J Pediatr Res 2023;10(1):1-7
DOI: 10.4274/jpr.galenos.2022.89106

Original Article

Lung Ultrasound - Can it be Potentially Painful for a Newborn?

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ABSTRACT

Aim: We aimed to analyze changes in vital parameters and assess behavioral responses to lung ultrasound (LUS) in preterm and term newborns hospitalized in a neonatal intensive care unit (NICU).

Materials and Methods: Three groups of neonates (term, $37^{0/7}$-$41^{6/7}$ weeks; moderate to late preterm, $32^{0/7}$-$36^{6/7}$; and very preterm, $<32^{0/7}$) were included. Response to LUS was assessed using heart rate (HR), blood oxygen saturation (SpO_2), and the neonatal infant pain scale (NIPS). Reactions to LUS, blood sampling, and nappy change were compared.

Results: Seventy-one infants were enrolled: 30 term, 21 moderate to late preterm, and 20 very preterm. An increase in mean HR and a decrease in median SpO_2 during LUS were observed ($p<0.001$) in all analyzed groups, whereas the median NIPS score was 3. During LUS, 38% of term infants experienced pain according to NIPS. The same was observed for 47% and 35% of infants in the moderate to late preterm and very preterm groups. The trend of NIPS increased along with the higher intensity of the stimulus. The highest NIPS values were related to blood sampling, moderate to LUS, and the lowest to nappy change ($p<0.001$).

Conclusion: As LUS affects vital parameters and may be perceived as potentially painful in >1/3 newborns, indications for each examination and adequate pain management should always be considered.

Keywords: Newborn, pain, lung ultrasound, response, neonatal infant pain scale

Introduction

In recent years, the importance of lung ultrasound (LUS) in neonatal intensive care units (NICUs) has increased significantly. It has been successfully used for the diagnosis, management, and monitoring of most pleural and pulmonary pathologies in newborns, such as pneumonia, pneumothorax, pleural effusion, and respiratory distress syndrome (1-5). In comparison with chest radiography, the main advantage of LUS is the absence of ionizing radiation, which guarantees the safety of serial imaging in extremely vulnerable neonates (6,7). Moreover, LUS is non-invasive, relatively low-cost, and can be performed at the point-of-care, which is especially important when managing critically ill, unstable patients in the NICU environment (8). Although ultrasound has been shown to cause harmful thermal and mechanical bio-effects in animal models (9-11), recent studies have not confirmed these findings in clinical settings (12). Therefore, neonates admitted to NICU are often subjected to numerous ultrasounds, especially in their first days of life.

Despite the many advantages of LUS, it remains unclear whether it can be undoubtedly classified as a neutral

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Received: 05.07.2022 **Accepted:** 07.12.2022

stimulus in infants. It is intuitively regarded as a non-painful procedure since it is not related to tissue damage. However, LUS may cause discomfort to infants, as it requires the use of gel, the pressure of the ultrasound probe, and regularly changing of the newborn's position. To date, an analysis of short-term behavioral reactions and changes in vital parameters in newborns undergoing LUS has not been performed. Moreover, whether premature infants respond differently to LUS compared with term newborns has not been evaluated.

The assessment of pain intensity in neonates remains challenging and is currently based on analyzing changes in their behavior and vital parameters, such as their heart rate (HR), respiratory rate, blood pressure, and blood oxygen saturation (SpO_2). Over 40 neonatal pain assessment tools, which include different combinations of these indicators, are available (13). One of the most popular is the Neonatal Infant Pain Scale (NIPS), which is dedicated to procedural pain assessment in both preterm and term neonates. The NIPS has been successfully tested for validity and reliability in the neonatal population (14,15).

The main aim of this study was to analyze changes in vital parameters and evaluate behavioral response in order to identify potential pain related to LUS in premature and term neonates hospitalized in the NICU. The other objective was to compare infants' reactions to LUS and other painful and neutral procedures.

Materials and Methods

Study Design

This study was conducted in a tertiary referral NICU. The research design is illustrated in Figure 1. Reactions to three different stimuli were analyzed for each patient: LUS and two control procedures. Blood sampling was chosen as the painful control procedure, while nappy change was used as the neutral control stimulus. The responses to each procedure were analyzed on different days in the morning between 6 and 9 a.m., before any other nursing or diagnostic actions, with at least a 1 hour interval after feeding. The procedure order was randomly established. The hospital stay was prospectively analyzed for the number of painful stimuli experienced by the newborns. The application of the methods of procedural pain management was also investigated.

Sample

Infants hospitalized in the NICU were enrolled into this study. The patients were divided into three groups based on their gestational age (GA): term ($37^{0/7}$-$41^{6/7}$ weeks), moderate to late preterm ($32^{0/7}$-$36^{6/7}$ weeks), and very preterm (<$32^{0/7}$ weeks). Patients were considered for this study if they were in a stable medical condition defined as the absence of invasive ventilation or cardiovascular support. The exclusion criteria included the occurrence of major malformations, severe intraventricular hemorrhage (IVH grade IV), and earlier hospitalization in neonatology units longer than 3 days. The analysis of the infants' responses was performed after the completion of 35 weeks of postmenstrual age (PMA) in preterm newborns and before discharge in term neonates.

Procedures

All ultrasounds were performed by one certified sonographer who was experienced in LUS using Phillips HD 11 (Philips, Amsterdam, the Netherlands) or Hitachi-Aloka Arietta v70 (Hitachi, Tokyo, Japan) scanners with a linear probe of 12-5 MHz. Five lung areas were routinely examined: anterior (midline), anterior (right), anterior (left), posterior (right), and posterior (left), using the transversal and longitudinal positions of the probe. If the infant was supine, the anterior parts of the lung were assessed first. Subsequently, the posterior fields were examined after changing to the prone position. If the patient was in the prone position, the LUS was started with posterior field scans. For all examinations, the ultrasound gel was warmed. The probe was disinfected before and after each LUS. The blood samples were obtained from each patient through a peripheral vein puncture or heel lance. The blood sampling was performed by qualified nurses. Each patient underwent a nappy change procedure, which included toileting of the recto-genital region using wet nappies. For standardization, the nappy change was carried out by the same researcher every time. In order not to increase exposure to procedural pain or stress, the analysis of the infants' response to each procedure was performed only on the occasion of routine daily care or when it was regarded as diagnostically necessary by the attending physicians.

Instruments

All infants were continuously monitored using pulse oximeters as part of their routine medical care in the NICU. Before beginning this study, the baseline HR and SpO_2 of the participants were noted. Subsequently, during the entire procedure, the infants' vital parameters were evaluated for maximal HR (HR max) and minimal SpO_2 (SpO_2 min) values. After completing the procedures, the recovery times were analyzed, which was defined as the time that elapsed

until HR and SpO_2 returned to their baseline values. It was measured separately for each parameter to an accuracy of 1 second using a stopwatch. The maximum observation time was 300 seconds. Additionally, ΔHR was calculated as the difference between the HR max and the baseline HR. Finally, ΔSpO_2 was estimated by subtracting SpO_2 min from the baseline SpO_2.

Continuous video recordings of the infants during each procedure were performed using a digital camera (SONY HDR-CX250E). Subsequently, all recordings were archived in order to assess pain intensity with the use of NIPS. The total pain score ranges from to 0-7. Values of >3 indicate pain (15). The videos were analyzed by two independent observers who were experienced in NIPS evaluation. The final NIPS result was calculated as the average of the results obtained from the two observers.

Statistical Analysis

Statistical analysis was performed using Statistica software, version 13.3 (TIBCO Software Inc. Palo Alto, CA, USA). The results are presented based on the parameters of descriptive statistics, including mean values and standard deviations (SD), or median values with first and third quartiles (Q1, Q3) for continuous variables and numbers with percentages for categorical variables. To confirm the normal distribution of continuous variables, the Shapiro-Wilk test was used. The assessed groups were compared using the Kruskal-Wallis test, and the ***t***-test was used for dependent samples. The Wilcoxon test was performed to analyze changes in vital parameters during LUS. Friedman's rank test was performed to compare the infants' responses during different procedures. The Jonckheere test was applied to evaluate the trend of NIPS changes with the increase in the intensity of the stimuli. The Spearman rank test was used to assess the correlation between HR, SpO_2, and NIPS values. The NIPS values were analyzed for inter-observer agreement using Kendall's coefficient of concordance. Statistical significance was set at p-values of <0.05.

Ethical Standards

Written formal consent was obtained from all of the legal guardians of the newborns. This study was approved by the Ethics Committee of Jagiellonian University (approval number: 1072.6120.112.2018).

Results

Study Population

Seventy-one infants hospitalized in the NICU between June, 2018 and March, 2021 were enrolled. The study cohort consisted of 30 term neonates (Group 1), 21 moderate to late preterm (Group 2), and 20 very preterm infants (Group 3). The detailed characteristics of the assessed groups are presented in Table I.

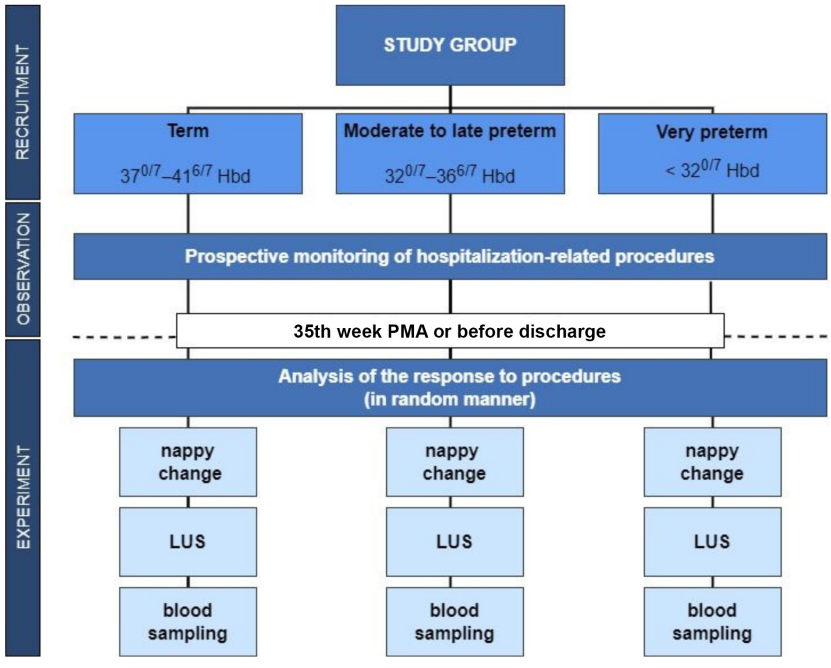

Figure 1. Study design
PMA: Postmenstrual age, LUS: Lung ultrasound

Procedural pain management

During the analyzed procedures, the patients received methods of non-pharmacological analgesia including non-nutritive sucking, oral glucose, maternal milk feeding, or a combination of those methods. Pharmacological treatment was not used for pain relief, but three patients received it for other reasons coincidentally.

Changes in vital parameters during LUS

In all analyzed groups, the mean HR increased significantly during LUS (Figure 2). A decrease in SpO_2 values was also observed in both term and preterm infants (Figure 3). The median change in the vital parameters during LUS in all the groups did not differ (p=0.08 for ΔHR and p=0.91 for ΔSpO_2). The median time of HR recovery was 27 seconds for term neonates, 72 seconds for moderate to late preterm neonates, and 66 seconds for late preterm neonates (p=0.11). Very preterm infants were characterized by the highest median time of SpO_2 recovery (14 seconds), whereas term and moderate to late preterm neonates obtained similar median values (8 seconds and 7 seconds, respectively; p=0.52).

Evaluation of pain intensity during LUS and control procedures

The values of Kendall's coefficient of concordance revealed a high level of agreement in NIPS evaluation between the two raters (Table II). During LUS, the median NIPS score was 3 in all analyzed groups. A total of 38% of term infants experienced pain according to NIPS (>3), whereas these rates were 47% of neonates from the moderate to late preterm group and 35% of neonates from the very preterm group. The analysis of perinatal history and hospitalization-related parameters did not reveal any predisposing factor to overreaction to LUS. In term and moderate to late preterm infants, NIPS scores were positively correlated with HR

Table I. Characteristics of the assessed groups

Group characteristics	Term	Moderate to late preterm	Very preterm
Male	17 (56.67%)	12 (57.14%)	9 (45%)
Gestational age (weeks)	39 (38-40)	33 (33-35)	28 (27-30)
Birth weight (g)	3,340±520	2,080±570	1,220±360
Cesarean section	13 (43.33%)	18 (85.71%)	17 (85%)
1st minute Apgar score (pt.),	7 (4-10)	7 (6-9)	6 (4-6)
Twin pregnancy	0 (0%)	6 (28.57%)	4 (20%)
Age at the admission (days)	1 (1-3)	1 (1-2)	1 (1-1)
Time from admission to analysis (days)	7±4	16±8	57±22

Data are presented as n (%), median (Q1-Q3), or mean ± SD
SD: Standard deviation

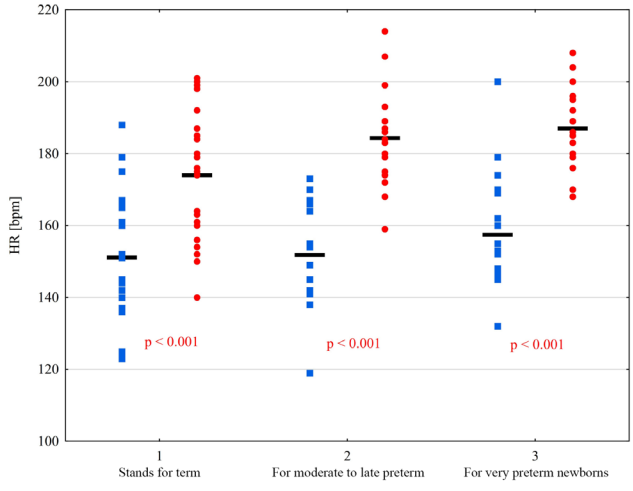

Figure 2. Change in HR during LUS
Black: Mean, Blue: HR before LUS, Red: HR max during LUS, HR: Heart rate

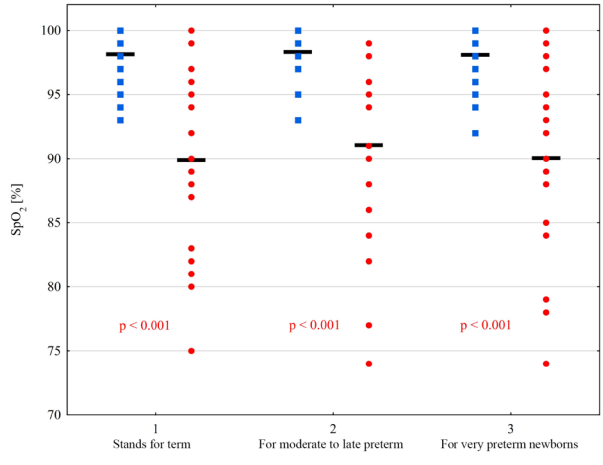

Figure 3. Change in SpO_2 during LUS
Black: Median, Blue: SpO_2 before LUS, Red: SpO_2 min during LUS, SpO_2: Blood oxygen saturation, LUS: Lung ultrasound

Table II. Inter-observer agreement in the NIPS assessment for different procedures		
Statistics procedure	**Kendall's coefficient of concordance**	**p-value**
Nappy change	0.94	**<0.001**
LUS	0.93	**<0.001**
Blood sampling	0.87	**0.001**
LUS: Lung ultrasound, NIPS: Neonatal infant pain scale		

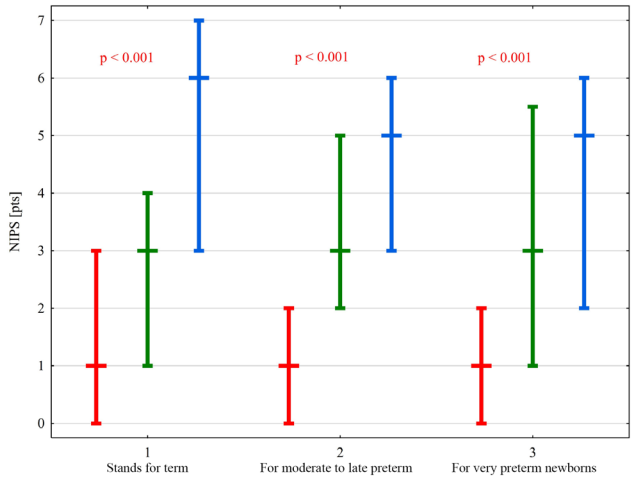

Figure 4. NIPS values during nappy change, LUS, and blood sampling. Data are presented as medians with quartiles
Red: Nappy change, Green: LUS, Blue: Blood sampling, NIPS: Neonatal infant pain scale, LUS: Lung ultrasound

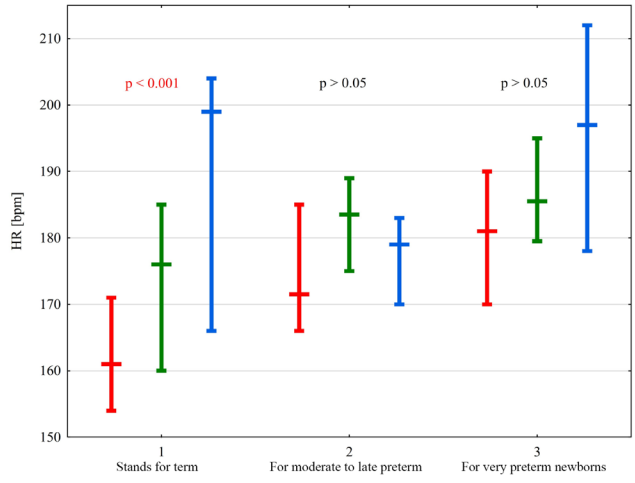

Figure 5. HR max during nappy change, LUS, and blood sampling. Data are presented as medians with quartiles
Red: Nappy change, Green: LUS, Blue: Blood sampling, HR max: Maximal heart rate, LUS: Lung ultrasound, HR: Heart rate

max (Rs=0.74, p<0.05, and Rs=0.47, p<0.05, respectively), while in very preterm neonates, a negative correlation with SpO_2 min was observed (Rs=-0.57, p<0.05). In all analyzed groups, the median NIPS values during LUS were higher than during nappy change and lower than during blood sampling (Figure 4). A significant trend of NIPS increase along with the higher intensity of the stimuli was observed in both preterm and term infants (p<0.001 for each group).

Change of vital parameters - comparison between procedures

In term neonates, the median value of HR max during LUS was significantly lower than during blood sampling (p<0.02) and did not differ from the median value during nappy change (p>0.05), whereas, in preterm infants, no difference in HR max was observed during the procedures (p>0.05) (Figure 5). SpO_2 min, HR, and SpO_2 recovery times were similar regardless of the type of procedure in all the analyzed groups.

Discussion

Despite major progress in neonatology, infants who are admitted to the NICU are still exposed to multiple painful procedures and require a long hospital stay. The systematic review by Cruz et al. (16) including six different studies revealed that hospitalized neonates experienced between 8 and 17 invasive procedures per day during their first two weeks of hospitalization. A study conducted by Lee et al. (17) showed that the median lengths of hospital stay in infants with low birth weight, very low birth weight, and extremely low birth weight were 21, 46, and 79 days, respectively. Thus, significant pain loads in newborns are an unquestionable problem. In particular, exposure to multiple painful procedures may have long-lasting effects on the postnatal process of central nervous system development. Repetitive pain cannot only prolong functional dysmaturity of the brain, but also induce permanent neuroanatomical changes, including reduced white matter and subcortical gray matter maturation, thalamic volume loss and decreased functional brain connectivity (18-21). The persistence of pathological reactions towards pain has also been observed (22,23). Thus, all procedures in the NICU environment should be carefully monitored for their indispensability and influence on neonates. Moreover, for all painful procedures, recommended evidence-based pain management should be implemented.

LUS has revolutionized the diagnosis of newborns. Its common use significantly decreases exposure to ionizing radiation and enables repetitive point-of-care lung imaging. Although the safety of ultrasound has been proven, the lack of side effects related to LUS should not be equated with painlessness. To the best of our knowledge, this is the first study to investigate changes in the vital parameters during LUS and to evaluate pain intensity using a scale dedicated to neonates.

Our study demonstrates that LUS is related to short-lasting disruption of crucial vital parameters, which can be observed in both term and premature infants of different GA. It seems that very preterm neonates require more time for SpO_2 and HR stabilization after stimulus, as they were characterized by the longest recovery times of the above parameters.

Although term and preterm infants obtained the same median NIPS values, which did not exceed 3 points, which was taken as the cut-off value for pain, in 35-47% of the evaluated neonates, the obtained scores were higher. The presence of procedural overreaction was previously reported by Chimello et al. (24) and was identified in approximately 1/3 of preterm infants. Further comparison of infants who experienced LUS as painful in comparison to those infants who did not experience the procedure as painful did not reveal any significant differences in their perinatal history or their course of hospital stay between the groups. The comparison between NIPS during LUS, nappy change, and blood sampling suggests that LUS should be classified as a procedure which moderately reduces the comfort of infants.

Premature babies were analyzed when they were stable and were already above 35 weeks PMA. We did not evaluate the reactions of babies in their first days of life; hence, we could not ascertain whether stimuli in this period may be even more dangerous. Our data cannot answer the above question, but they highlight its importance. The same question can be raised in unstable full-term newborns.

It may be argued whether the observed reactions are truly caused by pain, or they are rather manifestations of the stress experienced by newborns. Indeed, both stress and pain can manifest similarly in neonates. Behaviors associated with pain (such as grimace, changed breathing pattern, crying, and flexion of extremities) may accompany both painful and stressful procedures (25). Changes in vital parameters are also observed in both types of stimuli (25,26). The analysis of the pain concept by Fitri et al. (27) indicated tissue damage as the main attribute distinguishing neonatal pain from stress. As LUS is not related to the activation of nociceptors through tissue damage, it can therefore be intuitively classified as a stressful procedure rather than a painful one. However, The International Association for the Study of Pain emphasized that "pain and nociception are different phenomena" because pain is also strongly related to the psychological and subjective context (28). Moreover, not only can the clinical presentation of stress and pain be similar, but also their effects on the central nervous system can be similar. Studies based on functional magnetic resonance imaging showed that response to stress or pain may overlap in the amygdala, hippocampus, striatum, insula, and anterior cingulate cortex (29,30). Overall, as distinguishing between acute pain and stress in newborns is demanding in clinical and experimental settings and their effects may be similar, we postulate that strong stress and pain should be regarded as similar phenomena in newborns.

One of the main strengths of this study is the methodology of NIPS assessment, as it was based on video recordings and the evaluations were performed by two independent observers. A high inter-observer agreement guarantees good accuracy of these results and reduces observer bias.

Study Limitations

It should be emphasized that the group of term newborns assessed for this study was represented by a population with significant morbidity, which resulted in NICU admission. Hence, the main limitation of our study is that the obtained results cannot be generalized to healthy term neonates or those with only mild health issues.

Another limitation is related to the different time points of analysis of response to procedures in preterm and term newborns. Although all infants were examined in a similar PMA, the premature groups were characterized by an older chronological age. Hence, further studies are needed to compare reactions in the first days of life close to the expected date of delivery in preterm newborns.

Conclusion

Based on the obtained results, it should be emphasized that although LUS is a safe procedure, it should not be performed without limits and clinical indications, as it significantly affects critical vital parameters in neonates. Moreover, our study suggests that LUS can be classified as a procedure which is not neutral to infants. Some newborns may even perceive LUS as a potentially painful procedure, and the current level of knowledge does not allow them to be identified in advance, but in such cases, appropriate pain management should be always used.

Acknowledgements

We would like to thank Editage (www.editage.com) for English language editing.

Ethics

Ethics Committee Approval: This study was approved by the Ethics Committee of Jagiellonian University (approval number: 1072.6120.112.2018).

Informed Consent: Written formal consent was obtained from all of the legal guardians of the newborns.

Authorship Contributions

Concept: M.O., P.K., Design: M.O., P.K., Data Collection and/or Processing: S.P.T., Analysis and/or Interpretation: P.K., Writing: M.O., S.P.T., P.K.

Conflict of Interest: None of authors have any conflicts of interest to report.

Financial Disclosure: The author(s) received no financial support for the research, authorship, and/or publication of this article.

References

1. Raimondi F, Rodriguez Fanjul J, Aversa S, et al. Lung Ultrasound for Diagnosing Pneumothorax in the Critically Ill Neonate. J Pediatr 2016; 175:74-8.e1.
2. Szymański P, Kruczek P, Hożejowski R, Wais P. Modified lung ultrasound score predicts ventilation requirements in neonatal respiratory distress syndrome. BMC Pediatr 2021; 21:17.
3. Corsini I, Parri N, Ficial B, Dani C. Lung ultrasound in the neonatal intensive care unit: Review of the literature and future perspectives. Pediatr Pulmonol 2020; 55:1550-62.
4. Szymońska I, Wentrys Ł, Jagła M, et al. Lung ultrasound reduces the number of chest X-rays in newborns with pneumothorax. Dev Period Med 2019; 23:172-7.
5. Chen SW, Fu W, Liu J, Wang Y. Routine application of lung ultrasonography in the neonatal intensive care unit. Medicine (Baltimore) 2017; 96:e5826.
6. Gajski G, Milković D, Ranogajec-Komor M, Miljanić S, Garaj-Vrhovac V. Application of dosimetry systems and cytogenetic status of the child population exposed to diagnostic X-rays by use of the cytokinesis-block micronucleus cytome assay. J Appl Toxicol 2011; 31:608-17.
7. Verdun FR, Bochud F, Gundinchet F, Aroua A, Schnyder P, Meuli R. Quality initiatives* radiation risk: what you should know to tell your patient. Radiographics 2008; 28:1807-16.
8. Lichtenstein DA, Mauriat P. Lung Ultrasound in the Critically Ill Neonate. Curr Pediatr Rev 2012; 8:217-23.
9. Lalzad A, Wong F, Schneider M. Neonatal Cranial Ultrasound: Are Current Safety Guidelines Appropriate? Ultrasound Med Biol. 2017; 43:553-60.
10. Zhu J, Lin J, Zhu Z, Shou W, Bi D, Shi L. Effects of diagnostic levels of color Doppler ultrasound energy on the cell cycle of newborn rats. J Ultrasound Med 1999; 18:257-60.
11. O'Brien WD Jr, Yang Y, Simpson DG, et al. Threshold estimation of ultrasound-induced lung hemorrhage in adult rabbits and comparison of thresholds in mice, rats, rabbits and pigs. Ultrasound Med Biol 2006; 32:1793-804.
12. Jagła M, Krzeczek O, Buczyńska A, Zakrzewska Z, Kwinta P. The safety of pulmonary ultrasonography in the neonatal intensive care unit. Dev Period Med 2018; 22:75-80.
13. Maxwell LG, Fraga MV, Malavolta CP. Assessment of Pain in the Newborn: An Update. Clin Perinatol 2019; 46:693-707.
14. Gallo AM. The fifth vital sign: implementation of the Neonatal Infant Pain Scale. J Obstet Gynecol Neonatal Nurs 2003; 32:199-206.
15. Lawrence J, Alcock D, McGrath P, Kay J, MacMurray SB, Dulberg C. The development of a tool to assess neonatal pain. Neonatal Netw 1993; 12:59-66.
16. Cruz MD, Fernandes AM, Oliveira CR. Epidemiology of painful procedures performed in neonates: A systematic review of observational studies. Eur J Pain 2016; 20:489-98.
17. Lee HC, Bennett MV, Schulman J, Gould JB, Profit J. Estimating Length of Stay by Patient Type in the Neonatal Intensive Care Unit. Am J Perinatol 2016; 33:751-7.
18. Lavanga M, Bollen B, Caicedo A, et al. The effect of early procedural pain in preterm infants on the maturation of electroencephalogram and heart rate variability. Pain 2021; 162:1556-66.
19. Brummelte S, Grunau RE, Chau V, et al. Procedural pain and brain development in premature newborns. Ann Neurol 2012; 71:385-96.
20. Duerden EG, Grunau RE, Guo T, et al. Early Procedural Pain Is Associated with Regionally-Specific Alterations in Thalamic Development in Preterm Neonates. J Neurosci 2018; 38:878-86.
21. Tortora D, Severino M, Di Biase C, et al. Early Pain Exposure Influences Functional Brain Connectivity in Very Preterm Neonates. Front Neurosci 2019; 13:899.
22. Hohmeister J, Kroll A, Wollgarten-Hadamek I, et al. Cerebral processing of pain in school-aged children with neonatal nociceptive input: an exploratory fMRI study. Pain 2010; 150:257-67.
23. Vinall J, Grunau RE. Impact of repeated procedural pain-related stress in infants born very preterm. Pediatr Res 2014; 75:584-7.
24. Chimello JT, Gaspardo CM, Cugler TS, Martinez FE, Linhares MB. Pain reactivity and recovery in preterm neonates: latency, magnitude, and duration of behavioral responses. Early Hum Dev 2009; 85:313-8.
25. Morelius E. Stress in infants and parents. Studies of salivary cortisol, behaviour and psychometric measures [dissertation]. Linköping, Sweden: Linköping University; 2006.
26. Prevention and management of pain and stress in the neonate. Paediatr Child Health 2000; 5:31-47.
27. Fitri SYR, Lusmilasari L, Juffrie M, Rakhmawati W. Pain in Neonates: A Concept Analysis. Anesth Pain Med 2019; 9:e92455.
28. Raja SN, Carr DB, Cohen M, et al. The revised International Association for the Study of Pain definition of pain: concepts, challenges, and compromises. Pain 2020; 161:1976-82.
29. Sinha R, Lacadie CM, Constable RT, Seo D. Dynamic neural activity during stress signals resilient coping. Proc Natl Acad Sci U S A 2016; 113:8837-42.
30. Tracey I, Mantyh PW. The cerebral signature for pain perception and its modulation. Neuron 2007; 55:377-91.