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Research paper

Measurement of stress in stable neonates during ambulance transportation: A feasibility study

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ABSTRACT

Background: Stress during ambulance transportation has been described in adult healthy volunteers where indicators of stress such as heart rate, blood pressure, and cortisol increased significantly. In neonates, a few studies have described stress in ambulance with behavioural scales. However, there is no study in neonates assessing both behavioural and physiological indicators of stress simultaneously during ambulance transportation.
Objective: To assess the feasibility of a study aiming at identifying stress in clinically stable neonates during ambulance transportation in non-emergency situations.
Methods: Stable neonates transported by ambulance from September 2015 to January 2016 were eligible. Physiological and behavioural parameters of stress were measured during the entire transfer procedure, starting on the ward of departure until hospitalisation at destination. Physiological parameters included salivary cortisol concentration, heart rate, respiratory rate, and oxygen saturation. Behavioural parameters were measured with the Comfort Behavior and the Premature Infant Pain Profile-Revised scales.
Results: Twenty neonates were included. The study proved to be feasible, but collection of saliva for cortisol measurement was problematic. To reach a sufficient amount of saliva, the collection time had to be extended from 90 to 300 s. Physiological parameters demonstrated heterogeneous patterns of stress. Behavioural scores increased during the entire transfer procedure and did not return to baseline values, indicating discomfort, specifically during transfer from the cot into the transport incubator.
Conclusions: Salivary cortisol values were variable. Behavioural measurement of stress provided a more sensitive measure to detect low level of stress, as shown in our sample of stable neonates, during non-emergency transportation.

1. Introduction

In adults, emergency ambulance transportation has been shown to induce stress in healthy volunteers measured by increased heart rate, blood pressure, and stress hormones levels, including cortisol.[1,2] In neonates, although physical stressors caused by vibration, movement, acceleration, braking, noise, and shocks associated with ambulance transportation have been widely studied,[3–8] there is limited evidence of their effect on neonates' health wellbeing.

Stress is defined as "a physical, chemical, or emotional factor that causes bodily or mental tension and may be a factor in disease causation".[9] In neonates, stress is difficult to measure. There is no validated instrument available for its assessment, and stress can be either specific to a stressor or non-specific. Therefore, observational

Abbreviations: CB, The comfort behavior; PIPP-R, The premature infant pain profile-revised; GA, Gestational age; NICU, Neonatal intensive care unit.

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behavioural pain and discomfort scales have commonly been used as proxy measures of stress. The Premature Infant Pain Profile-Revised (PIPP-R) and the Comfort Behavior (CB) scales have strong psychometric properties and have been well implemented in acute hospital settings to assess pain and discomfort.[10] As a physiological measure, the salivary cortisol level has been reported to be a useful and non-invasive parameter of newborn infant's stress response.[11]

Only a few studies have evaluated neonatal stress experienced during transportation, by assessing either behavioural parameters with the PIPP scale[12] or physiological parameters, such as heart rate or heart rate variability.[5,13,14] To the best of our knowledge, no study has described neonatal stress by measuring simultaneously behavioural and physiological parameters during ambulance transportation to date.

This feasibility study focused, therefore, on planned ambulance transfers of clinically stable neonates no more in need of neonatal intensive care. Transfers were from the university level III hospital to a regional hospital close to parent's home. Although the patients were stable, these transfers are still potentially associated with physical stressors.[5,12,15] Clinically stable neonates were selected for reference baseline stress or discomfort measures in a low-risk population with no potential bias introduced by a pathology. We hypothesised that clinically stable neonates experience increased stress during transportation compared to baseline. The aim of this feasibility study was to measure stress in neonates during ambulance transportation, using both behavioural and physiological measures.

2. Methods

This study was conducted in the neonatal intensive care unit (NICU) of the Lausanne University Hospital, Switzerland, where around 500 neonatal transports per year are conducted. Approximately half of them (46%) include ambulance transfers of clinically stable neonates. All neonates transported by ambulance from the NICU to the ward of a regional hospital between September 2015 and January 2016 were eligible. A convenience sample of 20 neonates was included in the study, based on the guideline recommendations for sample size for pilot studies by Hertzog.[16] Exclusion criteria were infants over 44 weeks of corrected gestational age at the moment of transfer and/or whose parents did not speak French. Parents were approached for information and written consent the day before transport. The study was accepted by the Cantonal Ethics Committee for Human Research (Project: 321/15).

Each transport was carried out as per standard practice in a transport incubator (Voyager, Airbone® Life Support Systems, International Biomedical, Austin, Texas, USA) equipped with an isothermal cover. Two ground ambulances for neonatal transport were used (VW T5® Baby Rescue and IVECO®). The entire transfer procedure was divided into six time slots (Fig. 1): step 1—baseline situation before any transfer procedure at the university hospital; step 2—transfer of the newborn infant from the cot into the transport incubator; step 3—intra-hospital transport from the clinical ward to the ambulance; step 4—road transfer between the two hospitals; step 5—intra-hospital transport to the receiving ward; and step 6—transfer of the newborn infant to the cot in the regional hospital.

Clinical data of the participants were extracted from the patient's electronic database (Métavision®; iMDsoft, Tel Aviv, Israel) at the university hospital (baseline data) and from the transport records. The physiological and behavioural parameters of stress were measured at each step and every 15 min during the road transport (step 4). Heart rate, respiratory rate, and oxygen saturation were continuously recorded throughout the procedure with the Philips® IntelliVue X2 Transport Monitor (Boeblingen, Germany). All data were collected by the observing research nurse (MZ) who was not involved in the patient's care.

At step 1 and 6, two saliva samples were collected from each participant using a commercial device (SalivaBio Infant's Swab, SIS-Salimetrics®, Carlsbad, California, USA). Saliva was collected using an absorbent swab stick inserted into the neonate's mouth for 300 s without any additional stimulation of saliva production. To avoid contamination of samples, salivary collection was done 1 h after enteral feeding. All samples were centrifuged (15 min at 1500× g) within 4 h of sampling and stored at −80°C until assayed. Salivary cortisol levels were determined using a high sensitivity enzyme immunoassay kit (Salimetrics®, Newmarket, Suffolk, UK) according to the manufacturer's protocol.[17] Minimum salivary sample volume required for the assay was 40 μ_1, but samples were run in duplicate if >75 μ_1 were available. Samples were destroyed after analyses.

The PIPP-R is a well-established and clinically validated multidimensional scale to assess procedural pain response in premature

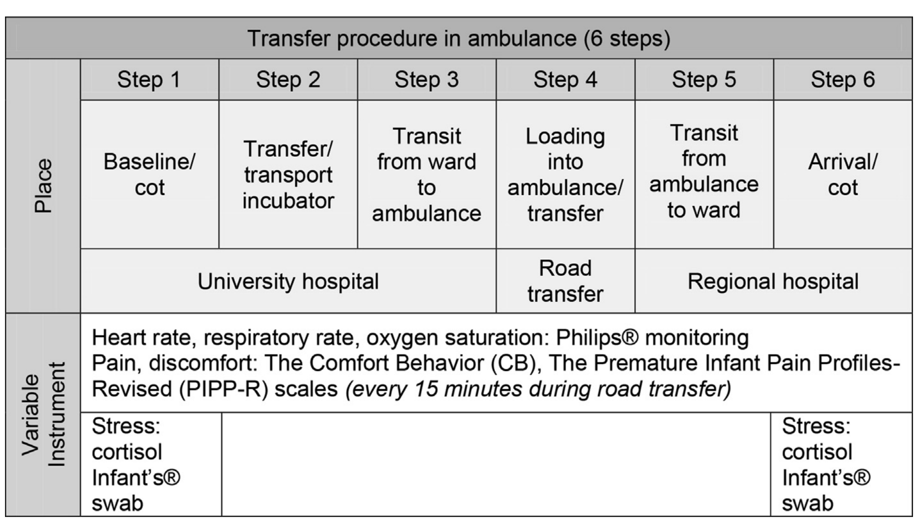

Fig. 1. Measures and instruments used for each step of the transfer procedure in ambulance transportation from the NICU university hospital to regional hospitals. NICU = neonatal intensive care unit.

or full-term infant.[18] It includes two physiological parameters (changes in heart rate and oxygen saturation), three behavioural parameters (duration of brow bulge, eye squeeze, and naso-labial furrow), and two contextual items [gestational age (GA) and behaviour]. Each item is numerically scored on a four-point Likert scale from 0 to 3. The total scores can range and depend on the weighting of GA (<28 weeks GA: 3 points, 28–31 + 6/7 weeks GA: 2 points, 32–35 + 6/7 weeks GA: 1 point and ≥ 36 weeks GA: 0). The PIPP-R scores between 0 and 6 indicate minimal or no pain, 7 to 12 reflect moderate pain, and 13 to 21 reflect severe pain. Behaviour was assessed by observing each participant for 45 s at each step.

The CB score is a reliable, valid, and well-established scale to assess pain and distress in infants and children.[19] The CB includes six behavioural items that are each scored from 1 to 5: alertness, calmness/agitation, respiratory response in mechanically ventilated patients or crying in spontaneously breathing neonates, physical movement, muscle tone, and facial tension. The total score varies from 6 (no pain) to 30 (maximum). Scores ≥ 17 indicate pain or discomfort and require pain medication or adjustment of the situation.[19] Rating was performed after the recommended 2-min observation at each step and every 15 min during the road transfer (step 4), first with the PIPP-R scale and secondly with the CB scale.

3. Results

Of the 93 screened neonates, 73 were eligible, and 20 were included in the study (Fig. 2). The majority of parents (96%) approached for consent agreed to participate in the study. Reasons for not being included were due to logistic and human resource constraints independent of the patients. Eight boys and 12 girls were included. The majority of participants were premature infants without ventilatory support. Demographic and clinical data are described in Table 1. To reach the target sample size of 20, data were collected over 17 weeks between September 2015 and January 2016.

The initial technique of saliva collection as recommended by the manufacturer proved to be inadequate due to insufficient volume of saliva. The recommended sample collection volume required a minimum of 200 μl for automated duplicates testing.[17] Volume of collected saliva remained below expectation, not allowing for automated testing. The duration of swabbing had thus to be extended from 90 to 300 s by soaking both sides of the absorbent swab stick, allowing for manual testing only (>75 μ_1). From the 40 collected saliva samples, 38 (95%) were analysed, and two (5%) did not have sufficient amount of saliva for analysis. Out of 38, 31 were analysed in manual duplicate (>75 μ_1) and seven in manual singlet (>40–45 μ_1).

The average salivary cortisol levels (μg/dL) were not statistically different between step 1 (baseline) and step 6 (end of procedure) [mean = 0.222, standard deviation (SD) ± 0.164 and mean = 0.259, SD ± 0.173, p = 0.663, respectively] (Fig. 3).

There were no significant changes in heart rate, respiratory rate, and oxygen saturation during the whole transfer procedure and between values at baseline and the end of the transfer (Table 2). In step 1, average heart rate beats per minute (bpm), respiratory rate (per minute), and oxygen saturation (%) varied between participants (Table 2).

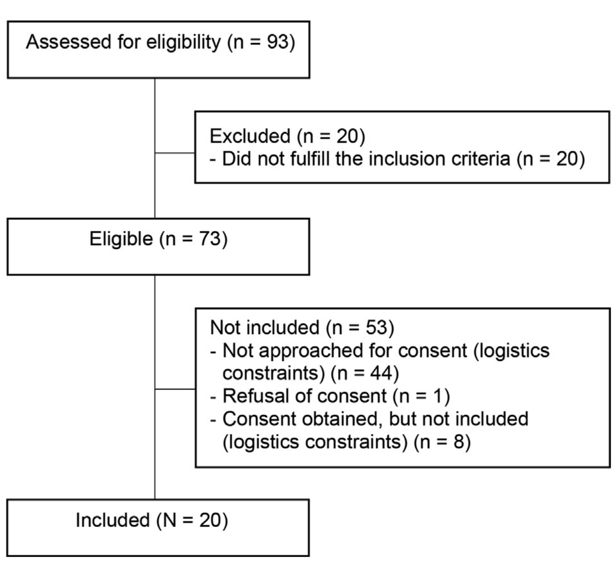

Fig. 2. Flow chart of participants included.

Table 1
Demographic and health characteristics of neonates (N = 20).

Characteristics	n	%
Gender		
Male	8	40
Female	12	60
Race		
Caucasian	16	80
Mixed (Caucasian/African or Caucasian/Latin America)	4	20
Age gestational week at birth		
<32	8	40
32–33	4	20
34–36	5	25
≥37	3	15
Age corrected week during transport		
32–33	5	25
34–36	8	40
≥37	7	35
Respiratory support (n = 4)		
Oxygen therapy (high flow nasal)	2	50
Nasal continuous positive airway pressure	2	50
Diagnosis during initial hospitalisation in NICU[a]		
Prematurity	17	85
Respiratory distress syndrome	16	80
Suspicion of neonatal infection	11	55
Intrauterine growth restriction	5	25
Neonatal asphyxia	1	5
Drug withdrawal syndrome	1	5

NICU = neonatal intensive care unit.
[a] Participants could have up to five diagnoses at admission; percentages are calculated out of N = 20 for each diagnosis.

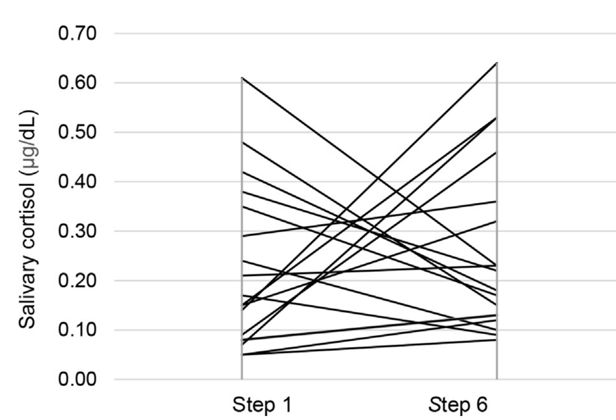

Fig. 3. Salivary cortisol levels (μg/dL) for neonates in step 1 (baseline) and in step 6 (end of the transfer procedure) (n = 18). Each line represents one individuum.

Table 2
Average of heart rate, respiratory rate, and oxygen saturation in neonates for each step of the transfer procedure (N = 20).

Transfer (step)	Heart rate	Respiratory rate	Oxygen saturation
	Mean (SD) (min–max)	Mean (SD) (min–max)	Mean (SD) (min–max)
Baseline, university hospital (1)	149/min (16.3) (111–182)	55/min (11.1) (36–82)	98% (1.6) (92–99)
Transfer of the neonate into the transport incubator (2)	151/min (20.9) (125–194)	55/min (9.9) (42–78)	96% (2.6) (88–100)
Intra-hospital transport (3)	149/min (19.9) (122–197)	55/min (8) (46–70)	96% (2.6) (89–100)
Road transfer (4)	149/min (14.4) (126–178)	52/min (9.4) (36–68)	95% (2.9) (89–100)
Intra-hospital transport in regional hospital (5)	152/min (17.1) (127–187)	52/min (10) (35–67)	95% (2.9) (90–100)
Transfer of the neonate into the cot at regional hospital (6)	155/min (17.8) (130–187)	54/min (11.2) (38–84)	96% (2.8) (91–100)

NICU = neonatal intensive care unit; SD = standard deviation.

The CB and the PIPP-R average scores nearly doubled between step 1 and step 6. The average pain scores increased by two-fold or more at step 2, CB (mean = 17.6, SD ± 6.8) and PIPP-R (mean = 6.5, SD ± 3.3), during transfer into the transport incubator and did not return to baseline values during the whole transport procedure (Table 3).

4. Discussion

To the best of our knowledge, this is the first feasibility study that has focused on stress in stable neonates during ambulance transportation, measuring simultaneously physiological parameters, including salivary cortisol levels and two behavioural pain scales. Although parental acceptance was high in this feasibility study, only 27% of selected patients could be included due to logistic and human resource limitations. This indicates that to undertake a larger study, more resources would be needed to increase capacity of research staff.[20]

Salivary cortisol levels showed not to be an adequate measure to identify stress in stable neonates during ambulance transportation. The choice for salivary cortisol collection in neonates was based on a commercial device adapted for this specific population.[17] Several studies have used the same instrument to get at least 75 μl in order to run the cortisol level analysis in duplicates.[11,21,22] However, this sampling method initially proved not to be sufficient in this study. Others have reported similar issues regarding insufficient volume of saliva for the cortisol assay, sometimes affecting for up to half of their samples.[11,22–24] To address this issue, some researchers have used additional stimulation of saliva production such as citric acid.[24] Others have performed the sampling with saliva suction.[23,25] In this study, to optimise saliva collection, the time of sampling was extended from 90 to 300 s, and both sides of the absorbent swab stick were used without using any stimulation of saliva production.

Salivary cortisol levels showed a high variability in neonates and did not exhibit significant changes between baseline and the end of the transfer procedure. Although there were no cortisol baseline reference values for neonates, salivary cortisol levels (in μg/dL) were comparable to other neonatal studies.[23,24] The interindividual variability of cortisol levels was comparable to that observed in other studies,[25,26] demonstrating difficulties in interpreting salivary cortisol responses in preterm infants and neonates. Nevertheless, several intervention studies have shown significantly decreased levels of salivary cortisol values in neonates or preterm infants during painful procedures in response to comfort interventions, such as prone position,[23] skin-to-skin care,[21] and music therapy.[27] One could conclude from these reports that cortisol measurement in neonates may still add some objective values to measure stress-induced procedures. Evidence suggests that cortisol levels are variable at birth and during the first week of life. It depends on the development of the nycthemeral cycle, the adrenal circadian rhythm, and the maturity of the hypothalamic–pituitary–adrenal axis.[28] The circadian rhythm influenced by the nycthemeral cycle is established between the second and third month postnatal of life.[28] In contrast, recent research suggests that the nycthemeral cycle is acquired in utero by exposure to maternal stimuli.[22,25] During the first few days of life, hormonal and placental factors, stress experienced at birth, and antenatal corticosteroids influence the cortisol level of the newborn.[21] In this study, participant's postnatal age was heterogeneous, ranging between four days and 12 weeks, which might explain the unreliable values of salivary cortisol.[28]

Our findings showed no significant changes in heart rate between baseline and the end of the transfer. These results are supported by other studies describing no significant impact on heart rate during ground and air neonatal transportation, respectively.[5,13] However, they found a heart rate variability which was significantly increased by the end of transport, suggesting it might be a more sensitive indicator of neonatal stress.[5,14] It is worth noting that in these studies also critically ill neonates were included, which contrasts with this study. Measurement of heart rate variability was considered when designing the study but was not included for logistic reasons.

In contrast to physiological measures, behavioural parameters scores measured by the CB and the PIPP-R scales were higher at each step compared to baseline. Both scales average scores doubled

Table 3
Average scores of the Comfort Behavior (CB) and the Premature Infant Pain Profile-Revised (PIPP-R) scales in neonates during transfer procedure for each step (N = 20).

Transfer (step)	CB Mean (SD)	PIPP-R Mean (SD)
Baseline, university hospital (1)	8.8 (2.7)	2.9 (1.3)
Transfer of the neonate into the transport incubator (2)	17.6 (6.8)	6.5 (3.3)
Intra-hospital transport (3)	12.4 (3.9)	5.4 (1.8)
Road transfer (4)	12 (2.4)	6 (1.6)
Intra-hospital transport in regional hospital (5)	13.4 (4.3)	5.8 (2.3)
Transfer of the neonate into the cot at regional hospital (6)	17.3 (4.5)	5.7 (1.9)

SD = standard deviation.
CB: cut-off score ≥ 17; PIPP-R: cut-off score ≥ 6.

between baseline and the end of the transfer procedure. The CB scores increased mainly during transfer of the newborn into the transport incubator before and after transportation, indicating pain or discomfort. The PIPP-R scores demonstrated a trend for increased pain or discomfort between baseline and the remaining steps of the transfer procedure, as performed by others.[12]

In adults, excess movements, surrounding noise, and vibration in ambulance transportation were recognised as uncomfortable experiences.[2] This appears to be similar in transported clinically stable newborn infants, as demonstrated by our findings that both pain and discomfort scores did not return to baseline values during the whole transfer procedure. One can assume similar, if not increased, pain and discomfort level in infants who are critically ill. It therefore would be sensible to further explore whether those particularly vulnerable infants experience stress and discomfort during ambulance transportation and to what extent. In the present sample, almost half of the neonates had high behavioural scores, suggesting moderate to severe pain or discomfort at least one step of the transfer procedure. It is indeed difficult to interpret those scores as pain is certainly stressful, but stress is not always related to pain. It is, therefore, important to take all possible stressors during transportation into account, including potential sources of pain, before making decision about treatment. Interventions to reduce noise and vibrations, by non-pharmacologic procedures,[29] should be favoured.

Using both scales in this study, it appeared the PIPP-R may be more sensitive and specific to pain as compared to the CB, as PIPP-R weights the total pain score by taking developmental neonatal age into account. In addition, the PIPP-R was specifically designed for premature and term infants, as opposed to the CB adapted for neonates with no distinction between premature and full-terms. Behavioural pain scales have been validated in the NICU;[18,19] however, adaptation of one of those instruments for neonatal transportation is warranted.

Limitations of this study were the small sample size and the heterogeneous characteristics of the patients with confounding factors, such as postnatal age, corrected gestational age, time of saliva collection, and duration of transport. Given the exploratory nature of this feasibility study, the sample size was adequate but too small to draw conclusive results. However, we were able to demonstrate a trend of increased behavioural scores between baseline and the end of the transfer procedure, suggesting some degree of discomfort or stress.

5. Conclusion

The results underline that the research protocol aiming to identify the stress measurement in stable neonates during ambulance transportation was feasible, except for the measurement of the salivary cortisol. This suggests that devices for sampling saliva should be developed for the newborn population. For its use as a potential stress indicator, reference values in the newborn population should be established. The two validated behavioural CB and PIPP-R scales have shown some degree of pain or discomfort in neonates during transfer procedure, indicating that transfer, especially handling of the patient may be stressful for the infant. Although we cannot draw any conclusive statement about salivary cortisol and the other physiological measures, further clinical research is needed to evaluate the impact of the physical stressors on healthy and sick neonates during ambulance transportation.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.aucc.2018.06.006.

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