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Course of Stress during the Neonatal Intensive Care Unit Stay in Preterm Infants

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Keywords
Prematurity · Neonatal stress · Neonatal intensive care

Abstract
Introduction: Understanding the course of stress during the neonatal intensive care unit stay may provide targets for interventions. Our aim was to describe the course of stress in preterm infants during the first 28 days of life, the influence of gestational age, and associations with clinical characteristics. ***Methods:*** In a single centre prospective cohort study, we included infants with a gestational age <30 weeks and/or birth weight <1,000 g. We measured stress over the first 28 days using the Neonatal Infant Stressor Scale (NISS). We plotted daily NISS total and subcategory scores by gestational age. The subcategories were (1) nursing, (2) skin-breaking, (3) monitoring and imaging, and (4) medical morbidity-related scores. We assessed associations of cumulative NISS scores over the first 7, 14, and 28 days with clinical characteristics using regression analyses. ***Results:*** We included 45 infants, with a median gestational age of 27 weeks. The mean daily NISS score was 66.5 (SD 8.7), with highest scores in the first 7 days of life. Scores decreased the slowest for the lowest gestational ages, in particular for nursing scores, rather than skin-breaking, monitoring and imaging, and medical morbidity-related scores. Adjusted for gestational age, infants with lower Apgar scores, sepsis, intraventricular haemorrhages, and on mechanical ventilation had significantly higher cumulative NISS scores at 7, 14, and 28 days. ***Conclusion:*** NISS scores varied greatly within infants and over time, with the highest mean scores in the first week after birth. The course of declining NISS scores in the first 28 days depended on gestational age at birth.

Introduction

Over the last decades, advances in neonatal care have led to improved survival rates in preterm infants [1]. Still, these children are at higher risk for short- and long-term morbidities, including infections, chronic lung disease, and brain injury, as well as cognitive and motor delays, socio-emotional delays, and regulatory issues [2–5]. A major part of these consequences is attributed to the course and length of the stay in the neonatal intensive care unit (NICU) [6].

Although a life-saving necessity, this NICU environment exposes infants to multiple stressors. These stressors include many painful procedures, for example, skin-breaking procedures. A recent meta-analysis reported that preterm infants undergo 7.5–17.3 skin-breaking pro-

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cedures per day, with heel sticks being one of the most common procedures [7]. Infants are furthermore exposed to several other physical, psychological, and sensorial stressors, including medical and nursing procedures, maternal separation, and excessive noise and light [8, 9]. All occur when the developing preterm brain is very sensitive to environmental stimuli [10]. Evidence is rapidly accumulating that neonatal stress associates with poorer early brain development and adverse neurodevelopmental outcomes [6, 11, 12].

Measuring, quantifying, and understanding the course of stress in neonates is difficult but important given its impact. Stress can be measured using clinical assessments or physiological signs, but these are non-specific in preterm infants and may reflect a variety of other causes. In recent studies, the focus has mainly been on pain-related stress, measured by counting skin-breaking procedures [13]. Nonetheless, research indicates that preterm infants react similarly to other stressors that may not necessarily be painful [8], thereby justifying broadening the burden of neonatal stress beyond skin-breaking procedures alone. The Neonatal Infant Stressor Scale (NISS), developed by Newnham et al. [8], was specifically designed to encompass this broader burden of stressors in a quantitative and specific measure.

With this validated quantitative measure of neonatal stress, it is possible to investigate which stressors are experienced by the infants. Understanding the detailed course of neonatal stress may provide us with insight into the cumulative exposure to stressors, as well as the timing of that exposure. Being aware of this timing may provide opportunities for guided and targeted early interventions, whether they are pharmacological or non-pharmacological in nature, and contribute to neonatal care guidelines. Therefore, our aim was firstly to describe the course of neonatal stress in extremely and very preterm infants during the first 28 days of life in the NICU; secondly, to describe this course by the week of gestational age; and thirdly, to explore associations with clinical characteristics.

Methods

Setting and Population

We included infants born between September 2019 and December 2020 with a gestational age of <30 weeks and/or a birth weight below 1,000 g, who were admitted to the level III–IV NICU of the University Medical Center Groningen. Parents of eligible infants were invited to participate in this study shortly after birth. Infants were included after written informed consent was obtained from both parents. The study was approved by the Medical Ethical Review Board of the University Medical Center Groningen (METc 2019/128) and was registered online (ISRCTN62164166).

Procedure and Measures

To quantify neonatal stress, we used the validated NISS [8]. The NISS incorporates skin-breaking procedures but goes beyond that to encompass a broad range of stressors. The NISS includes both acute and chronic items. Scores for individual items are assigned points based on expert opinions, from 2 (a little stressful) to 5 (extremely stressful). The weighted items result in a sum score per day. Sum scores over multiple days reflect cumulative stress exposure during the NICU stay. In addition, to determine whether we could identify which stressors reflected observed differences, we constructed 4 subcategories within the NISS. These categories were (1) nursing, (2) skin-breaking, (3) monitoring and imaging, and (4) medical morbidity-related.

Statistical Analyses

Firstly, we described participant characteristics using descriptive statistics and compared the characteristics with non-participating infants using χ^2 tests for binary variables and Mann-Whitney U tests for continuous variables. Secondly, we present the course of NISS scores for the first 28 days after birth, both cumulative at days 7, 14, and 28, as well as daily. We performed multilevel modelling, using MLWiN (University of Bristol, Bristol, UK) and built 2 separate models. The first model for the cumulative NISS scores, specified with timepoints, that is, 7 days, 14 days, and 28 days, nested within gestational age. In the second model, NISS scores were specified with days nested within the gestational-age week. This second model assumed a linear function of gestational age, which visually seemed to fit the data well. Thirdly, we constructed a detailed pattern of the subcategories by the week of gestational age. Finally, we assessed the associations between the cumulative NISS scores over the first 7, 14, and 28 days after birth and clinical characteristics using linear regression analyses adjusted for gestational age.

Results

Participant Characteristics

In total, 93 infants were eligible to participate in this study. We eventually included 45 infants in the study. Reasons for exclusion were decease before informed consent could be asked (*n* = 6), language barriers (*n* = 2), declined participation (*n* = 15), and logistical reasons, including the COVID-19 research stop at our department (*n* = 25). The infants had a gestational age of a median (interquartile range) 27 (26–28) weeks with a birth weight of 1,000 (790–1,248) g. Non-participating infants more often were part of multiple births, deceased during the NICU stay, were admitted to the NICU for a shorter period, and suffered from necrotizing enterocolitis (NEC) more often. We present all participant characteristics in Table 1.

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Table 1. Participant characteristics

	Participating infants (n = 45)	Non-participating infants (n = 48)
Gestational age, weeks	27 (26–28)	27 (26–29)
Birth weight, g	1,000 (790–1,248)	1,030 (791–1,253)
Male sex	22 (48.9)	24 (50.0)
Multiple births	9 (20.0)	21 (43.8)*
Apgar 1 min	5.5 (3.0–7.0)	5.0 (2.0–7.0)
Apgar 5 min	7.0 (6.0–8.0)	7.5 (6.0–8.0)
NICU admission for surviving infants, days	35 (24–49)	18 (11–31)***
Deceased during NICU admission	2 (0.4)	13 (27.1)**
Delivery via caesarean section	23 (51.1)	20 (41.7)
Antenatal steroids	38 (84.4)	43 (89.6)
Complete course	23 (51.1)	31 (64.6)
IVH grade ≥ grade 3	6 (13.3)	5 (10.4)
Mechanical ventilation	30 (66.7)	32 (66.7)
Days (n = 30)	7.0 (2.8–20.5)	4.0 (2.0–9.0)
NEC	4 (8.9)	12 (25.0)*
Sepsis	16 (35.6)	15 (31.3)
Circulatory insufficiency	5 (11.1)	9 (18.8)
PDA	20 (44.4)	15 (31.3)
Cumulative NISS score present		
At day 7	44 (97.8)	N/A
At day 14	43 (95.6)	N/A
At day 28	29 (64.4)	N/A

Data are presented as median (25th–75th percentile) or n (%) where appropriate. The complete course of antenatal steroids was defined as birth >48 h after the first dose. The presence of intestinal pathologies was based on clinical and radiographical examinations. Sepsis was defined as clinical signs of infection, combined with a positive blood culture and requiring antibiotic treatment. Circulatory insufficiency was defined as requiring fluid therapy and/or treatment with an inotropic agent such as dopamine or dobutamine. Reasons for missing NISS scores were one child being born outside our hospital and transferred on day 3 after birth and one infant being discharged to a post-IC unit on day 12 after birth, and 14 other infants were discharged to a post-IC unit before day 28 after birth. In the non-participating group, 6 of these infants were already deceased before consent could be asked, 6 of them deceased during the COVID-19 research stop, and for one other infant, there was a language barrier for consent. NICU, neonatal intensive care unit; IVH, intraventricular haemorrhage; NEC, necrotizing enterocolitis; PDA, patent ductus arteriosus; NISS, Neonatal Infant Stressor Scale. * $p < 0.05$, ** $p < 0.01$ *** $p < 0.001$ for non-participating versus participating infants.

Course of Neonatal Stress during the NICU Stay

In Figure 1, we present the distribution of NISS scores over the first 28 days after birth. The median daily NISS scores ranged between 50 and 85, with a large variation. On average, the mean daily NISS scores were 66.4 (SD 8.7). Several infants had extreme outlying NISS scores. The cumulative NISS scores were median (interquartile range) 559 (163), 1,057 (239), and 1,857 (604) at 7, 14, and 28 days, respectively. In Figure 2 and Table 2, we show the increase of cumulative NISS scores over time with averages per gestational-age week. Compared with the 24 weeks of gestational age, scores were consistently lower for almost all higher gestational ages at each timepoint ($p < 0.05$), except for infants born at 26 weeks on days 7 and 14.

We present the course of neonatal stress for each week gestational age in Figure 3a. Upon visual inspection, NISS scores were highest for all infants in the first 7 days after birth. Thereafter, decrease of NISS scores depended highly on gestational age, with infants with a gestational age of 24 weeks still having high NISS scores at 28 days, while others had declined. Our second multilevel model confirmed this and showed a significant daily decrease of stress scores of −0.443 points at 24 weeks (p = 0.029), −0.930 points at 25 weeks (p = 0.067), −1.522 points at 26 weeks ($p < 0.001$), −1.080 points at 27 weeks (p = 0.012), −1.261 points at 28 weeks (p = 0.002), and −1.836 points at 29 weeks ($p < 0.001$).

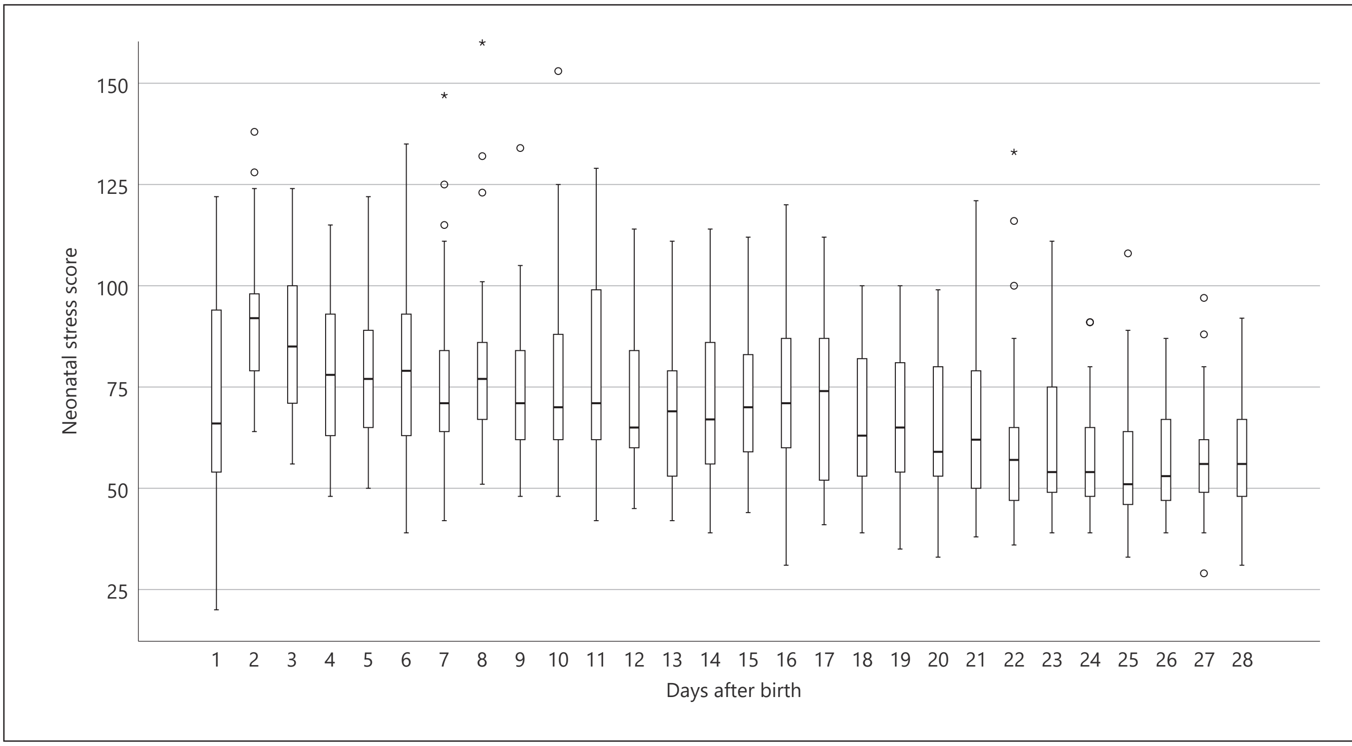

Fig. 1. Box-whisker plots of weighted neonatal stress scores per day during the first 28 days after birth. In these box-whisker plots, the boxes represent the 25th–75th percentiles, the whiskers represent the range of all stress scores, and the dots and stars represent outliers. Variability in day 1 may be explained by the time interval being shorter than 24 h, and all other days are 24-h intervals.

In Figure 3b–e, we present the course of the 4 subcategories. For nursing, we found a similar pattern as for the total NISS score, where the means for the various gestational-age weeks visually seemed different. Nursing contributed most to the cumulative NISS score throughout the entire period. Skin-breaking showed incidental peeks for the different gestational age groups, but the means were similar. A trend of declining scores was found, with averages of one to five skin-breaking procedures per day. This pattern of similar means with incidental peeks was also observed for both monitoring and imaging, as well as for medical morbidity-related categories. Moreover, skin-breaking and medical morbidity-related categories declined fast, while nursing as well as monitoring and imaging categories showed a more gradual decline.

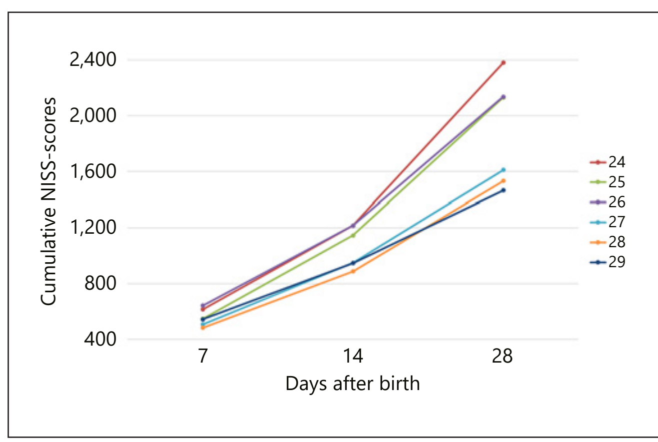

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Fig. 2. Pattern of cumulative NISS scores at days 7, 14, and 28 after birth for each week of gestational age. NISS, Neonatal Infant Stressor Scale.

Associations of Neonatal Stress with Clinical Characteristics

We present associations between cumulative NISS scores and clinical characteristics for all infants in Table 3. Cumulative NISS scores were negatively associated with Apgar scores. Moreover, infants who were mechanically ventilated, infants with a blood culture-proven sepsis, and infants with a haemodynamically significant patent ductus arteriosus had significantly higher cumulative NISS scores at all 3 timepoints in univariable analyses.

Table 2. Cumulative NISS scores at days 7, 14, and 28 after birth for each week gestational age

Mean (SD) cumulative NISS score	Gestational age at birth					
	24 weeks	25 weeks	26 weeks	27 weeks	28 weeks	29 weeks
7 days	616 (129)	559 (82)[a]	627 (83)	507 (96)[a]	481 (53)[a]	543 (71)[a]
14 days	1,216 (183)[b]	1,150 (111)[a,b]	1,191 (178)[b]	950 (213)[a,b]	875 (105)[a,b]	947 (142)[a,b]
28 days	2,381 (273)[b]	2,152 (192)[a,b]	2,114 (371)[a,b]	1,760 (345)[a,b]	1,729 (112)[a,b]	1,661 (118)[a]

[a] $p < 0.05$ compared with 24 weeks of gestational age. [b] $p < 0.001$ for change in the cumulative NISS score compared to the 7-day cumulative score for each gestational-age week.

After adjusting for gestational age, infants who were mechanically ventilated, infants with a sepsis, and infants with intraventricular haemorrhages (IVHs) had significantly higher cumulative NISS scores. Of note, infants with NEC did not have higher NISS scores.

Discussion

In this study, we describe the course of neonatal stress in extremely and very preterm infants during the first 28 days of life in the NICU. Stress scores were highly variable between infants and between days, with the highest mean scores in the first week after birth. We observed a strong inverse association of neonatal stress with gestational age, even within the group of extremely and very preterm infants. Visually, differences between weeks of gestational age were mainly due to variations in nursing scores, rather than skin-breaking, monitoring and imaging, or medical morbidity-related scores. Higher neonatal stress was also strongly associated with several clinical characteristics after adjustment for gestational age, including lower Apgar scores, mechanical ventilation, blood culture-proven sepsis, and IVHs, but not NEC.

We found the course of neonatal stress to show highest scores in the first 7 days after birth. To the best of our knowledge, ours is one of the first studies examining neonatal stress, encompassing more than skin-breaking procedures. Cong et al. [14] reported on chronic and acute items of the NISS without applying the weighing. They found on average 23 times acute items per day, seemingly stable over the first 28 days. The chronic items however recorded in hours showed declining durations across the first 28 days. In our study, acute and chronic items were combined, and the NISS weighing was applied to establish a daily NISS score that captured the full burden of neonatal stress per day, which hampers one-to-one comparisons between the two studies. Strikingly, in comparison with studies on skin-breaking procedures alone, with reported averages of 7.5–17.3 skin-breaking procedures per day [7], our population was subjected to relatively fewer of such procedures.

Importantly, we identified different courses of NISS scores by the week of gestational age, with scores declining faster with higher gestational ages. Visually, the different courses were mainly explained by differences in NISS-nursing scores and not by skin-breaking, monitoring and imaging, or medical morbidity-related scores. The identified pattern by the gestational-age week has, to the best of our knowledge, not been reported before. Moreover, we found no studies that investigated nursing as a subcategory of the NISS. At our NICU, all staff members, including neonatal nurses and attending neonatologists, have been trained to adhere to the Family and Infant Neurodevelopmental Education programme [15], which is based on the Newborn Individualised Developmental Care and Assessment Program principles. Minimal touch by medical staff, especially for infants born after 24 and 25 weeks, is one of the implemented guidelines within this philosophy. These principles have proven beneficial in the short term [16]. Therefore, we do not believe that the style of nursing in our NICU could explain this pattern. Instead, we hypothesize that several of the nursing procedures, that is, suctioning of the nose and mouth or flushing IV lines, are related to illness severity, thereby reflecting immaturity at birth. Immaturity of the organ systems may therefore be a critical underlying mechanism that explains our findings [10, 11, 17].

We also identified several clinical characteristics that were associated with higher NISS scores, including mechanical ventilation, blood culture-proven sepsis, and IVHs. These characteristics are often associated with more nursing procedures, that is, suctioning of the mouth, nose, or tube, while being mechanically ventilated and infusion treatment during sepsis, with subsequent circulatory insufficiency. Therefore, this strengthens the afore-

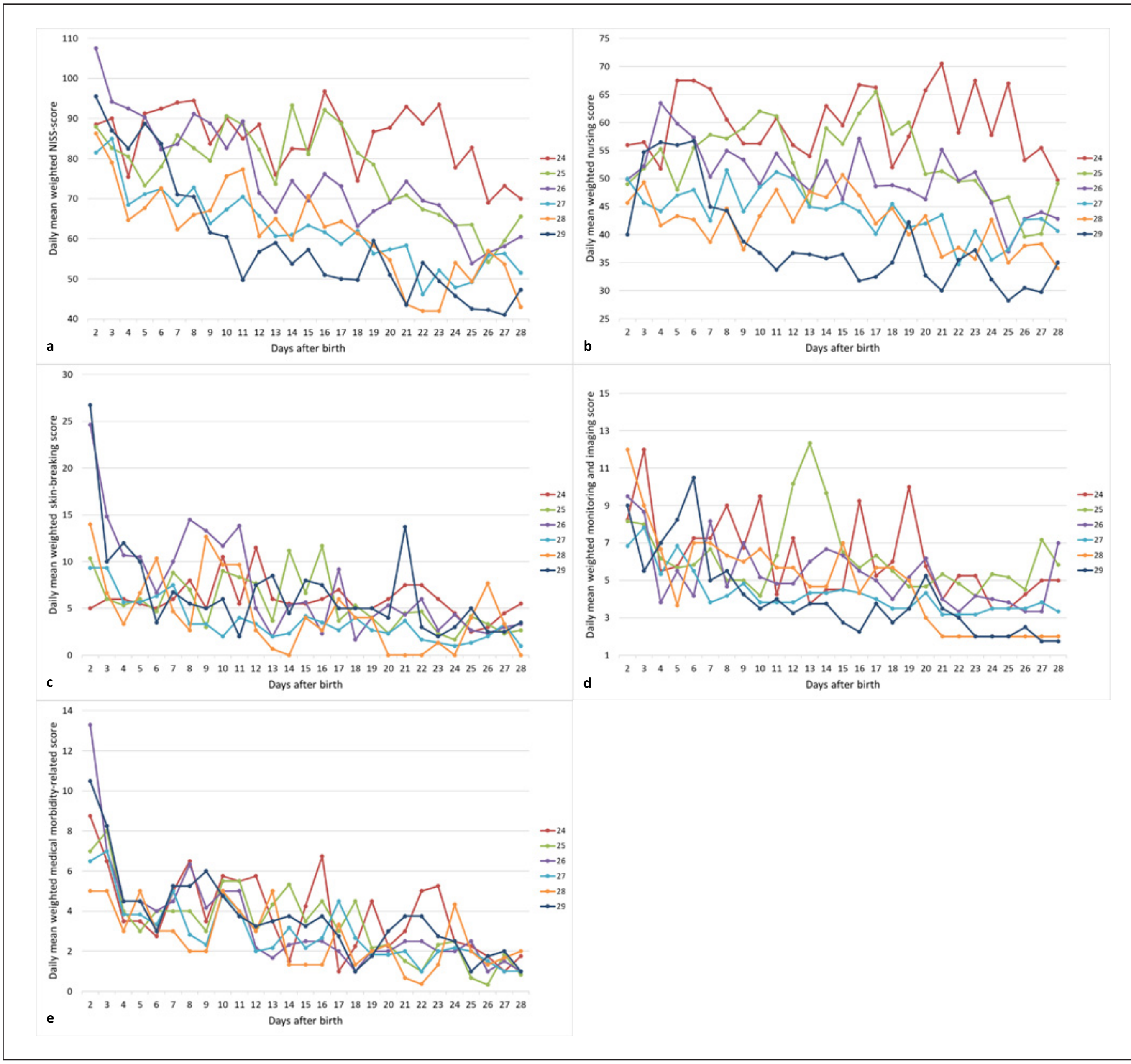

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Fig. 3. Pattern of NISS scores per day for (**a**) the NISS scores and the scores for its subcategories nursing (**b**), that is, nappy changes, cleaning of the eyes and mouth, suctioning of the nose and mouth, flushing IV lines, weighing infants, inserting feeding tubes, and removing infants from an incubator; skin-breaking (**c**), that is, inserting IV lines, blood sampling, inserting drains, heel pricks, lumbar punctures, and surgery; monitoring and imaging (**d**), that is, electrocardiograms, ultrasounds, scans, X-rays, and application of sensors for cerebral function monitoring, electroencephalograms, or near-infrared spectroscopy; and medical morbidity-related procedures (**e**), that is, suffering from a local or systemic infection, receiving phototherapy, intubation, ventilation (both mechanical and non-invasive), and eye examinations, during the first 28 days after birth for each week of gestational age. NISS, Neonatal Infant Stressor Scale.

mentioned hypothesis that illness severity is an important determinant for the NISS. Neonatal stress may therefore be one of the mechanisms underlying associations between these clinical characteristics and adverse neurodevelopmental outcomes. Preterm infants are known to react to simply touch with more pronounced reflexes than full-term infants [18]. These more pronounced reflexes may be the result of altered development of peripheral af-

Table 3. Associations between cumulative NISS scores at days 7, 14, and 28 and clinical characteristics: results of linear regression analyses

Factor	Cumulative NISS score 7 days (n = 44)		Cumulative NISS score 14 days (n = 43)		Cumulative NISS score 28 days (n = 29)	
	univariable B (95% CI)	multivariable B (95% CI)	univariable B (95% CI)	multivariable B (95% CI)	univariable B (95% CI)	multivariable B (95% CI)
Apgar score 1 min	−16.2 (−28.8; −3.6)*	−10.7 (−24.6; 3.3)	−31.7 (−59.3; −4.2)*	−7.3 (−33.9; 19.2)	−45.2 (−104.6; 14.3)	4.6 (−48.3; 57.4)
Apgar score 5 min	−33.2 (−51.6; −14.8)**	−28.4 (−50.2; −6.9)*	−73.3 (−111.8; −34.7)***	−42.6 (−84.5; −0.7)*	−120.8 (−200.0; −41.5)**	−33.8 (−124.4; 56.9)
Male sex	−18.9 (−76.5; 38.5)	−23.8 (−78.0; 30.4)	−51.8 (−174.9; 71.2)	−67.9 (−168.7; 32.8)	111.5 (−162.4; 385.4)	−33.4 (−251.0; 184.3)
Multiple births	−4.0 (−75.5; 67.6)	−19.7 (−88.2; 48.7)	−31.2 (−183.1; 120.7)	−84.5 (−209.8; 40.8)	−47.5 (−389.3; 294.2)	−185.1 (−437.5; 67.2)
Delivery via caesarean section	−14.2 (−71.8; 43.3)	24.0 (−37.9; 86.0)	−79.8 (−201.1; 41.5)	41.1 (−75.9; 158.2)	−129.4 (−410.5; 151.7)	59.7 (−169.7; 289.0)
Complete course of antenatal steroids	−47.7 (−103.8; 8.1)#	−75.7 (−146.3; −5.0)*	−71.7 (−193.5; 50.1)	−119.6 (−253.3; 13.0)#	−46.2 (−323.0; 230.6)	−77.8 (−353.8; 198.2)
Mechanical ventilation	116.0 (67.0; 165.1)***	105.9 (50.5; 161.3)***	265.1 (162.9; 367.4)***	195.1 (88.3; 301.9)**	483.6 (170.2; 797.0)**	265.8 (−18.0; 549.7)#
NEC	51.0 (−48.1; 150.2)	25.2 (−72.0; 122.5)	197.2 (−17.6; 392.0)#	102.1 (−76.5; 280.7)	327.1 (−109.4; 763.6)	165.1 (−178.4; 508.6)
Sepsis	78.4 (23.6; 133.2)**	63.8 (8.3; 119.4)*	218.4 (110.4; 326.4)***	165.6 (68.5; 262.7)**	354.3 (110.1; 598.6)**	224.0 (20.5; 427.4)*
IVH ≥ grade 3	95.8 (17.1; 174.4)*	134.9 (63.7; 206.1)***	137.7 (−35.6; 311.0)	252.9 (121.1; 384.6)***	45.5 (−356.1; 447.1)	332.9 (39.4; 626.3)*
Circulatory insufficiency	112.8 (28.9; 196.7)*	98.5 (17.2; 180.0)*	203.4 (21.2; 385.5)*	151.1 (−3.6; 305.6)#	318.1 (−63.8; 700.0)#	259.9 (−24.2; 544.0)#
PDA	70.6 (16.9; 124.2)*	48.7 (−13.8; 111.2)	199.1 (92.0; 306.1)**	110.0 (−6.0; 225.9)#	358.3 (110.1; 606.5)**	205.3 (−10.0; 420.5)#

Apgar scores were treated as continuous variables. The complete course of antenatal steroids was defined as birth >48 h after the first dose. The presence of intestinal pathologies was based on clinical and radiographical examinations. Sepsis was defined as clinical signs of infection, combined with a positive blood culture and requiring antibiotic treatment. Circulatory insufficiency was defined as requiring fluid therapy and/or treatment with an inotropic agent such as dopamine or dobutamine. NISS, neonatal infant stressor scale; IVH, intraventricular haemorrhage; NEC, necrotizing enterocolitis; PDA, patent ductus arteriosus. # $p < 0.10$, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

ferents of the somatosensory circuitry in the spinal cord due to early life experiences [19]. Thus, repeated handling, painful procedures, and all other stressors in their totality may be responsible for the sensitization of the central nervous system [13]. One other route may be altered programming of the hypothalamic-pituitary-adrenal axis due to stress exposure, with studies reporting altered cortisol responses in later life [20], in turn associated with adverse neurodevelopmental outcomes [21]. Thus, early environmental factors may programme hormonal and behavioural responses later in life, as was suggested already several decades ago [22].

Strengths and Limitations

Our study has several strengths. Firstly, we systematically collected the neonatal stress data prospectively with trained neonatal nurses completing the electronic patient files. In addition, we were able to study neonatal stress in great detail by splitting the score into 4 categories. We also acknowledge some limitations. Most importantly, we only included 45 participants whom we split into gestational-age weeks. This resulted in small numbers of infants per gestational-age week. Additionally, non-participating infants differed from participating infants on a few characteristics, which may have led to underestimation of the association between the NISS scores and, for example, NEC. Secondly, we quantified the cumulative exposure to neonatal stress rather than how an infant experiences this stress. Still, the NISS correlates well with cortisol measurements [23], which have proven to be one of the most precise physiological measures of changes in the HPA axis, that are associated with infant stress [24].

Implications

Because neonatal stress is associated with a variety of adverse neurodevelopmental outcomes, it is important to reduce neonatal stress as much as possible. While the stressors that the NISS encompasses are life-saving, we speculate that it may be appropriate to weigh the necessity of every individual stressor. Moreover, besides the Newborn Individualised Developmental Care and Assessment Program principles, non-pharmacological interventions, such as kangaroo care, may be of interest to facilitate a more natural habitat and parental involvement. Future research should try to encompass neonatal stress in a broader perspective than skin-breaking procedures alone. For the NISS specifically, reference norms for stress severity could contribute to interpretation of findings, and the interrelation between exposure to stress and experience of stress is highly warranted. Research should also determine whether it would be beneficial to limit daily stress levels and further clarify the burden of neonatal stress for neurodevelopmental outcomes, brain development, and parent-infant bonding.

Conclusion

Neonatal stress is highly variable within infants and over time, with highest average scores in the first week after birth and a different course, depending on the gestational age at birth. NISS scores strongly associate with several clinical characteristics, possibly reflecting illness severity during the NICU stay.

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Statement of Ethics

This study was conducted according to the guidelines for human studies in accordance with the World Medical Association Declaration of Helsinki. This study was approved by the Medical Ethics Committee of the University Medical Center Groningen (METc 2019/128). All parents provided written informed consent. Presented data were anonymized.

Conflict of Interest Statement

The authors have no conflicts of interest to declare.

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Author Contributions

Ms. N.H. van Dokkum conceptualized and designed the study, collected and analysed the data, and drafted the first and final manuscript. Dr. M.L.A. de Kroon contributed to the study design and critically reviewed and revised the manuscript. Dr. P.H. Dijk contributed to the interpretation of the findings and critically reviewed and revised the manuscript. Drs. K.E. Kraft contributed to the interpretation of the findings and critically reviewed and revised the manuscript. Prof. S.A. Reijneveld contributed to the study design and critically reviewed and revised the manuscript. Prof. A.F. Bos conceptualized and designed the study, supervised the collection, analysed the data, contributed to the interpretation of the findings, and critically reviewed and revised the manuscript. All the authors gave final approval of the version to be published and agree to be accountable for all aspects of the work.

Data Availability Statement

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

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