Assessment of pain during application of nasal-continuous positive airway pressure and heated, humidified high-flow nasal cannulae in preterm infants

Article *in* Journal of perinatology: official journal of the California Perinatal Association · November 2014
DOI: 10.1038/jp.2014.206 · Source: PubMed

3 authors, including:

Ashraf A Elsharkawy
Mansoura University
19 PUBLICATIONS **176** CITATIONS
SEE PROFILE

Hesham Abdel-Hady
Mansoura University
64 PUBLICATIONS **620** CITATIONS
SEE PROFILE

Journal of Perinatology (2015) 35, 263–267

www.nature.com/jp

ORIGINAL ARTICLE

Assessment of pain during application of nasal-continuous positive airway pressure and heated, humidified high-flow nasal cannulae in preterm infants

M Osman[1], A Elsharkawy[2] and H Abdel-Hady[1]

OBJECTIVE: To assess pain and compare its severity in preterm infants during application of nasal-continuous positive airway pressure (nCPAP) and heated, humidified high-flow nasal cannulae (HHHFNC).

STUDY DESIGN: An observational cross-sectional study. Sixty preterm infants, categorized into nCPAP ($n = 37$) and HHHFNC groups ($n = 23$). Pain response was assessed using Premature Infant Pain Profile (PIPP), duration of first cry and salivary-cortisol concentrations.

RESULT: The PIPP scores were significantly higher in the nCPAP compared with HHHFNC group (10 (7–12) vs 4 (2–6), $P < 0.01$). None of the infants in the HHHFNC group had severe pain defined as a PIPP score >12, compared with 5 (13.5%) infants in the nCPAP group. Salivary-cortisol concentrations were significantly higher in nCPAP group compared with the HHHFNC group (5.0 (3.6–5.9) vs 1.6 (1.0–2.3) nmol l^{-1}, $P < 0.01$). A lower incidence of cry was observed for infants in the HHHFNC group compared with the nCPAP group (11 (47.8%) vs 30 (81.1%), $P < 0.001$), however, the duration of first cry was not significantly different between groups. The respiratory rate was significantly lower after application of HHHFNC compared with nCPAP ($P < 0.001$). There were no significant differences between groups with regard to fraction of inspired oxygen (FiO_2), oxygen saturation by pulse oximeter (SpO_2) and heart rate.

CONCLUSION: The application of HHHFNC in preterm infants is associated with less pain compared with nCPAP, as it is associated with less PIPP scores and lower salivary-cortisol concentrations.

Journal of Perinatology (2015) **35,** 263–267; doi:10.1038/jp.2014.206; published online 27 November 2014

INTRODUCTION

Neonates admitted to neonatal intensive care units (NICUs) experience multiple stressful and painful procedures.[1] Despite guidelines from professional societies for the management of procedural pain in neonates,[2] most of these procedures are performed without pharmacological or non-pharmacological analgesia.[3] Untreated pain during this critical period of brain development is associated with both immediate and long-term consequences.[4]

Nasal-continuous positive airway pressure (nCPAP) is frequently used as a respiratory support strategy for preterm infants. Several nCPAP devices are available, but all can cause nasal trauma which can result in permanent deformities.[5–7] Moreover, many preterm infants do not tolerate the nCPAP prongs, which must be tightly affixed to the nose and face. Heated, humidified high-flow nasal cannulae (HHHFNC) is increasingly used as an alternative means of providing non-invasive respiratory support in preterm infants.[8,9] Because HHHFNC have a simpler interface with the infant and smaller prongs than nCPAP, it is considered a more convenient and "gentler" way to provide nCPAP, the HHHFNC are perceived as easier to use, more comfortable for the infant, less traumatizing to the nose,[10–12] advantageous for mother–infant bonding,[11] and reduces cost.[12] No previous study has assessed and compared objectively the pain response during the application of nCPAP and HHHFNC in preterm infants. We hypothesized that the application of HHHFNC is less painful compared with the application of nCPAP in preterm infants requiring respiratory support.

METHODS

Subjects

This is an observational cross-sectional study performed on 60 preterm infants who are categorized into two groups; the first group received nCPAP, the second group received HHHFNC. This study was conducted in the NICU at Mansura University Children's Hospital in the period between December 2012 and January 2014. The study was approved by the local Medical Research Ethics Committee and written informed consent was obtained from parent(s) or guardian(s) before the study. We enrolled preterm infants at the time of application of nCPAP or HHHFNC. The decision of putting the baby on nCPAP or HHHFNC was according to the attending neonatologist's decision, both modalities are used in our NICU for neonates requiring respiratory support. We excluded the infants: (1) with signs of serious, life-threatening malformations, (2) who had undergone any surgical intervention, (3) who had undergone any painful procedures, such as venipuncture, intubation, suctioning, blood sampling, heel-prick, catheterization, and so on, 30 min before assessment, (4) in whom salivary samples could not be obtained or were contaminated by blood and (5) with signs of nasal injuries at the time of application of nCPAP or HHHFNC.

[1]Neonatal Intensive Care Unit, Mansoura University Children's Hospital, Egypt and [2]Pediatric Endocrinology and Diabetes Unit, Mansoura University Children's Hospital, Egypt. Correspondence: Professor H Abdel-Hady, Department of Neonatology, Neonatal Intensive Care Unit, Mansoura University Children's Hospital, Gomhoria street, Mansoura, Dakahlia 35516, Egypt.
E-mail: hehady@yahoo.com
Received 6 July 2014; revised 17 September 2014; accepted 9 October 2014; published online 27 November 2014

Nasal-continuous positive airway pressure

In total, 37 preterm infants were included in the nCPAP group. The need for nCPAP was based on: the presence of signs of respiratory distress ($n = 23$, 62%), recurrent apnea ($n = 3$, 8%), or as a method of weaning from mechanical ventilation ($n = 11$, 30%). The INCA Infant Nasal CPAP Assembly (Cooper Surgical, Trumbull, CT, USA) was used to deliver nCPAP. The prong size was chosen from five different available sizes, for appropriate nasal prong fitting, the INCA sizing gauge was used. The nasal prongs should fill the nasal opening completely without stretching the skin or putting undue pressure on the nares. The bridge of the prong was not allowed to press up against the septum, a small space was left between the tip of the septum and the bridge between the prongs. The mean initial nCPAP pressure was 5.0 cm H_2O in all infants, the pressure was altered at the physician's discretion in a stepwise fashion during the course of NICU admission.

High-flow nasal cannulae

A total of 23 infants received HHHFNC. The need for HHHFNC was based on the presence of signs of respiratory distress ($n = 15$, 65%), recurrent apnea ($n = 2$, 9%), or as a method of weaning from mechanical ventilation ($n = 6$, 26%). Bi-nasal cannulae, 2.4-mm external diameter (Ultramed Med Healthcare, Cairo, Egypt) were used. The HHHFNC therapy system was composed of oxygen and air source, blender, flow meter, humidifier (MR850 humidifier, Fisher & Paykel Healthcare, Aukland, New Zealand). The inspired gas temperature was set at 37 °C. Nasal cannulas were applied according to manufacturer' suggestions with recommendations that the prong-outer diameter occupy ~50% of the nares internal diameter. The typical starting flow rate was 4 l min^{-1}. Flow rates were altered at the attending physician's discretion in a stepwise fashion, with mandated limits between 2 l min^{-1} and the maximum 8 l min^{-1}.

None of the infants in both groups received pharmacological or non-pharmacological pain alleviating interventions and no topical anesthetics or hydrocolloid gel were used at the time of application of nCPAP or HHHFNC. Patients were monitored for heart rate and respiratory rate using Draeger Infinity Delta cardio-respiratory monitor (Draeger Medical Systems, Danvers, MA, USA), and preductal oxygen saturation by pulse oximeter (SpO_2) was measured using Nellcor OxiMax N-600 Oximeter (Covidien-Nellcor and Puritan Bennett Boulderm, Boulder, CO, USA). The baseline heart rate and SpO_2 before application of nCPAP/ HHHFNC and the maximum heart rate and the minimum SpO_2 in the 30 s after the application of nCPAP/ HHHFNC were used to calculate the physiological indices in the Premature Infant Pain Profile (PIPP) score. The initial fraction of inspired oxygen (FiO_2) was 30%, the FiO_2 was adjusted to keep SpO_2 (90–95%).

Measurements

Pain assessment was based on PIPP, presence of crying, duration of first cry and salivary-cortisol concentrations. The duration of the first cry was defined as audible distressed vocalizations with a continuous pattern before a quiet interval of 5 s soon after application of nCPAP or HHHFNC.

Premature infant pain profile

The PIPP[13] was used to measure pain at bedside, directly after application of nCPAP/HHHFNC. PIPP comprises three behavioral variables (time of brow bulge, eye squeeze and naso-labial furrow), two physiologic variables (changes in heart rate and SpO_2) and two contextual variables (gestational age and behavioral state). Behavioral state ranges from "active/awake, eyes open, facial movements" to "quiet/sleep, eyes closed, no facial movements." Every variable is scored on a scale from 0 to 3. A total score, the sum of total of points, indicating: lack of pain (0–6), mild–moderate pain (6–12) and severe pain (above 12). PIPP has documented reliability and validity and has been used previously in several studies in neonates.[14,15] PIPP score measurement was based on video recording the infant for 45 s. Three different DVDs were compiled with the sets in random order. Three different nurses from NICU were recruited to evaluate the segments. They were not informed of the nature of the study. All three nurses were trained in performing the PIPP. The facial expression component of the PIPP was re-scored by two observers in 15 of of the 60 (25%) videos to assess intra-rater and inter-rater reliability using the Bland–Altman test. Bland–Altman plots showed good reliability with little bias (intra-rater bias 0.08; inter-rater bias 0.76). The limits of agreement for the intra-rater re-test were ± 1.71. The limits of agreement for the inter-rater comparison were ± 1.52.

Salivary cortisol

Salivary samples were obtained 30 min after application of nCPAP/ HHHFNC using sterile-single channel 500 µl pipette (Dragon Laboratory Instruments, Beijing, China). All salivary samples were obtained before the introduction of feeds to avoid contamination of saliva samples with milk. After collection, the saliva was centrifuged, frozen and stored at − 70 °C. The samples were later analyzed using enzyme linked immunosorbent assay technique; IBL kits (IBL International GmbH, Hamburg, Germany). Intra-assay coefficients of variation were 12% at 2.0 nmol l^{-1} and 6.0% at 10.0 nmol l^{-1}. Samples were run neat in duplicate, and all samples from an individual were run in the same assay.

Statistical analysis

Statistical analyses were performed using SPSS statistical software (version 16; SPSS, Chicago, IL, USA). Kolmogrov–Smirnov test was done to examine the distribution of data. Student's-*t* test was used to compare continuous parametric variables; Mann–Whitney *U*-test was used to compare continuous non-parametric variables; X^2 test or Fisher's exact test were used for categorical variables, when appropriate. Bland–Altman test was used to assess intra-rater and inter-rater reliability for PIPP. A P-value of < 0.05 was considered to be statistically significant.

RESULTS

A total of 79 infants were screened for participation and 60 infants qualified on the basis of inclusion and exclusion criteria. Nineteen infants were not included in the study owing to the failure to obtain consent ($n = 9$), failure to obtain salivary-cortisol ($n = 6$) or saliva samples were contaminated with blood ($n = 2$), complex congenital heart disease ($n = 1$) and surgical interventions ($n = 1$).

There were no significant differences between the groups regarding demographic data, clinical characteristics and respiratory outcomes (Table 1). The mean ± s.d.-initial nCPAP pressure was 5.0 ± 0.82 cm H_2O and the mean ± s.d.-initial HHHFNC flow rate was 4.0 ± 1.88 l min^{-1}. The PIPP scores were significantly higher in the nCPAP compared with HHHFNC group (10 (7–12) vs 4 (2–6), $P < 0.01$)); Figure 1). None of the infants in the HHHFNC group had severe pain defined as a PIPP score >12, compared with 5 (13.5%) infants in the nCPAP group (Table 2).

Table 1. Demographic data, clinical characteristics and respiratory outcome of the study groups

	nCPAP (n = *37*)	*HHHFNC* (n = *23*)	P-*value*
Gestational age (weeks)[a]	31.9 ± 3.2	32.3 ± 1.8	0.58
Birth weight (g)[a]	1687 ± 555	1797 ± 561	0.46
Postnatal age (days)[b]	6 (3.5–12)	5 (3–11)	0.66
Male sex[c]	15 (40.5%)	12 (52.2%)	0.38
Cesarean delivery[c]	32 (86.5%)	20 (87%)	0.96
Inborn[c]	11 (29.7%)	7 (30.4%)	0.99
Mechanical ventilation[c]	21 (56.8%)	9 (39.1%)	0.18
Antenatal steroids[c]	23 (62.2%)	14 (60.9%)	0.57
Surfactant therapy[c]	4 (10.8%)	3 (13.0%)	0.55
Caffeine therapy[c]	24 (64.9)	18 (78.3)	0.21
Total parentral nutrition[c]	6 (16.2%)	3 (13.0%)	0.52
Central venous access[c]	6 (16.2%)	4 (17.4%)	0.59
Pneumothorax[c]	1 (2.7%)	1 (4.3%)	0.62
Duration of oxygen therapy (days)[b]	8 (1–9)	7 (1–9)	0.73
Duration of respiratory support (days)[b]	16 (3–32)	12 (2–34)	0.89
Duration of hospitalization (days)[b]	27 (18–38)	24 (17–36)	0.48

Abbreviations: HHHFNC, heated, humidified high-flow nasal cannulae; nCPAP, nasal-continuous positive airway pressure. [a]Data expressed as mean +s.d. [b]Median (interquartile range). [c]Number (%).

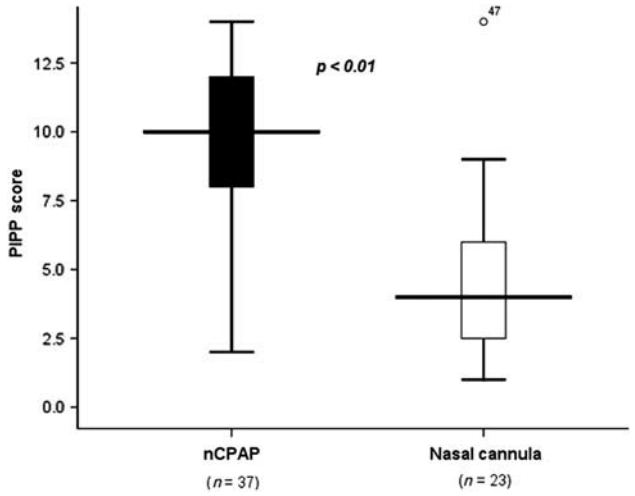

Figure 1. PIPP scores in study groups.

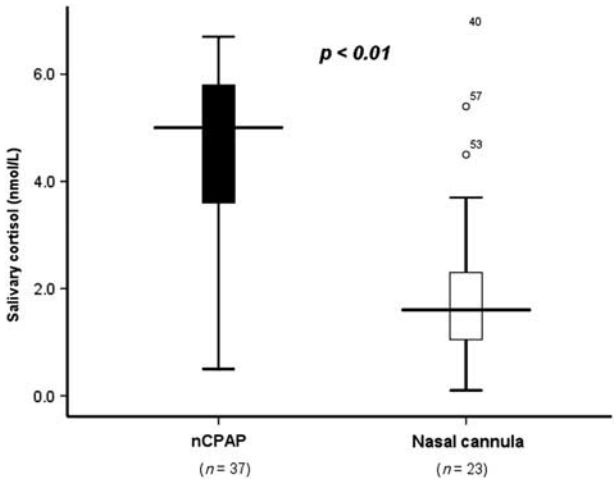

Figure 2. Salivary cortisol in study groups.

Table 2. PIPP scores, presence of cry, and duration of first cry in study groups

	nCPAP (n = *37*)	*HHHFNC* (n = *23*)	P-*value*
PIPP score[a]	10 (7–12)	4 (2–6)	< 0.01
PIPP score[b]			
< 6	13 (35.1%)	16 (69.6%)	
6–12	19 (51.4%)	7 (30.4%)	0.03
> 12	5 (13.5%)	0	
Cry[b]			
Yes	30 (81.1%)	11 (47.8%)	0.007
No	7 (18.9%)	12 (52.2%)	
Duration of first cry (seconds)[a]	49 (34–63)	22 (15–40)	0.58

Abbreviations: HHHFNC, heated, humidified high-flow nasal cannulae; nCPAP, nasal-continuous positive airway pressure; PIPP, Premature Infant Pain Profile. [a]Data expressed as median (interquartile range). [b]Data expressed as as number (%).

Table 3. Respiratory rate, fraction of inspired oxygen (FiO_2) and oxygen saturation by pulse oximeter (SpO_2) in the study groups

	nCPAP (n = *37*)	*HHHFNC* (n = *23*)	P-*value*
Respiratory rate (breath per minute)[a]	46 (40–55)	40 (36–44)	< 0.001
Heart rate (beat per minute)[b]	166 ± 23	148 ± 15	0.08
FiO_2 (%)[b]	37.5 ± 6.8	29.7 ± 2.1	0.06
SpO_2 (%)[b]	94.5 ± 4.0	93.5 ± 3.7	0.33

Abbreviations: FiO_2, fraction of inspired oxygen; HHHFNC, heated, humidified high–flow nasal cannulae; nCPAP, nasal-continuous positive airway pressure; SpO_2, oxygen saturation by pulse oximeter. [a]Median (interquartile range). [b]Data expressed as mean ± s.d.

Salivary-cortisol concentrations were significantly higher in the nCPAP group compared with HHHFNC group (5.0 (3.6–5.9) vs 1.6 (1.0–2.3) nmol l^{-1}, $P < 0.01$) (Figure 2).

A lower incidence of cry was observed for infants in the HHHFNC group compared with the nCPAP group ($P = < 0.001$), however, the duration of first cry was not significantly different between groups (Table 2). We recorded the respiratory rate, heart rate, FiO_2 and SpO_2 5 min after application of nCPAP/HHHFNC (Table 3). The respiratory rate was significantly lower after application of HHHFNC compared with nCPAP ($P < 0.001$). There were no significant differences between groups as regard FiO_2, SpO_2 and heart rate (Table 3).

DISCUSSION

nCPAP and HHHFNC therapy are the most commonly used respiratory support strategies in NICUs. As far as we know, no previous study have assessed and compared objectively the pain response during the application of nCPAP and HHHFNC in preterm infants.

We have demonstrated that the pain response was less during the application of HHHFNC than during application of nCPAP, as evidenced by lower PIPP scores, salivary-cortisol concentrations and less incidence of cry. The nCPAP prongs are designed to seal within the nares to maintain a constant-airway pressure. During nCPAP nasal injuries from prongs and masks are common and may result in permanent deformity.[5–7] HHHFNC, on the other hand, are smaller, lighter, do not need to be snuggly inserted into the baby's nostrils. This may lead to less discomfort during the insertion of the HHHFNC and less nasal trauma compared with nCPAP as reported in our study and in previous studies.[10–12]

A recent randomized cross-over trial,[16] compared patient comfort (defined as absence of prolonged pain assessed by EDIN scale),[17] in 20 preterm infants < 34 weeks gestation with HHHFNC vs nCPAP. They have found no differences between HHHFNC and nCPAP in mean-cumulative EDIN score. However, parents of enrolled infants preferred HHHFNC to CPAP.[16]

We opted to use the PIPP in our study. Many infant pain measures have been developed over the past two decades.[14,18] The majority of these measures have limited validation. The PIPP score is a well-developed and -studied composite measure of procedural pain and frequently used as an effective outcome measure in pain intervention studies in neonates.[13–15] We have assessed the PIPP scores using 45 s video recordings. As per the PIPP scoring guidelines, the behavioral state is scored by observing the infant for 15 s before and for 30 s immediately following the painful event.[13] Many studies used similar time frames in assessing PIPP scores using video-recodings.[19–21] More infants in the nCPAP had cried during the application of nCPAP

compared with HHHFNC group; although, the duration of first cry was not significantly different between groups. Assessment of pain through measuring isolated parameters such as the duration of cry has been criticized for being neither sensitive, nor specific.[22]

In our study, the salivary cortisol was significantly higher in infants receiving nCPAP compared with HHHFNC. Many investigators have previously shown the advantages of using salivary cortisol as a marker of stress and pain in newborn infants.[23,24] Measuring cortisol in saliva rather than in plasma is easy to perform, painless and non-invasive. Moreover, salivary-cortisol reflects free cortisol rather than total cortisol that can be affected by plasma-binding protein concentrations,[25] and it has been validated against serum cortisol in preterm infants.[26]

Despite these advantages, only a few studies assessed salivary-cortisol responses in preterm babies during painful procedures,[23,24] basically, as a result of methodological problems in obtaining sufficient amounts of saliva.[16,27,28] We managed to collect saliva by a novel technique using a pipette with a high success rate (95.3%) without using salivary-flow stimulants, such as citric acid, which may potentially result in inaccurately-high levels of salivary cortisol.[29] Another factor that may have increased our success in obtaining salivary-cortisol samples is that infants receiving nCPAP and HHHFNC often have increased salivation during the first few hours of therapy.[30]

Nursing staff should asses the pain response during application of nCPAP and HHHFNC. Intense pain should be managed with pharmacological agents, whereas lesser pain can be managed by means of non-pharmacological pain alleviating interventions.[31] According to our results, pharmacological and non-pharmacological analgesia will be frequently required during application of nCPAP; on the other hand, fewer infants receiving HHHFNC will require non-pharmacological interventions only. Enders *et al.*[32] has advocated the use of low-dose morphine (single intravenous dose of 0.01 mg kg^{-1}) for analgosedation in preterm infants receiving nCPAP, however, 9.3% of infants receiving low-dose morphine in their study developed considerable delayed apnea. Controversy exists regarding the safety and long-term impact of opioids analgesia in mechanically ventilated neonates.[33] A number of non-pharmacological techniques have been advocated as pain-relieving interventions in neonates, integration of these interventions into routine clinical practice will alleviate neonatal distress and provide greater satisfaction to both the parents and clinical staff. These non-pharmacological techniques include non-nutritive sucking, kangaroo care, facilitated tucking, swaddling and rocking/holding.[34] Oral sucrose is recommended by the American Academy of Pediatrics and the Canadian Pediatric Society,[2] to be used as a routine pain relief in NICUs during invasive and painful procedures; however, studies into its use in repeat doses remain inconclusive and merit further investigation.[35]

We have demonstrated that infants receiving HHHFNC have significantly lower respiratory rate compared with those receiving nCPAP. Similarly, Klingenberg *et al.*[16] have demonstrated lower respiratory rates in infants on HHHFNC compared with nCPAP, they attributed this to the washout effect of HHHFNC leading to the lower CO_2 levels, and HHHFNC being less painful than nCPAP. Giving the perceived benefit of HHHFNC compared with nCPAP in deceasing pain and discomfort, does not necessarily mean that we should shift from using nCPAP to HHHFNC in preterm infants requiring respiratory support until the safety and efficacy of HHHFNC is proven in adequately randomized-controlled trials.[36]

We acknowledge that there are limitations to this study like small sample size, lack of double-blind randomized controls and lack of data on baseline salivary-cortisol concentrations. Further, adequately powered randomized-controlled trials are required to compare the pain response on using different nCPAP systems and interfaces. Also, evaluation of the effect of pharmacological and non-pharmacological interventions in alleviating the pain associated with the application of nCPAP and HHHFNC should be considered.

CONCLUSIONS

The application of HHHFNC in preterm infants is associated with less pain compared with nCPAP, as it is associated with less PIPP scores and lower concentrations of salivary cortisol.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ACKNOWLEDGEMENTS

We thank Sabah Abdelnaser and Saber Elsheikh for their help in laboratory assessment of salivary cortisol, nursing staff of NICU, Mansoura University Children's Hospital, for their help in assessment of PIPP scores and patient recruitment, and all the parents and infants who took part in this study.

REFERENCES

1 Vitaliti SM, Costantino G, Li Puma L, Re MP, Vergara B, Pinello G. Painful procedures in the NICU. *J Matern Fetal Neonatal Med* 2012; **25**(Suppl 4): 146–147.
2 American Academy of Pediatrics Committee on Fetus and Newborn; American Academy of Pediatrics Section on Surgery; Canadian Paediatric Society Fetus and Newborn Committee, Batton DG, Barrington KJ, Wallman C. Prevention and management of pain in the neonate: an update. *Pediatrics* 2006; **118**: 2231–2241.
3 Simons SH, van Dijk M, Anand KS, Roofthooft D, van Lingen RA, Tibboel D. Do we still hurt newborn babies? A prospective study of procedural pain and analgesia in neonates. *Arch Pediatr Adolesc Med* 2003; **157**: 1058–1064.
4 Grunau RE, Holsti L, Peters JW. Long-term consequences of pain in human neonates. *Semin Fetal Neonatal Med* 2006; **11**: 268–275.
5 Fischer C, Bertelle V, Hohlfeld J, Forcada-Guex M, Stadelmann-Diaw C, Tolsa J-Fo. Nasal trauma due to continuous positive airway pressure in neonates. *Arch Dis Child-Fetal Neonatal Ed* 2010; **95**: F447–F451.
6 Yong SC, Chen SJ, Boo NY. Incidence of nasal trauma associated with nasal prong versus nasal mask during continuous positive airway pressure treatment in very low birthweight infants: a randomized control study. *Arch Dis Child Fetal Neonatal Ed* 2005; **90**: F480–F483.
7 Collins CL, Barfield C, Horne RS, Davis PG. A comparison of nasal trauma in preterm infants extubated to either heated humidified high-flow nasal cannulae or nasal continuous positive airway pressure. *Eur J Pediatr* 2014; **173**: 181–186.
8 Haq I, Gopalakaje S, Fenton AC, McKean MC, JO'Brien C, Brodlie M. The evidence for high flow nasal cannula devices in infants. *Pediatr Respir Rev* 2014; **15**: 124–134.
9 Yoder BA, Stoddard RA, Li M, King J, Dirnberger DR, Abbasi S. Heated humidified high-flow nasal cannula versus nasal CPAP for respiratory support in neonates. *Pediatrics* 2013; **131**: e1482–e1490.
10 Collins CL, Holberton JR, Barfield C, Davis PG. A randomized controlled trial to compare heated humidified high-flow nasal cannulae with nasal continuous positive airway pressure postextubation in premature infants. *J Pediatr* 2013; **162**: 949–954.
11 Manley BJ, Owen LS, Doyle LW, Andersen CC, Cartwright DW, Pritchard MA *et al.* High-flow nasal cannulae in very preterm infants after extubation. *N Eng J Med* 2013; **369**: 1425–1433.
12 Fernandez-Alvarez JR, Gandhi RS, Amess P, Mahoney L, Watkins R, Rabe H. Heated humidified high-flow nasal cannula versus low-flow nasal cannula as weaning mode from nasal CPAP in infants $\leq$ 28 weeks of gestation. *Eur J Pediatr* 2014; **173**: 93–98.
13 Stevens B, Johnston C, Petryshen P, Taddio A. Premature infant pain profile: development and initial validation. *Clin J Pain* 1996; **12**: 13–22.
14 Maxwell LG, Malavolta CP, Fraga MV. Assessment of pain in the Neonate. *Clin Perinatol* 2013; **40**: 457–459.
15 Ballantyne M, Stevens B, McAllister M, Dionne K, Jack A. Validation of the premature infant pain profile in the clinical setting. *Clin J Pain* 1999; **15**: 297–303.
16 Klingenberg C, Pettersen M, Hansen EA, Gustavsen LJ, Dahl IA, Leknessund A *et al.* Patient comfort during treatment with heated humidified high flow nasal cannulae versus nasal continuous positive airway pressure: a randomized cross-over trial. *Arch Dis Child Fetal Neonatal Ed* 2014; **99**: F134–F137.
17 Debillon T, Zupan V, Ravault N, Magny J, Dehan M. Development and initial validation of the EDIN scale, a new tool for assessing prolonged pain in preterm infants. *Arch Dis Child-Fetal Neonatal Ed* 2001; **85**: F36–F41.

18 Arias MC, Guinsburg R. Differences between uni-and multidimensional scales for assessing pain in term newborn infants at the bedside. *Clinics (Sao Paulo)* 2012; **67**: 1165–1170.
19 South MM, Strauss RA, South AP, Boggess JF, Thorp JM. The use of non-nutritive sucking to decrease the physiologic pain response during neonatal circumcision: a randomized controlled trial. *Am J Obstet Gynecol* 2005; **193**: 537–542.
20 Slater R, Cornelissen L, Fabrizi L, Patten D, Yoxen J, Worley A *et al.* Oral sucrose as an analgesic drug for procedural pain in newborn infants: a randomised controlled trial. *Lancet* 2010; **376**(9748): 1225–1232.
21 Cohen AM, Cook N, Harris MC, Ying GS, Binenbaum G. The pain response to mydriatic eyedrops in preterm infants. *J Perinatol* 2013; **33**: 462–465.
22 Gallo AM. The fifth vital sign: implementation of the Neonatal Infant Pain Scale. *J Obstet Gynecol Neonatal Nurs* 2003; **32**: 199–206.
23 Cabral DM, Antonini SR, Custódio RJ, Martinelli CE Jr, da Silva CA. Measurement of salivary cortisol as a marker of stress in newborns in a neonatal intensive care unit. *Horm Res Pediatr* 2013; **79**: 373–378.
24 Herrington CJ, Olomu IN, Geller SM. Salivary cortisol as indicators of pain in preterm infants: a pilot study. *Clin Nurs Res* 2004; **13**: 53–68.
25 Aardal E, Holm AC. Cortisol in saliva-reference ranges and relation to cortisol in serum. *Eur J Clin Chem Clin Biochem* 1995; **33**: 927–932.
26 Calixto C, Martinez FE, Jorge SM, Moreira AC, Martinelli CE Jr. Correlation between plasma and salivary cortisol levels in preterm infants. *J Pediatr* 2002; **140**: 116–118.
27 Ben-Aryeh H, Lapid S, Szargel R, Benderly A, Gutman D. Composition of whole unstimulated saliva of human infants. *Arch Oral Biol* 1984; **29**: 357–362.
28 Joyce BA, Keck JF, Gerkensmeyer J. Evaluation of pain management interventions for neonatal circumcision pain. *J Pediatr Health Care* 2001; **15**: 105–114.
29 Talge NM, Donzella B, Kryzer EM, Gierens A, Gunnar MR. It's not that bad: error introduced by oral stimulants in salivary cortisol research. *Dev Psychobiol* 2005; **47**: 369–376.
30 Bonner KM, Mainous RO. The nursing care of the infant receiving bubble CPAP therapy. *Adv Neonatal Care* 2008; **8**: 78–95.
31 Stevens B, Gibbins S, Franck LS. Treatment of pain in the neonatal intensive care unit. *Pediatr Clin North Am* 2000; **47**: 633–650.
32 Enders J, Gebauer C, Pulzer F, Robel-Tillig E, Knupfer M. Analgosedation with low-dose morphine for preterm infants with CPAP: risks and benefits. *Acta Pediatrica* 2008; **97**: 880–883.
33 Schuurmans J, Benders M, Lemmers P, van Bel F. Neonatal morphine in extremely and very preterm neonates: its effect on the developing brain—a review. *J Matern Fetal Neonatal Med* 2014 (e-pub ahead of print 29 April 2014).
34 Pillai Riddell RR, Racine NM, Turcotte K, Uman LS, Horton RE, Din Osmun L *et al.* Non-pharmacological management of infant and young child procedural pain. *Cochrane Database Syst Rev* 2011; (10): CD006275.
35 Holsti L, Grunau RE. Considerations for using sucrose to reduce procedural pain in preterm infants. *Pediatrics* 2010; **125**: 1042–1047.
36 Shetty S, Greenough A. Review finds insufficient evidence to support the routine use of heated, humidified high-flow nasal cannula use in neonates. *Acta Pediatr* 2014; **103**(9): 898–903.