antiangiogenic state and microvascular abnormalities in adult life. *Hypertension.* (2015) 65:607–14. doi: 10.1161/HYPERTENSIONAHA.114.04662
52. Sutherland MR, Bertagnolli M, Lukaszewski MA, Huyard F, Yzydorczyk C, Luu TM, et al. Preterm birth and hypertension risk: the oxidative stress paradigm. *Hypertension.* (2014) 63:12–8. doi: 10.1161/HYPERTENSIONAHA.113.01276

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's Note: All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

frontiers in Pediatrics

ORIGINAL RESEARCH
published: 06 January 2022
doi: 10.3389/fped.2021.684576

Neonates in the Intensive Care Unit: Maternal Health-Related Quality of Life and Depression After Term and Preterm Births

Eva Mautner[1], *Christina Stern*[1]*, *Alexander Avian*[2], *Maria Deutsch*[1], *Wolfgang Schöll*[1] and *Elfriede Greimel*[1]

[1] Department of Obstetrics and Gynecology, Medical University of Graz, Graz, Austria, [2] Institute for Medical Informatics, Statistics and Documentation, Medical University of Graz, Graz, Austria

OPEN ACCESS

Edited by:
Lynette Kay Rogers,
The Research Institute at Nationwide Children's Hospital, United States

Reviewed by:
Fahri Ovalı,
Istanbul Medeniyet University, Turkey
Chutima Roomruangwong,
Chulalongkorn University, Thailand

***Correspondence:**
Christina Stern
christina.stern@medunigraz.at

Specialty section:
This article was submitted to Neonatology, a section of the journal Frontiers in Pediatrics

Received: 23 March 2021
Accepted: 06 December 2021
Published: 06 January 2022

Citation:
Mautner E, Stern C, Avian A, Deutsch M, Schöll W and Greimel E (2022) Neonates in the Intensive Care Unit: Maternal Health-Related Quality of Life and Depression After Term and Preterm Births. Front. Pediatr. 9:684576. doi: 10.3389/fped.2021.684576

Background/Objective: To examine maternal physical and mental health-related quality of life (HRQoL) and depression after early and late preterm and term births in the early postpartum period.

Method: In a prospective pilot study, three groups of women whose newborns had to be treated in the neonatal ward during the immediate postpartum period were established and compared with each other: 20 women with extremely to very preterm birth, 20 with moderate to late preterm birth and 20 women with term birth. All participants completed the Short Form-12 Health Survey (SF-12) to measure HRQoL, and the Edinburgh Postnatal Depression Scale (EPDS) to detect depressive symptoms combined with independently developed questions to evaluate anxiety and psychological distress.

Results: Maternal psychological HRQoL was significantly worse in the very preterm birth group compared to moderate to late preterm birth ($p < 0.001$) and full-term birth groups ($p = 0.004$). There were no differences between the birth groups in depressive symptoms ($p = 0.083$), anxiety ($p = 0.238$), perceived stress ($p = 0.340$) and the general psychological distress values ($p = 0.755$). In the EPDS, the depression screening instrument 30 to 65% were beyond the cut-off-value to detect major depression.

Conclusions: During the early postpartum period, an extensive medical care focussing on acute stress, HRQoL parameters and depression may be a good step to improving maternal well-being.

Keywords: health-related quality of life, depression, preterm birth, neonates, intensive care unit

INTRODUCTION

Worldwide preterm births and the treatment of neonates in the neonatal ward have an impact on the emotional well-being of women. Preterm births are defined as occurring before 37 weeks of gestation. Under-five-year-olds are at the forefront of child mortality worldwide and are associated with serious morbidities and long hospital admissions (1, 2). There is a differentiation between early gestational age infants, in which the risks of mortality and morbidity are much higher than in late preterm infants and infants born at term (3, 4). Several psychological reactions to preterm deliveries include parental acute stress, anxiety, depression, along with poorer family functioning (5–13). Postpartum depression (PPD) is a disorder which frequently affects women in the postnatal period and has consequences for the infant and the whole family. Since there are several treatment options for PPD (14), the recommendation for obstetric care providers is to accomplish an assessment of mood and emotional well-being during the postpartum period for each patient (15, 16). Another option to evaluate mood or well-being besides depression is HRQoL, which includes various dimensions of health and functioning. According to the World Health Organization (WHO) HRQoL represents "an individual's perception of their position in life in the context of the culture and value systems in which they live and in relation to their goals, expectations, standards and concerns (17)." HRQoL scores are implemented in several settings, such as conducting research, monitoring, and reporting or establishing quality improvement strategies. HRQoL is inspired by personal and environmental factors and their interactions, and can be enhanced through individualized support and personal growth opportunities (18–20).

The purpose of this study was to explore differences in maternal HRQoL and depression of mothers after preterm birth whose newborns had to be treated at the NICU, distinguishing between (1) very preterm and (2) moderate to late preterm, compared to (3) term births. We hypothesized that early preterm birth is associated with more depressive symptoms and decreased HRQoL compared to moderate to late preterm and term birth during the early postpartum period.

MATERIALS AND METHODS

This prospective questionnaire pilot survey was conducted in the Obstetrics Department of a major teaching hospital in Austria, which serves as a tertiary referral center. The study was carried out in agreement with the guidelines recommended by the World Medical Association of Helsinki and was endorsed by the local Ethical Committee (Nr.: 28-269 ex 15/16). There were no monetary or other incentives for women's contribution. Recorded informed consent was obtained from all the women.

Inclusion criteria were German-speaking women who had undergone preterm or term birth according to the intended groups (extremely to very preterm 23+0 until 31+6, moderate to late preterm 32 + 0 to 36 + 6 gestational week) or women who had full-term births (>37 weeks of gestation) and were separated from their neonates because of the newborns' need for intensive care at the neonatal ward (NICU). Exclusion criteria were the inability to speak or understand the local language. Inpatient women were contacted during their postnatal stay in the hospital from December 2016 to December 2018. After agreeing to participate, women given the informed consent form and the questionnaire set. Minimum to maximum intended time interval from birth to study inclusion was three to 10 days postpartum.

Ten persons (out of 70) refused to take part in the study: one woman of the very preterm birth group, three of the moderate to late preterm group and six of the term birth group. We compared accessible characteristics between participants (n = 60) and non-participants (n = 10) and found no statistically significant differences on age (p = 0.932) and study groups (p = 0.199).

TABLE 1 | Demographic and medical characteristics of the study population (N = 60).

Maternal characteristics	Mean (SD)	Range
Maternal age	31.45 (5.2)	17–43
Number of children	1.4 (0.7)	1–4
Gestational week at birth	34.2 (5.1)	25–42
Maternal hospitalization before birth	12.6 (15.2)	0–54
Weight of the infants	2,076.4 (1,024.5)	550–3,985
Hours of sleep during a day	5.86 (1.39)	3–10
Perceived participation and control*	43.15 (29.96)	0–100
	Numbers	Percentages
Maternal highest educational level		
Primary school	3	5
Secondary school	17	28.3
Higher education, not university	19	31.7
Higher education, university	21	35
Living situation		
With children	2	3.3
With partner and children	34	56.7
With partner, children and grandparents	7	11.7
With partner	17	28.3
Employment status		
Not employed	7	11.7
Full-time employed	36	60
Part-time employed	17	28.3
Financial situation		
Very satisfying	12	20
Satisfying	44	73.3
Less satisfying	4	6.7

SD, standard deviation.
**Scale from 0 to 100.*

Instruments (Questionnaires)

Women's HRQoL in the past month was evaluated by the German edition of the Short-Form-12 Health Survey (SF-12). It is a worldwide standardized instrument to measure self-assessed HRQoL (21–23). Twelve questions measure the general health perception and general mental condition, physical and social functioning, role limits due to physical health problems

or emotional problems and queries on vitality and bodily pain. Scoring guidelines are available to create standard values with a mean of 50 and a standard deviation of 10 (22, 23). The standardization enables cross-cultural comparison (22, 24). The reliability of all SF-12 scales was 0.88(22).

The Edinburgh Postnatal Depression Scale (EPDS) was applied to determine postnatal depression (25). It is an internationally established 10-item questionnaire. Women rate how they have felt during the past seven days. Each item is scored from 0–3 (resulting range 0–30). In many countries the EPDS is routinely administered to detect postnatal depression. For Austrian perinatal and postnatal women, a cut-off value of 10/11 is suggested with a sensitivity of 0.87 and specificity of 0.87 to detect all cases of major depression (26). We chose this depression screening instrument because it is widely endorsed and gives a clear overview.

To minimize the completion time for the participating women, postnatal stress and anxiety was measured with short independently developed single Likert scales. Women were asked how much stress and anxiety they have perceived during the previous 3 days (from 0%, which is not at all, to 100%, which is very much). General psychological distress was assessed with a single Likert scale from zero, which is not at all, to five, which is the most distress experienced.

On a separate sheet we asked about demographic and medical data including questions about number of children, maternal age, gestational week at birth, maternal hospitalization before birth, weight of the newborn, hours of sleep during the last day, perceived participation and control, education level, living situation, employment status and financial situation.

Statistical Analysis

Continuous data are shown as median and interquartile range (IQR) or mean and standard deviation. Categorical data are presented in absolute and relative frequencies. Differences among the three preterm groups were computed with Kruskal-Wallis test or ANOVA for continuous data and Fischer's exact test or χ^2—Test for categorical data. Since we performed a pilot study *p*-values of *post hoc* analyses were not corrected for multiple comparisons. A p-value < 0.05 was considered statistically significant. Statistical evaluations were implemented using IBM SPSS 24.0 (IBM Corp., Armonik, NY, USA).

RESULTS

Sample Characteristics

The study group consisted of 60 women, who gave birth preterm or at term within the last 9 days. The medical reasons for extremely to very preterm births were progressive preterm labor (i.e., preterm contractions, preterm rupture of membranes, prolapsing amniotic membranes, ($n = 9$), pre-eclampsia ($n = 6$), and complicated twin pregnancies ($n = 5$). The medical reasons for moderate to late preterm birth were progressive preterm labor (i.e., preterm rupture of membranes, preterm contractions, $n = 8$), complicated twin pregnancies ($n = 7$), pre-eclampsia or HELLP-syndrome ($n = 2$), fetal hydronephroses ($n = 1$), placenta praevia ($n = 1$) and fetal bradycardia ($n = 1$). The term infants were treated in the neonatal ward because of perinatal infection ($n = 9$), infant respiratory distress syndrome ($n = 5$), fetal hydronephroses ($n = 1$), fetal anal atresia ($n = 1$), intrapartum fetal bradycardias ($n = 1$), cleft lip and palate ($n = 1$), small-for-dateness ($n = 1$) and congenital ichthyosis ($n = 1$).

As shown in **Table 1**, median maternal age of the whole sample ($N = 60$) was 32.0 years. The median birth weight of the infants was 1,953 grams (range from 550 to 3,985 grams), and the median week of gestation at birth was 34 weeks (range from 25 to 42 week of gestation). The intended time interval between birth and day of the survey was day three to 10 postpartum. On average, women responded on the fifth day (range from three to nine). All maternal information and characteristics are presented in **Table 1**.

Demographic data did not differ between study groups, including age, number of children, mode of delivery, hours of sleep during the last day, perceived participation and control, maternal education level, living situation, employment status and the financial situation (see **Table 2**). However, regarding the time of hospitalization before birth the groups varied significantly ($p = 0.015$). Women with moderate to late preterm births spent significantly more days (median: 16 days, IQR: 6-32) in the hospital before birth compared to mothers of the very preterm (4 days, IQR: 1-19; $p = 0.025$) and mothers of the term group (median: 2, IQR: 1-7; $p = 0.009$).

Main Results

Comparisons of maternal mental HRQoL between the three birth groups (very preterm to term) demonstrated statistically significant differences during the early postpartum period (presented in **Table 2**). Women from the very preterm group showed significantly decreased mental HRQoL (36.8 ± 6.3) compared to those in the moderate to late preterm group (46.6 ± 7.2; $p<0.001$) and term group (43.3 ± 6.2; $p = 0.004$). Correspondingly, comparisons of physical HRQoL between the three subgroups presented also significant differences ($p = 0.046$). *Post hoc* analyses showed that women in the moderate to late preterm group (35.7 ± 7.4) had significantly lower physical HRQoL compared to those of the very preterm group (41.6 ± 7.2; $p = 0.020$).

Regarding depressive symptoms we found no significant differences among the three birth groups ($p = 0.083$). However, 45 % of the women in the very preterm group presented an at-risk EPDS score above the cut-of-value of 10 (11, ±6), 65% in the moderate to late preterm birth group (12, ±6) and 30% of women giving full-term birth (8, ±6).

Women showed no differences in anxiety level during the last 3 days ($p = 0.238$). Additionally, women did not differ regarding acute stress ($p = 0.340$) and the general psychological distress values ($p = 0.755$).

DISCUSSION

Key Results

The goal of this questionnaire survey was to investigate HRQoL and depression in women after being separated from their newborns immediately after birth in two preterm birth groups

TABLE 2 | Differences between the three birth groups in psychological outcomes and maternal characteristics.

Psychological outcomes	Early preterm <32 weeks	Moderate to late preterm 32–37 weeks o	Term birth >37 weeks	
	Mean ± SD Median (IQR)	Mean ± SD Median (IQR)	Mean ± SD Median (IQR)	*p*
SF-12 Physical componentsummary (PCS)	41.62 ± 7.23 [A]	35.71 ± 7.43 [B]	37.02 ± 6.90	0.046*
SF-12 Mental componentsummary (MCS)	36.82 ± 6.32 [A.]	46.58 ± 7.20[B]	43.31 ± 6.20[B]	<0.001**
EPDS Depression	11 ± 6.	12 ± 6.	8 ± 6	0.083
Perceived stress during thelast three days	70 (50–85)	55 (35–75)	70 (70–80)	0.340
Perceived anxiety duringthe last 3 days	80 (50–90)	55 (35–80)	80 (60–80)[A.B]	0.238
General psychologicaldistress	3 (2–4)	3 (2–4)	3 (3–4)	0.755
Maternal characteristics				
Maternal age (years, range)	30.55 (21–38)	31.8 (24–43)	32 (17–42)	0.642
Number of children (median IQR)	1 (1–2)	1 (1–2)	1 (1–2)	0.666
Hospitalization before birth (Median IQR days)	4 (1–19)	16 (6–32)***	2 (1–7)***	0.015
Weight of the infants (Median ± SD)	1,006 ± 342***	1,972 ± 294***	3,313 ± 464***	<001
Mode of birth				0.081
Spontaneous vaginal	5	4	9	
Planned cesarean	7	13	7	
Emergency cesarean	8	2	4	
Hours of sleep during a day (Median IQR)	6 (5.5–6)	6(5–7)	5 (4–6)	0.288
Perceived participation and control (Median IQR)	40(10–60)	50(20–70)	40(20–70)	0.441
Maternal educational				0.568
Primary school	2	1	0	
Secondary school	8	5	4	
Higher education, not university	5	6	8	
Higher education, university	5	8	8	
Living situation				0.629
With children	1	1	0	
With partner and children	10	10	14	
With partner, children and grandparents	2	4	1	
With partner	7	5	5	
Employment status				0.528
Not employed	4	1	2	
Full-time employed	10	12	14	
Part-time employed	6	7	4	
Financial situation				0.589
Very satisfying	5	3	4	
Satisfying	13	17	14	
Less satisfying	2	0	2	

*Statistically significant group differences at the 0.05 level between A and B.
**Statistically significant group differences at the 0.01 level between A and B.
***Significant difference between the groups.

compared to mothers of hospitalized full-term neonates. As hypothesized, the study results show that maternal psychological HRQoL was significantly worse in the early gestational birth group compared to moderate to late preterm birth and full-term birth group. Remarkably women with moderate to late preterm births showed significantly the worst physical HRQoL compared to others with very preterm births and term births. The whole study group showed high at-risk EPDS scores. Unexpectedly, women in the moderate to late preterm group showed the highest percentage of depressive symptoms with 65%. All women experienced similar high anxiety, acute stress, and general psychological distress values.

Interpretation

Early prematurity has, as expected, a serious effect on women's mental HRQoL in the early postpartum period. Parents, especially mothers, are usually not prepared for a preterm birth as it often occurs suddenly, and women describe this event

as a stressful and sometimes even traumatic experience. The arrival of an unexpected early premature infant, as described earlier, leads to maternal feelings of helplessness and fears that she will not be able to care and protect her newborn (27). Furthermore, women are confronted with very long hospital stays of their infants at the NICU, sometimes lasting months, and this is accompanied by alterations in normal life (work, family life, financial situation). During the first days women receive information from different sources about their newborns' health and perspectives, for example about risks of neurodevelopmental or general developmental delays, motor, visual, and hearing problems or learning difficulties (1, 2, 12). Consequently having a very preterm infant and apprehensions about short-term and long-term problems increases maternal stress and anxiety as found before (12, 27) but also alters mental HRQoL.

According to maternal physical HRQoL, our study specifies that moderate to late prematurity was accompanied with most impaired physical HRQoL. We speculate that the prenatal hospitalization of women of the moderate to late preterm group has an apparent influence on physical mobility. Women of this group were hospitalized longer prenatally than the women in the other groups to prevent early prematurity (on average 16 days). Most of them were confronted during pregnancy with the diagnosis of a severe pregnancy complication, such as hypertensive disorder, complicated twin pregnancies, intrauterine growth restriction, preterm premature ruptured membranes or cervical insufficiency. Receiving the diagnosis of a high-risk pregnancy combined with the longer inpatient treatment seem to be serious stressors for women and caused the lowest women's physical HRQoL and more depressive symptoms. However, the prevention of early prematurity was possible in this group, since all of them delivered between the 32nd and 37th week of gestation. Therefore, it appears reasonable that mental HRQoL was better in these women compared to women after extremely to very preterm births.

Maternal at-risk depression rates were high in our whole study population. It ranged from 45% in the early preterm group, 65% in the moderate to late preterm group and 30% in the term birth group. Correspondingly, other studies found that mothers of infants born prematurely have high PPD rates (28–40%), particularly in the early postpartum period in the NICU (7, 13). Roomruangwong et al. (13) found that a history of depression in first week postpartum was associated with depression 4–6 weeks postpartum among mothers of infants at the NICU. Therefore it is important how women feel immediately after preterm and term births. Vigod et al. (7) describes in their systematic review that "sustained depression was associated with earlier gestational age, lower birth weight, ongoing infant illness/disability, and perceived lack of social support." In general, postpartum depression (PPD) is the most frequent complication in all childrearing women. The general expected prevalence of PPD ranges according to Steward and Vigod (14) from 6.5 to 12.9 percent.

There are several studies which give inconsistent findings about how the mother-infant relationship develops following a preterm birth, concluding that the mother-preterm infant relationship is complex (27). Recent promotions of family-centered neonatal care seems to be promising. Both parents are inspired to be present and have the opportunity to be involved in the care of their newborns (27–29). However first it seems to be important to reestablish the maternal emotional well-being. Brecht et al. (5) published a review about the effectiveness of therapeutic behavioral interventions for parents of premature babies. The authors found a trend "toward early, brief interventions that are theoretically based, specifically target parent trauma, and utilize cognitive behavioral techniques." It might be a good approach to provide maternal well-being and HRQoL immediately after preterm births, or after the diagnosis of severe pregnancy complications earlier during pregnancy. Women need to understand their first stress reactions after severe pregnancy complications and preterm births. Early brief intervention can lead to more competence, reduce depressive symptoms, and improve HRQoL. Women could be better prepared for the adventure of having a preterm or a term baby at the NICU. They need to understand their reactions to the NICU environment and their infant's situation to develop coping strategies and take an active role in care. Brief interventions will improve self-efficacy, will reduce depressive symptoms and improve maternal physical and mental HRQoL.

Strengths and Limitations

The strength of the survey is the insight it gives into maternal postpartum HRQoL and depression with international validated questionnaires. The SF-12, an abridged version of the SF-36, is a reliable and valid tool for examining health-related quality of life, and has shown to be sensitive in various populations (22, 30). The EPDS remains an internationally widely applied reliable measure for depression (26). Maternal psychological outcomes are relevant for the treatment in the early postpartum period. The current study improves understanding of maternal HRQoL and depression after preterm births, especially in comparison with mothers of hospitalized term neonates, and enables a realistic assessment of the family's situation as well as allowing for improvements in clinical care.

This study was designed as a pilot study; therefore, it consists of quite a small number of participants, which might be a limiting factor for data interpretation. However, it can be assumed that the study cohort represents a sample of the standard population, as shown in the demographic data (see **Table 1**). Furthermore, patients with poor or absent German language abilities were excluded and might even have exacerbated the study results due to aggravated life conditions. A further larger study might verify our results and could focus on specific interventions and communication strategies in the NICU. Additional physical and psychological exams or inquiries would have enriched our findings. These might be topics for subsequent research.

Implications for Practice

Obstetricians, neonatologists, clinical psychologists, and nurses are the primary experts providing support for women experiencing pregnancy complications leading to

the need for their neonates' treatment in the neonatal care unit. In particular, extreme prematurity is accompanied by reduced mental HRQoL in the early postpartum period. Acknowledgment of concerns and fears and awareness of the needs of women may reduce prolonged psychological distress and improve self-efficacy for women and their families. Our findings specify that motherhood of preterm and full-term newborns requiring treatment in the neonatal ward is associated with high rates of depressive symptoms, reduced HRQoL and psychological distress. Adequate information and support from all health professionals in the obstetric and neonatal ward might be effective to reduce acute stress and anxiety. The goal of comprehensive care is to increase coping possibilities and resilience to reduce depression, anxiety and improve quality of life until the time of hospital discharge.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Ethics Commitee of the Medical University of Graz. The patients/participants provided their written informed consent to participate in this study. Written informed consent was obtained from the individual(s) for the publication of any potentially identifiable images or data included in this article.

AUTHOR CONTRIBUTIONS

EM, MD, EG, and AA: contributed substantial to conception and design, acquisition of data and analysis, and interpretation of data. EM, MD, EG, WS, AA, and CS: contributed to drafting the article and revising it critically for important intellectual content. All authors gave final approval of the version to be published.

ACKNOWLEDGMENTS

We thank all women who kindly contributed to the survey and Andrew Peaston for English language editing.

REFERENCES

1. Chawanpaiboon S, Vogel JP, Moller AB, Lumbiganon P, Petzold M, Hogan D, et al. Global, regional, and national estimates of levels of preterm birth in 2014: a systematic review and modelling analysis. *Lancet Glob Heal.* (2019) 7:e37–46.doi: 10.1016/S2214-109X(18)30451-0
2. Chang HH, Larson J, Blencowe H, Spong CY, Howson CP, Cairns-Smith S, et al. Preventing preterm births: analysis of trends and potential reductions with interventions in 39 countries with very high human development index. *Lancet.* (2013) 381:223–34.doi: 10.1016/S0140-6736(12)61856-X
3. Saigal S, Doyle LW. Preterm Birth 3 An overview of mortality and sequelae of preterm birth from infancy to adulthood. *Lancet.* (2008) 261–9.doi: 10.1016/S0140-6736(08)60136-1
4. Teune MJ, Bakhuizen S, Gyamfi Bannerman CG, Opmeer BC, Van Kaam AH, Van Wassenaer AG. A systematic review of severe morbidity in infants born late preterm. *AJOG.* (2011) 205:374.doi: 10.1016/j.ajog.2011.07.015
5. Brecht C, Shaw RJ, John NH, Horwitz SM. Effectiveness of therapeutic behavioral interventions for parents of low birth weigth premature infants: a review. *Infant Ment Health J.* (2014) 33:651–65. doi: 10.1002/imhj.21349
6. Helle N, Barkmann C, Ehrhardt S, Bindt C. Postpartum posttraumatic and acute stress in mothers and fathers of infants with very low birth weight: cross-sectional results from a controlled multicenter cohort study. *J Affect Disord.* (2018) 235:467–73. doi: 10.1016/j.jad.2018.04.013
7. Vigod SN, Villegas L, Dennis CL, Ross LE. Prevalence and risk factors for postpartum depression among women with preterm and low-birth-weight infants: a systematic review. *BJOG An Int J Obstet Gynaecol.* (2010) 117:540–50.doi: 10.1111/j.1471-0528.2009.02493.x
8. Enke C, Oliva y Hausmann A, Miedaner F, Roth B, Woopen C. Communicating with parents in neonatal Intensive Care Units: the impact on parental stress. *Patient Educ Couns.* (2017) 100:710–9.doi: 10.1016/j.pec.2016.11.017
9. Gangi S, Dente D, Bacchio E, Giampietro S, Terrin G, De Curtis M. Posttraumatic stress disorder in parents of premature birth neonates. *Procedia Soc Behav Sci.* (2013) 82:882–5. doi: 10.1016/j.sbspro.2013.06.365
10. Cook N, Ayers S, Horsch A. Maternal posttraumatic stress disorder during the perinatal period and child outcomes: a systematic review. *J Affect Disord.* (2018) 225:18–31. doi: 10.1016/j.jad.2017.07.045
11. Amorim M, Silva S, Irving MK, Alves E. Quality of life among parents of preterm infants : a scoping review. *Qual Life Res.* (2018) 27:1119–31. doi: 10.1007/s11136-017-1771-6
12. Singer LT, Salvator A, Collin M, Lilien L, Baley J, Page P. Psychological distress and parenting stress after the birth of a very low-birth-weight infant. *JAMA.* (2019) 281:799–805. doi: 10.1001/jama.281.9.799
13. Roomruangwong C, Tangwongchai S, Kantula A. Prevalence of depression in 4-6 weeks postpartum period and related factors among mothers of infants in Neonatal Intensive Care Unit (NICU), King Chulalongkorn Memorial Hospitatl. *Chula Med J.* (2006) 50:777–87. Available online at: http://clmjournal.org/_fileupload/journal/198-2-4.pdf
14. Stewart DE, Vigod SN. Postpartum depression. *n Engl J Med.* (2016) 375:2177–86. doi: 10.1056/NEJMcp1607649
15. Rafferty J, Mattson G, Earls MF, Yogman MW. Incorporating recognition and management ofpPerinatal depression into pediatric practice. *Pediatrics.* (2018) 143:e20183260. doi: 10.1542/peds.2018-3260
16. The American College of Obstetricians and Gynecologist. Screening for Perinatal Depression. Replace Comm Opin (2018) 132:208–12. doi: 10.1097/AOG.0000000000002927
17. Whoqol Group. The World Health Organization Quality of Life assessment (WHOQOL): position paper from the World Health Organization. *Soc Sci Med.* (1995) 41:1403–9. doi: 10.1016/0277-9536(95)00112-K
18. Schalock RL, Baker A, Claes C, Gonzalez J, Malatest R, van Loon J, et al. The use of quality of life scores for monitoring and reporting, quality improvement, and research. *J Policy Pract Intellect Disabil.* (2018) 15:176–82. doi: 10.1111/jppi.12250
19. Schalock RL, Van Loon J, Mostert R. A systematic approach to enhancing the personal well-being of children and adolescents. *Int J Child, Youth Fam Stud.* (2018) 9:188–205. doi: 10.18357/ijcyfs94201818647
20. Verdugo MA, Fernández M, Gómez LE, Amor AM, Aza A. Predictive factors of quality of life in acquired brain injury. *Int J Clin Heal Psychol.* (2019) 19:189–97. doi: 10.1016/j.ijchp.2019.06.004
21. Ware, JE., Kosinski M, Keller SD. *SF-12: How to score the SF-12 Physical and Mental healTh Summary Scales.* Boston, MA: Health Institute, New England Medical Center (1995).
22. Gandek B, Ware JE, Aaronson NK, Apolone G, Bjorner JB, Brazier JE, et al. Cross-validation of item selection and scoring for the SF-12 Health survey in nine countries: results from the IQOLA project. *J Clin Epidemiol.* (1998) 51:1171–8. doi: 10.1016/S0895-4356(98)00109-7

23. Morfeld MK, Bullinger M. *SF-36 Fragebogen zum Gesundheitszustand. Deutsche Version des Short Form-36 Health Survey. 2. ergänzte und überarbeitete Version*. Göttingen: Hogrefe (2011). p. 35.
24. Bai G, Korfage IJ, Hafkamp-De Groen E, Jaddoe VWV, Mautner E, Raat H. Associations between nausea, vomiting, fatigue and health-related quality of life of women in early pregnancy: The generation r study. *PLoS One*. (2016) 11:e1311. doi: 10.1371/journal.pone.0166133
25. Cox JL, Holden JM, Sagovsky R. Detection of postnatal depression: development of the 10-item Edinburgh postnatal depression scale. *Br J Psychiatry*. (1987) 150:782–6. doi: 10.1192/bjp.150.6.782
26. Muzik M, Klier CM, Rosenblum KL, Holzinger A, Umek W, Katschnig H. Are commonly used self-report inventories suitable for screening postpartum depression and anxiety disorders? *Acta Psychiatr Scand*. (2000) 102:71–3. doi: 10.1034/j.1600-0447.2000.102001071.x
27. Korja R, Latva R, Lehtonen L. The effects of preterm birth on mother - infant interaction and attachment during the infant's first two years. *Acta Obstet Gynecol Scand*. (2012) 91:164–73. doi: 10.1111/j.1600-0412.2011.01304.x
28. Dunn MS, Reilly MC, Johnston AM, Hoopes RD, Abraham MR. Development and dissemination of potentially better practices for the provision of family-centered care in neonatology?: The family-centered care map. *Pediatrics*. (2006) 118:S95–S107. doi: 10.1542/peds.2006-0913F
29. Lv B, Gao X, Sun J, Li T, Liu Z, Zhu L. Family-centered care improves clinical outcomes of very-low-birth-weight infants?: A quasi-experimental study. *Front Pediatr*. (2019) 7:1–6. doi: 10.3389/fped.2019.00138
30. Vinturache A, Stephenson N, McDonald S, Wu M, Bayrampour H, Tough S. Health-related quality of life in pregnancy and postpartum among women with assisted conception in Canada. *Fertil Steril*. (2015) 104:188–195.e1. doi: 10.1016/j.fertnstert.2015.04.012

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's Note: All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

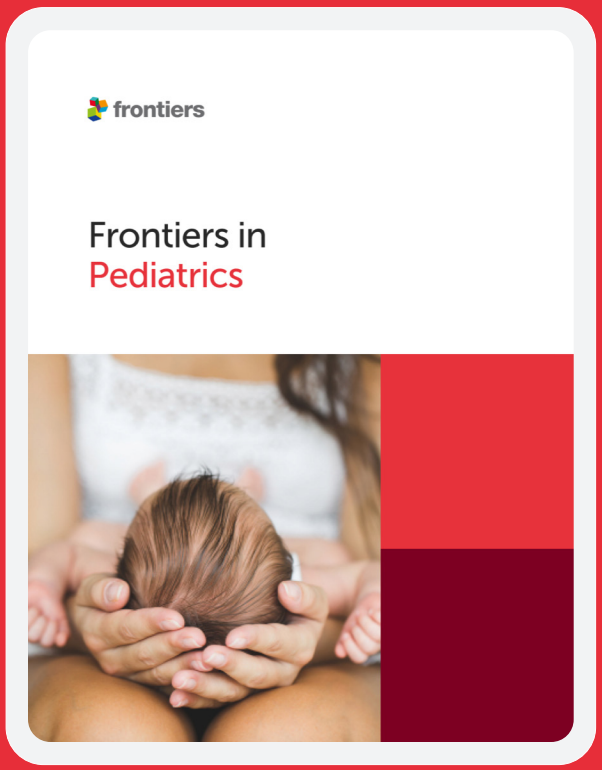
Frontiers in Pediatrics

Addresses ongoing challenges in child health and patient care

Explores research that meets ongoing challenges in pediatric patient care and child health, from neonatal screening to adolescent development.

Discover the latest Research Topics

See more →



Frontiers

Avenue du Tribunal-Fédéral 34
1005 Lausanne, Switzerland
frontiersin.org

Contact us

+41 (0)21 510 17 00
frontiersin.org/about/contact