Paracetamol and Preterm infants: a painless liaison?

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Paracetamol en premature kinderen:
een pijnloos verbond?

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Chapter 1

General Introduction

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Introduction

In the late 1980s there was a true turnabout on the important issue of neonatal pain. Then, Anand and co-workers [1, 2], published a trial on preterm infants randomly allocated to fentanyl with a muscle relaxant or muscle relaxant only during surgical patent ductus arteriosus (PDA) closure. This provided evidence, for the first time, that preterm infants have a capacity to feel pain from a very early age (24-26 weeks gestation) and that early repetitive pain gives rise to short-term and long-term consequences [3, 4].

Nowadays pain management has become an essential part of the standard of care in NICUs worldwide, and pain is considered 'the fifth vital sign'. The degree of distress associated with the treatment of preterm infants is reflected by the, on average, 11.2 painful procedures per day at our NICU [5]. Studies from NICUs in other countries have shown a similar trend with 6 to 17.3 procedures per newborn per day [6-11]. It has been found also that neonates receive comparatively less pain medication than older children and adults in similar painful procedures [12]. Caregivers may be reluctant to prescribe analgesics to neonates for fear of adverse effects, drug tolerance and dependence [13]. Moreover, there are few dosing guidelines and pharmacokinetic data on common drugs for neonates of different gestational ages and birth weights. On top of this, more than 90% of the currently prescribed drugs to neonates are not licensed for or are used in an off-label manner in this age group — and pain medication is no exception.

Animal and human studies have shown significant risks for neurological impairment, besides adverse learning, cognitive and behavioral effects after exposure to pain early in life [14, 15]. Painful skin-breaking procedures in very preterm infants, for example, have been related to impaired brain development characterized by reduced maturation of white matter and subcortical grey matter [16], and reduced brain size in the frontal en parietal regions [17]. The necessity to carefully manage painful procedures in very preterm infants is not debated, but the question how to do this most effectively and safely is still open.

Analgesia is necessary to protect the newborn against the negative short and long term consequences of pain. Long acting opioids, such as morphine, are not effective to reduce procedural pain, such as that from heel sticks, in preterm infants [18]. Even anaesthesia is not without risks as neuroapoptosis is described in human and rodent studies [19-21]. Routine administration of morphine in ventilated neonates did not show a benefit in terms of neurological outcome and mortality and was associated with a higher incidence of hypotension and other adverse effects [22-24]. Opioid administration even has more long-term consequences. De Graaf et al. showed negative effects on cognitive functioning in 5-year-old children who had been treated with morphine as a neonate

[25] and magnetic resonance imaging (MRI) in these children at the age of 14-17 years showed less brain volume [26]. The effectiveness of sucrose to reduce procedural pain has also been questioned although it is still standard of care in many NICUs [27-29].

Safe and adequate dosing of analgesic medication is inextricably linked to proper pain assessment. Quite a few multidimensional pain assessment tools are available but only a limited number is recommended for neonates. The most used ones are the Premature Infant Pain Profile (PIPP) and the Neonatal Infant Pain Scale (NIPS). In 2001, a prospective study in our NICU found that neonates were subjected to a mean of 14.3 procedures per day with the highest exposure to painful procedures (63.6%) during the first day of admission [6]. This was reason to implement an individualized pain management protocol providing for pain assessment with the COMFORTneo scale and pain treatment (both pharmacological and non-pharmacological such as NIDCAP and sucrose) guided by a decision tree.
Many painful procedures are performed without analgesics, not only in the Netherlands [5] but also in Europe [30]. Our quest for new analgesic opportunities in neonatal pain management continues. Apart from the classic approach with opioids such as morphine, fentanyl, studies suggest a potential role for alternative drugs such as paracetamol. In other words, Paracetamol and Preterm infants: a painless liaison?

Paracetamol (N-acetyl-p-amino-phenol) was introduced by von Mering in 1893 but not widely used until the 1950s. Nowadays it is the most used analgesic and antipyretic drug in children and is even prescribed to neonates to treat mild to moderate pain. In addition it is increasingly used in combination with opioids to treat severe pain [31-33]. Paracetamol is less potent than opioids and probably induces fewer side effects, although hepatotoxicity has been described [15, 34-37]. In Europe there is no labeled use of paracetamol for neonates younger than 10 days of age. Term neonates and older children usually receive paracetamol via the intravenous, oral or rectal route, and there is limited evidence on the use of intravenous paracetamol in preterm neonates, especially those with a gestational age of less than 28 weeks [38]. Developmental changes in distribution, metabolism and elimination determine the appropriate dosing regimen of paracetamol for neonates with different gestational and postnatal ages [39]. Currently there are no dosing guidelines of intravenous paracetamol for very low birth weight (VLBW) infants.

Working mechanism

The mechanism of action of paracetamol analgesia is multifactorial: not only does it influence the central nervous system by inhibiting the prostaglandin synthesis (COX3, COX 2b) but it also acts peripherally through metabolites of paracetamol that stimulate TPRA1 (Transient receptor potential ankyrin1), a protein found on the surface of nerve cells [40]. When TPRA1 is activated, it interferes and reduces the transmission of information from pain-sensing nerves to the brain.

Furthermore paracetamol exerts its analgesic effects by inhibitory action on spinal nitric oxide mechanisms and serotonergic pathways by antagonizing N-methyl-D-aspartate and substance P induced spinal hyperalgesia [33].

Metabolism

The three pathways by which paracetamol is metabolized in the liver are sulphation, glucuronidation and oxidation. In neonates the most important route of metabolism is sulphation and the rate constant of sulphate formation in neonates is larger than in adults

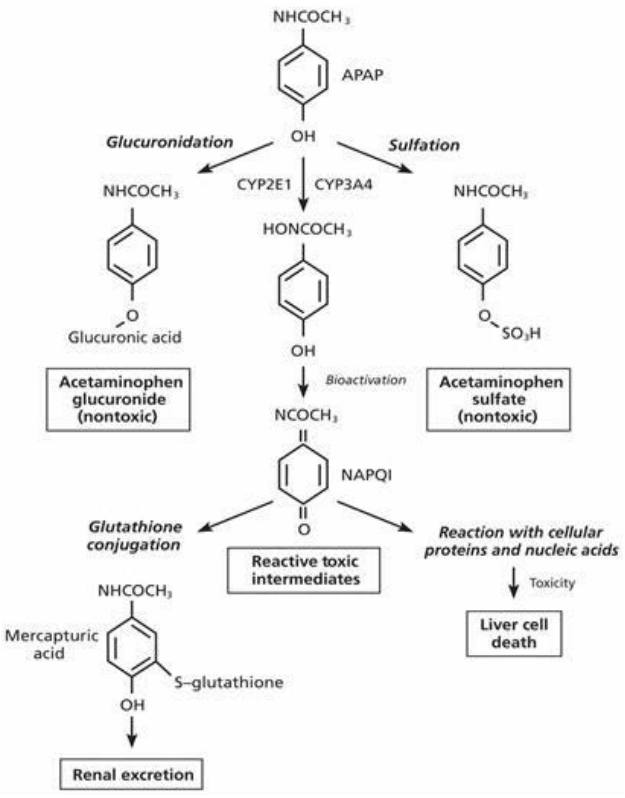

Figure 1. Metabolic pathways of paracetamol

[41]. Neonates have an immature glucuronide conjugation system. When paracetamol is administered in therapeutic doses the major part is excreted as non-toxic metabolites in the urine after sulphation or glucuronidation. Approximately 5% is excreted unchanged in the urine and the remainder is metabolized by cytochrome P-450 in the liver, of which CYP2E1 is the most important iso-enzyme. The product of oxidation is a highly reactive metabolite, N-acetyl-p-benzoquinone imine (NAPQI).

Dosing

Maturational changes in developmental physiology require age-specific doses, intervals and serum drug concentrations: the distribution volume of paracetamol is probably larger in preterm infants compared to term infants but seems to be similar in term neonates and older children [32, 42-44]. Reduced clearance in preterm infants necessitates multiple doses treatment of paracetamol with a longer time interval or adjusted total daily doses to prevent progressive increasing plasma concentrations. Still, the optimal loading dose of paracetamol for VLBW infants is unknown and simply extrapolating dose recommendations for less preterm infants is inadvisable. We should remain cautious, especially since high dose paracetamol treatment in preterm infants for PDA in the first days of life seems to win ground as drug of choice.
Paracetamol hepatotoxicity is caused by NAPQI, produced by the oxidative enzyme CYP2E1 and normally detoxified by glutathione reserves. Paracetamol concentrations associated with increased NAPQI in neonates have not been reported, and the activity of CYP2E1 is still not quantified [45, 46]. Whether hepatotoxicity by formation of NAPQIs and other metabolites in neonates is of lesser importance needs to be elucidated.

Aims and outline of this thesis

The studies described in this thesis aim to improve our knowledge on the use, indications, effects and adverse effects of paracetamol in VLBW infants (pharmacokinetics). Furthermore they also address the use of pain assessments tools to quantify the pain-relieving effect of paracetamol (pharmacodynamics).
In **Chapter 2** we tested the psychometric qualities of a modified version of the COMFORT behavioral scale tailored for use in premature neonates, called the COMFORTneo scale.
In 2011 we prospectively evaluated the degree of compliance of our NICU medical and nursing staff with the protocolized pain assessments and treatment. As secondary outcome reasons for non-compliance were investigated (**Chapter 3**).
In **Chapter 4** we evaluated whether the introduction of a new pain assessment protocol and pharmacological guidelines lowered the number of painful procedures and

changed the amount and frequency of analgesic therapy as compared to the results of our previous study in 2001.
To gain more insight in the pharmacokinetic and pharmacodynamics aspects of intravenous administered paracetamol, we conducted a multicenter, blinded and randomized trial in preterm infants (n= 60) with a gestational age between 24 and 32 weeks who were randomly allocated to three different single loading doses of intravenous paracetamol (10, 15, 20 mg/kg) before central venous catheter insertion in the first week of life. Plasma concentrations of paracetamol and its non-toxic and toxic metabolites were determined. Furthermore we investigated the relation between an infant's DNA and the formation of toxic metabolites of paracetamol with a possible threat to hepatotoxicity. The target was a polymorphism of CYP2E1, an important oxidative enzyme that can form a toxic metabolite of paracetamol called N-acetyl-p-benzoquinone imine (NAPQI) (**Chapter 5**). The effects of the three different loading doses of intravenous paracetamol were analysed with two different pain assessment tools: the PIPP score and the COMFORTneo scale. Scores in the study population were compared with scores in age-matched neonates (n = 20) who underwent central venous line placement with only sucrose as analgesia (**Chapter 6**).

More recently paracetamol has been suggested to be effective for patent ductus arteriosus (PDA) closure [47]. More than 10 observational and retrospective studies have described oral or intravenous high dose paracetamol treatment with varying effectiveness [48-50]. In **Chapter 7** we describe, in a prospective observational single center study, the evaluation of the efficacy of intravenous paracetamol on PDA closure. We included 33 VLBW infants with hemodynamically significant PDA (hsPDA) who did not respond to ibuprofen or had a contraindication for ibuprofen treatment and received high dose paracetamol (15mg/kg/6h) for 3-7 days.

The results of our studies are addressed in the general discussion in **Chapter 8**, followed by future perspectives in research.

Chapter 9 contains the summary of this thesis.

References:

1. Anand, KJ, et al., Pain, anaesthesia, and babies. Lancet 1987;2:1210.
2. Anand, KJ and PR Hickey, Pain and its effects in the human neonate and fetus. N Engl J Med 1987;317:1321-1329.
3. Taddio, A, et al., Effect of neonatal circumcision on pain response during subsequent routine vaccination. Lancet 1997;349:599-603.
4. Weisman, SJ, et al., Consequences of inadequate analgesia during painful procedures in children. Arch Ped Adol Med 1998;152:147-149.
5. Roofthooft, DW, et al., Eight years later, are we still hurting newborn infants? Neonatology 2014;105:218-226.
6. Simons, SH, et al., Do we still hurt newborn babies? A prospective study of procedural pain and analgesia in neonates. Arch Pediatr Adolesc Med 2003;157:1058-1064.
7. Carbajal, R, et al., Epidemiology and treatment of painful procedures in neonates in intensive care units. JAMA 2008;300:60-70.
8. Cignacco, E, et al., Neonatal procedural pain exposure and pain management in ventilated preterm infants during the first 14 days of life. Swiss Med Wkly 2009;139:226-232.
9. Johnston, C, et al., Pain in Canadian NICUs: have we improved over the past 12 years? Clin J Pain 2011;27:225-232.
10. Lago, P, et al., Guidelines for procedural pain in the newborn. Acta Paediatr 2009;98:932-939.
11. van Dijk, M, et al., Taking up the challenge of measuring prolonged pain in (premature) neonates: the COMFORTneo scale seems promising. Clin J Pain 2009;25:607-616.
12. Purcell-Jones, G, et al., Paediatric anaesthetists' perceptions of neonatal and infant pain. Pain 1988;33:181-187.
13. Berde, CB and NF Sethna, Analgesics for the treatment of pain in children. N Engl J Med 2002;347:1094-1103.
14. Grunau, R, Early pain in preterm infants. A model of long-term effects. Clin Perinatol 2002;29:373-394, vii-viii.
15. Duhrsen, L, et al., Effects of repetitive exposure to pain and morphine treatment on the neonatal rat brain. Neonatology 2013;103:35-43.
16. Brummelte, S, et al., Procedural pain and brain development in premature newborns. Ann Neurol 2012;71:385-396.
17. Smith, GC, et al., Neonatal intensive care unit stress is associated with brain development in preterm infants. Ann Neurol 2011;70:541-549.
18. Carbajal, R, et al., Morphine does not provide adequate analgesia for acute procedural pain among preterm neonates. Pediatrics 2005;115:1494-1500.
19. Zhu, C, et al., Isoflurane anesthesia induced persistent, progressive memory impairment, caused a loss of neural stem cells, and reduced neurogenesis in young, but not adult, rodents. J Cereb Blood Flow Metab 2010;30:1017-1030.
20. Jevtovic-Todorovic, V, et al., Early exposure to common anesthetic agents causes widespread neurodegeneration in the developing rat brain and persistent learning deficits. J Neurosci 2003;23:876-882.
21. Creeley, CE and JW Olney, Drug-Induced Apoptosis: Mechanism by which Alcohol and Many Other Drugs Can Disrupt Brain Development. Brain Sci 2013;3:1153-1181.
22. Anand, KJ, et al., Effects of morphine analgesia in ventilated preterm neonates: primary outcomes from the NEOPAIN randomised trial. Lancet 2004;363:1673-1682.

23. Simons, SH, et al., Routine morphine infusion in preterm newborns who received ventilatory support: a randomized controlled trial. JAMA 2003;290:2419-2427.
24. Bellu, R, et al., Opioids for neonates receiving mechanical ventilation. Cochrane Database Syst Rev 2008:CD004212.
25. de Graaf, J, et al., Long-term effects of routine morphine infusion in mechanically ventilated neonates on children's functioning: Five-year follow-up of a randomized controlled trial. Pain 2011.
26. van den Bosch, GE, et al., Prematurity, Opioid Exposure and Neonatal Pain: Do They Affect the Developing Brain? Neonatology 2015;108:8-15.
27. Wilkinson, DJ, et al., Sugaring the pill: ethics and uncertainties in the use of sucrose for newborn infants. Arch Pediatr Adolesc Med 2012;166:629-633.
28. Slater, R, et al., Oral sucrose as an analgesic drug for procedural pain in newborn infants: a randomised controlled trial. Lancet 2010;376:1225-1232.
29. Lasky, RE and W van Drongelen, Is sucrose an effective analgesic for newborn babies? Lancet 2010;376:1201-1203.
30. Guedj, R, et al., Does neonatal pain management in intensive care units differ between night and day? An observational study. BMJ Open 2014;4:e004086.
31. Agrawal, S, et al., Intravenous paracetamol for postoperative analgesia in a 4-day-old term neonate. Pediatric Anesthesia 2007;17:70-71.
32. Anderson, BJ, et al., Size, myths and the clinical pharmacokinetics of analgesia in paediatric patients. Clin Pharmacokinet 1997;33:313-327.
33. Allegaert, K, et al., The pharmacokinetics of a high intravenous dose of paracetamol after caesarean delivery: the effect of gestational age. Eur J Anaesthesiol 2012;29:484-488.
34. Hopchet, L, et al., Does intravenous paracetamol administration affect body temperature in neonates? Arch Dis Child 2011;96:301-304.
35. Isbister, GK, et al., Paracetamol overdose in a preterm neonate. Arch Dis Child Fetal Neonatal Ed 2001;85:F70-72.
36. de la Pintiere, A, et al., Intravenous propacetamol overdose in a term newborn. Arch Dis Child Fetal Neonatal Ed 2003;88:F351-352.
37. Walls, L, et al., Acetaminophen-induced hepatic failure with encephalopathy in a newborn. J Perinatol 2007;27:133-135.
38. Autret, E, et al., Pharmacokinetics of paracetamol in the neonate and infant after administration of propacetamol chlorhydrate. Dev Pharmacol Ther 1993;20:129-134.
39. Anderson, BJ, et al., Scattering for mixtures of hard spheres: comparison of total scattering intensities with model. Phys Rev E Stat Nonlin Soft Matter Phys 2006;73:031407.
40. Andersson, DA, et al., TRPA1 mediates spinal antinociception induced by acetaminophen and the cannabinoid Delta(9)-tetrahydrocannabiorcol. Nat Commun 2011;2:551.
41. Arana, A, et al., Treatment with paracetamol in infants. Acta Anaesthesiol Scand 2001;45:20-29.
42. Allegaert, K, et al., Pharmacokinetics of single dose intravenous propacetamol in neonates: effect of gestational age. Arch Dis Child Fetal Neonatal Ed 2004;89:F25-28.
43. Anderson, BJ and GM Palmer, Recent developments in the pharmacological management of pain in children. Curr Opin Anaesthesiol 2006;19:285-292.
44. Allegaert, K, et al., Renal drug clearance in preterm neonates: relation to prenatal growth. Ther Drug Monit 2007;29:284-291.
45. Manyike, PT, et al., Contribution of CYP2E1 and CYP3A to acetaminophen reactive metabolite formation. Clin Pharmacol Ther 2000;67:275-282.
46. Gonzalez, FJ, The 2006 Bernard B. Brodie Award Lecture. Cyp2e1. Drug Metab Dispos 2007;35:1-8.

47. Oncel, MY, et al., An alternative drug (paracetamol) in the management of patent ductus arteriosus in ibuprofen-resistant or contraindicated preterm infants. Arch Dis Child Fetal Neonatal Ed 2013;98:F94.
48. Oncel, MY, et al., Intravenous paracetamol treatment in the management of patent ductus arteriosus in extremely low birth weight infants. Neonatology 2013;103:166-169.
49. Hammerman, C, et al., Ductal closure with paracetamol: a surprising new approach to patent ductus arteriosus treatment. Pediatrics 2011;128:e1618-1621.
50. Dang, D, et al., Comparison of oral paracetamol versus ibuprofen in premature infants with patent ductus arteriosus: a randomized controlled trial. PLoS One 2013;8:e77888.

Chapter 2

Taking Up the Challenge of Measuring Prolonged Pain in (Premature) neonates The COMFORTneo Scale Seems Promising

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Clin J Pain. 2009 Sep; 25(7): 607-16

Abstract

Objectives

Pain assessment is essential to tailor intensive care of neonates. The present focus is on acute procedural pain; assessment of pain of longer duration remains a challenge. We therefore tested a modified version of the COMFORT-behavior scale – named COMFORTneo – for its psychometric qualities in the Neonatal Intensive Care Unit setting.

Methods

In a clinical observational study, nurses assessed patients with COMFORTneo and Numeric Rating Scales (NRS) for pain and distress, respectively. Interrater reliability, concurrent validity, and sensitivity to change were calculated as well as sensitivity and specificity for different cut-off scores for subsets of patients.

Results

Interrater reliability was good: median linearly weighted Cohen κ 0.79. Almost 3600 triple ratings were obtained for 286 neonates. Internal consistency was good (Cronbach α 0.84 and 0.88). Concurrent validity was demonstrated by adequate and good correlations, respectively, with NRS-pain and NRS-distress: r = 0.52 (95% confidence interval 0.44-0.59) and r = 0.70 (95% confidence interval 0.64-0.75). COMFORTneo cut-off scores of 14 or higher (score range is 6 to 30) had good sensitivity and specificity (0.81 and 0.90, respectively) using NRS-pain or NRS-distress scores of 4 or higher as criterion.

Discussion

The COMFORTneo showed preliminary reliability. No major differences were found in cut-off values for low birth weight, small for gestational age, neurologic impairment risk levels, or sex. Multicenter studies should focus on establishing concurrent validity with other instruments in a patient group with a high probability of ongoing pain.

Introduction

Daily pain management in the Neonatal Intensive Care Unit (NICU) continues to be far from ideal according to surveys in Australia,[1] France,[2-4] and Italy.[5] Yet, pain should be considered the 5th vital sign, recommends the American Pain Society, and accordingly be given due attention. Adequate dosing of analgesics in neonates must inevitably rely on proper pain assessment. The available pain measurement instruments mostly target acute procedural pain, such as caused by heel lance or postoperative pain. An international expert group recommended the Premature Infant pain Profile (PIPP), Neonatal Infant Pain Scale, Neonatal Facial Coding Scale, and Crying; requires increased oxygen administration; increased vital signs; Expression; Sleeplessness for procedural pain assessment.[6]

Acute pain measures include behavioral indicators of pain, of which facial expression is considered the most sensitive.[7] Its sensitivity may be questioned however. Slater et al recently compared PIPP scores with cortical pain responses detected by near infrared spectrograph during 33 heel lance procedures in 12 infants. Surprisingly, in 10 out of these 33 procedures scores on the facial expression did not indicate pain, whereas cortical pain responses were present. Physiological indicators such as increased heart rate and decreased oxygen saturation also proved useful for acute, procedural pain assessment.[8] Heart rate changes and oxygen saturation changes are both incorporated in the PIPP.[9] This instrument has been extensively applied in studies comparing pain treatments for procedural pain[10-13] and in psychometric studies comparing PIPP with other instruments.[14-16]

Prolonged pain, for example, due to extended artificial ventilation or diseases such as necrotizing enterocolitis (NEC), is much more difficult to assess. Because it is not easy to differentiate it from other sources of distress. In 2006 the Neonatal Pain task Force, (appointed by the Food and Drug Administration and the National Institutes of Health), pointed out that pain instruments for ongoing pain in preterm and term infants are lacking. Assessing pain of longer duration remains a challenge as Stevens and Pillai Riddell[18] and Anand[19] recently emphasized. Boyle et al[20] asked clinical staff first to guess whether preterm ventilated babies were receiving morphine or placebo, and next asked what made them think so. As staff associated facial expressions, high activity levels, poor response to handling, and poor ventilator synchrony with the effects of placebo, the authors suggest these factors are useful markers for persistent pain in preterm infants. But are they indeed?

To date, 2 instruments designed to measure prolonged pain in neonates are available, the neonatal Pain, Agitation, and Sedation Scale (N-PASS)[15] and Echelle Douleur Inconfort Nouveau-Né (EDIN).[21] Hummel et al[15] recently validated the N-PASS, analyzing

72 paired assessments before and after intervention in 46 infants. The N-PASS aims to assess both pain and sedation. It includes vital signs as 1 of 5 items. Interrater reliability between nurses was good and both beginning construct and concurrent validity were demonstrated. Internal consistency was good. There are no other studies investigating the usefulness of vital signs for prolonged pain assessment in neonates. The EDIN, a behavioral pain instrument widely used in French NICU's, was validated in 76 neonates.[21] Interrater reliability, has been internal consistency, and sensitivity to change was established. The EDIN incorporates "a blank face" and "paucity of movements," features often seen in neonates, for instance those with NEC. Both instruments include consolability (the extent to which a child can be consoled), an aspect that goes beyond clinical observation we feel, and may require nursing interventions.

The Neonatal Task Force furthermore elaborated on behavioral distress, as shown during for instance mechanical ventilation. The creators of the COMFORT scale provided the following definition: behavioral distress encompasses all behaviors of negative affect associated with pain, anxiety, and fear.[22] they added that distress may occur in the absence of pain. The question is how can we tell if a child is in distress, pain, or both? A neonate is like an "emotional black box" and caregivers find it very difficult to decide whether it is in pain or distressed, or both.

Disentangling both states requires emphasis on contextual factors as well. This is a universal challenge in all preverbal and nonverbal human beings, for example, neonates, cognitively impaired children and demented elderly. Although difficult, we have to make a serious attempt to address both.

In an earlier randomized controlled trial comparing morphine and placebo in ventilated premature neonates we used PIPP, Neonatal Infant Pain Scale, and COMFORT behavioral scale to assess pain/distress around endotracheal suctioning.[23] After this trial, which run for almost 3 years, we asked nurses to choose an instrument for future application in situations other than acute pain. They were given a choice of 15 validated pain instruments and chose the COMFORT behavior scale, provided it was slightly modified to make it more useful for preterm neonates.[24] The COMFORT behavior scale had earlier been validated for postoperative pain in 0 to 3-year-old infants in our institute[25] and for distress and sedation in children with a median age of 17 months.[26]

In this study, we tested the modified scale, which we named COMFORTneo scale. Two research questions were addressed: (1) Are the psychometric properties of the COMFORTneo scale adequate in terms of reliability, validity, and sensitivity to change? (2) Are different cut-off scores needed for different subpopulations in the NICU?

Materials and methods

Patients and Settings

In this clinical observational study, all neonates admitted to the Erasmus MC-Sophia NICU in the period October 2005 to August 2006 were eligible, except those treated with continuous neuromuscular blocking agents. This NICU is a regional 28-bed tertiary care unit with 650 admissions yearly and a Neonatal Individualized Developmental Care and Assessment Program training center since 2003.

Procedure

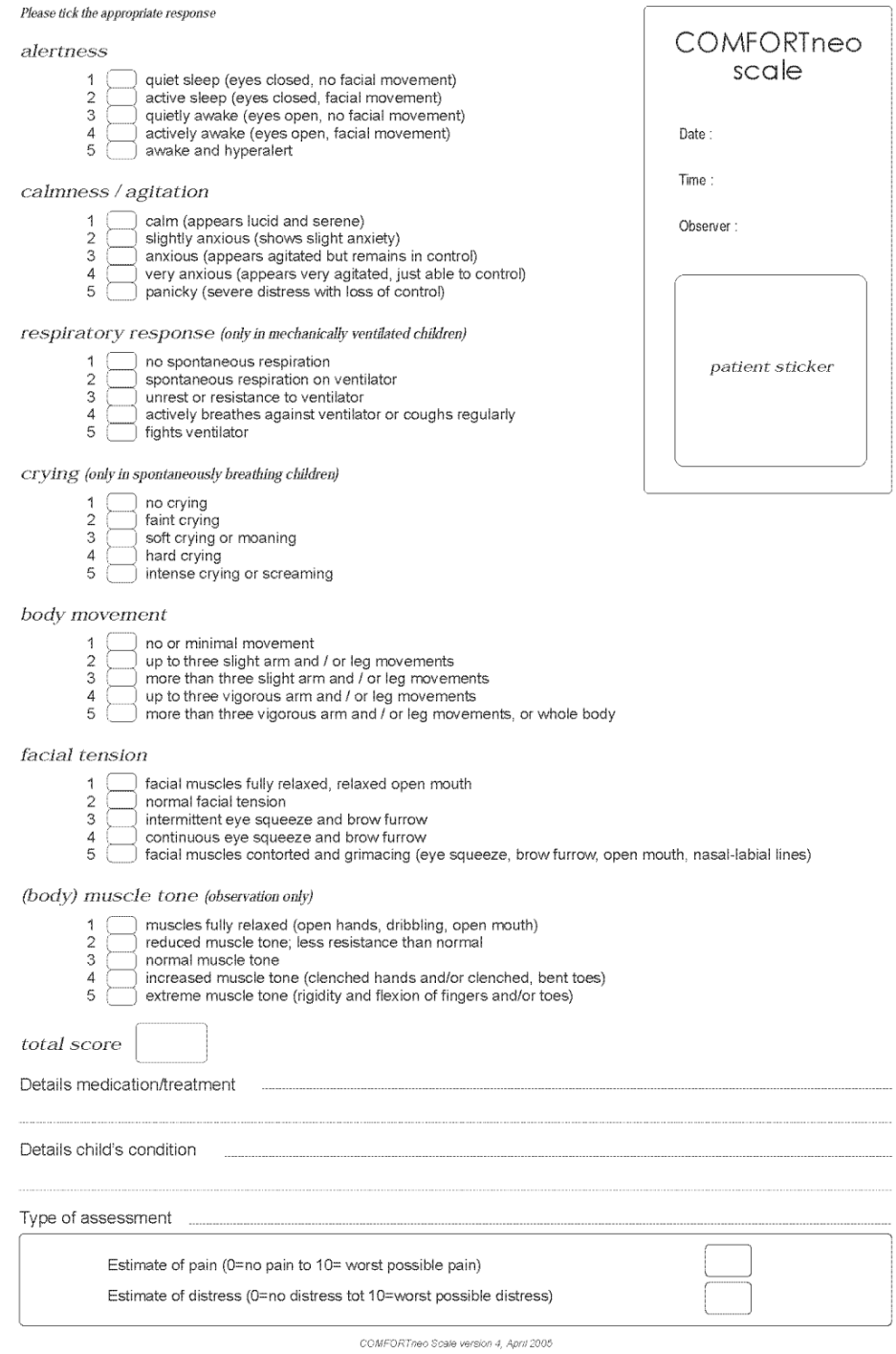
In October 2005, the digital Patient Data Management System (PDMS) replaced paper recording. COMFORTneo scores and numeric rating scores for pain and distress, data on analgesics and sedatives were entered and later retrieved from the system. Nurses indicated if a patient's assessment was "standard," or performed on suspicion of pain or on suspicion of oversedation. Standard assessments were those performed at least once every shift to monitor the patient.

Acute painful procedures are not scored. Pain assessment is part of daily care and because of its noninvasive and observational character institutional review board approval of the study was waived.

Scales

The COMFORTneo scale was modified from the COMFORT behavior scale[25] as follows. The item alertness was rewritten to bring it in line with Prechtl's[27] behavioral state criteria. Muscle tone was no longer scored by touch, but by observation of toes and fists (clenched or not), fingers played or not, and legs or arms (stretched or relaxed). The "no movement" response to body movement became "no or minor movement" because premature neonates are seldom without movement.[28,29] Like the COMFORT behavior scale, the COMFORTneo scale is composed of 7 behavioral dimensions (Fig. 1). As respiratory response applies to ventilated neonates only, and crying to spontaneously breathing neonates only (including those requiring continuous positive airway pressure), the rater will actually rate 6 dimensions. Alertness, calmness/agitation, facial tension, muscle tone, body movement are rated for all neonates. As responses are on a 1 to 5 Likert scale, total scores ranges from 6 to 30–with higher scores indicating more pain. Before scoring, the rater will observe the patient for 2 minutes and next rate each individual item for its most extreme manifestation observed during this period.

The COMFORTneo was introduced in 2004 and its effectiveness was evaluated 6 months later by 6 nurses, a neonatologist (D.R.) and psychologist (M.v.D.). It was then decided to include a Numeric rating Scale (NRS) for distress as well because nurses felt that high COMFORTneo scores were often due to distress rather than pain. Nurses also were con-



Please tick the appropriate response

COMFORTneo scale

Date :

Time :

Observer :

patient sticker

alertness

1 ☐ quiet sleep (eyes closed, no facial movement)
2 ☐ active sleep (eyes closed, facial movement)
3 ☐ quietly awake (eyes open, no facial movement)
4 ☐ actively awake (eyes open, facial movement)
5 ☐ awake and hyperalert

calmness / agitation

1 ☐ calm (appears lucid and serene)
2 ☐ slightly anxious (shows slight anxiety)
3 ☐ anxious (appears agitated but remains in control)
4 ☐ very anxious (appears very agitated, just able to control)
5 ☐ panicky (severe distress with loss of control)

respiratory response (only in mechanically ventilated children)

1 ☐ no spontaneous respiration
2 ☐ spontaneous respiration on ventilator
3 ☐ unrest or resistance to ventilator
4 ☐ actively breathes against ventilator or coughs regularly
5 ☐ fights ventilator

crying (only in spontaneously breathing children)

1 ☐ no crying
2 ☐ faint crying
3 ☐ soft crying or moaning
4 ☐ hard crying
5 ☐ intense crying or screaming

body movement

1 ☐ no or minimal movement
2 ☐ up to three slight arm and / or leg movements
3 ☐ more than three slight arm and / or leg movements
4 ☐ up to three vigorous arm and / or leg movements
5 ☐ more than three vigorous arm and / or leg movements, or whole body

facial tension

1 ☐ facial muscles fully relaxed, relaxed open mouth
2 ☐ normal facial tension
3 ☐ intermittent eye squeeze and brow furrow
4 ☐ continuous eye squeeze and brow furrow
5 ☐ facial muscles contorted and grimacing (eye squeeze, brow furrow, open mouth, nasal-labial lines)

(body) muscle tone (observation only)

1 ☐ muscles fully relaxed (open hands, dribbling, open mouth)
2 ☐ reduced muscle tone; less resistance than normal
3 ☐ normal muscle tone
4 ☐ increased muscle tone (clenched hands and/or clenched, bent toes)
5 ☐ extreme muscle tone (rigidity and flexion of fingers and/or toes)

total score ☐

Details medication/treatment .....

Details child's condition .....

Type of assessment .....

Estimate of pain (0=no pain to 10= worst possible pain) ☐

Estimate of distress (0=no distress tot 10=worst possible distress) ☐

COMFORTneo Scale version 4, April 2006

Figure 1. Scoring form for COMFORTneo.

cerned that without a treatment algorithm pain assessment would be less effective and pain and distress would stay untreated. The data of 1164 assessments in 209 infants in the period March 2004 until September 2005 were used to calculate cut-off scores for a treatment algorithm.[24-30] Internal consistency was good and concurrent validity with the NRS pain and NRS distress adequate.

A NRS for pain replaced the Visual Analogue Scale used with the COMFORT behavioral scale, as it fits in better with PDMS. Both NRS were used for ongoing validity testing.[24] Both NRS pain and NRS distress are scored after the COMFORTneo score by the caregiving nurse as representing the expert opinion of the nurse. Rating is on a scale of 0 to 10, with 0 representing no pain or distress and 10 worst imaginable pain or distress. Scores 4 to 6 are considered to reflect moderate pain or distress and 7 to 10 severe pain or distress.[31]

Reliability

All nurses followed a 2-hour training program. The program includes 10 assessments in different patients, together with but independently from a qualified nurse. Agreement is assessed from the weighted Cohen[32] κ calculated from these 10 paired assessments. If it was below 0.65, the nurse was asked to repeat 10 assessments until the required κ value had been reached. The linearly weighted κ corrects for chance agreement and gives greater weight to larger disagreements. As a rule of thumb, both Cohen and Fleiss[33] indicate that a κ of 0.61 or higher is adequate.

Internal consistency was calculated separately for spontaneous breathing versus mechanically ventilated neonates, using Cronbach α and minimum and maximum corrected item – total correlation. Because the COMFORTneo scale is highly comparable with the COMFORT behavior, we did not repeat structural equation modeling. The previous analysis had shown that the COMFORT behavior scale could be represented by a single factor which was stable across repeated assessments.[25]

Concurrent Validity

Concurrent validity of the COMFORTneo scale was determined on the basis of the NRS-pain and NRS-distress scores. The Pearson product moment correlation coefficients between the COMFORTneo and the 2 NRS scores are presented. As these correlations were found to violate the assumption of independence (repeated measurements), we also calculated the mean COMFORTneo, mean NRS-pain, and mean NRS-distress scores for each patient.[34] The Pearson product moment correlation coefficients of these mean values are presented with 95% confidence intervals (CI) and p values.

Sensitivity to Change

Sensitivity to change was determined by comparing scores before and after a pain or distress reducing intervention. Secondly, assessments indicated as standard in PDMS were compared with "suspected for pain" assessments in the same patients.

Patients with at least 1 assessment for each condition were included in the analysis. In addition, mean scores for the 2 conditions per patient were calculated. And finally, assessments on "suspected for oversedation" were compared with standard COMFORTneo scores for the same patients. For all 3 types, sensitivity to change was tested using the paired *t* test for parametric data.

Cut-off Scores

The expert opinion of the nurses represented by both NRS-distress and NRS-pain was used as the gold standard against which cut-off scores for COMFORTneo were evaluated. Cut-off scores are useful for clinical practice, because they can guide analgesic treatment. When either NRS-pain or NRS-distress scores were 4 or higher, a value of 1 was assigned. Values below 4 were assigned 0. Different cut-off scores COMFORTneo were tested against this classification.

Derivation of cut-off scores was as follows. First the combinations of specificity and sensitivity for different cut off scores of COMFORTneo were calculated and presented in a receiver operating characteristic curve. Then, of the cut-off scores closest to the top left-hand corner in the chart, the most "conservative" one of 14 was chosen as to prevent too rapid pharmacologic interventions. Sensitivity and specificity for specific cut-off scores were also calculated for subpopulations of extreme low birth weight (ELBW <1000 grams), very low birth weight (1000 to 1499 grams), different gestational age groups (24 to 28 weeks, etc.), and small for gestational age (SGA ≤ 2SD) infants. We also classified the sample by the level of risk of neurologic impairment (NI) into 3 groups according to Stevens et al.[35] At high risk for NI are neonates with perinatal asphyxia, intraventricular hemorrhage grade III or IV, congenital syndrome, or chromosomal anomaly (group A). At moderate risk for NI are neonates with persistent pulmonary hypertension, severe meconium aspiration, meningitis, and NEC (group B). At low risk for NI are neonates with respiratory distress requiring ventilation and sepsis (group C). Finally, boys and girls were compared, because sex may influence pain response.[36-38]

Statistical methods

Three aspects of analgesic and sedative treatment are presented. First, the maximum hourly dosages per kilogram of body weight for continuous midazolam and morphine; second, duration of administration in days; and third, number of dosages for oral or intermittent bolus medication.

Nominal variables of different groups were compared with the w2 test or Fisher Exact tests when cell values were small. Kruskal-Wallis test was used to compare nonparametric continuous variables between more than 2 groups. Data are given as median (interquartile range, IQR) for nonparametric data and as mean (SD) for parametric data. Correlational analyses were done with the mean of repeated assessments per patient as summary statistic[39] to avoid statistical testing across nonindependent observations. In addition, the summary statistics were correlated with each other to determine the level of association.

Results

Patients and Assessments

In the study period, 488 neonates were admitted to the NICU. One or more COMFORTneo observations were performed in 286 neonates (gestational ages from 24.6 to 42.6 weeks). Twenty-one percent were (ELBW <1000 gram) and 18.2% were SGA infants. Seventy-seven infants (26.8%) were at high or moderate risk for NI. The median number of assessments was 6 (IQR 2 to 13). Characteristics for the sample and for the infants not assessed are listed in Table 1. This latter group left the NICU significantly sooner (median stay 2 d opposed to 9 for the assessed patients) A significantly lower proportion of patients in the non assessed group received analgesics or sedatives.

COMFORTneo scores of 14 or higher were recorded for 23.1% of all 3596 assessments. Nurses sometimes failed to record NRS ratings in PDMS, so we could retrieve no more than 2684 and 2668 NRS ratings for NRS pain and NRS distress respectively. Moderate pain (NRS 4 to 6) was seen in 5.8% and severe pain in 1.3% (NRS 7 to 10) of assessments. Moderate distress (NRS 4 to 7) was scored in 12.6% and severe distress in 5.4% of assessments (NRS 7 to 10). Figure 2 gives a boxplot with COMFORTneo scores grouped by type of assessment. For 143 assessments (4% of all assessments) made, as nurses suspected the neonate to be oversedated the median score was 10 (IQR 9 to 11). For standard assessments, the median score was 11 (IQR 9 to 13), with 16.5% of the scores 14 or higher, requiring intervention. The highest median score is seen for the 290 assessments on suspicion of pain that is 18 (IQR 15 to 21).

Pharmacologic treatment

Forty-one percent of the assessed neonates received no analgesics (opioids or paracetamol at all during NICU stay. Almost half of the assessed patients (46.9%) received continuous morphine at a maximum dosage of 15 mg/kg/h (IQR 10.0 to 19.9) for a median duration of 5 days. Midazolam was given to 26.6% of all patients with a maximum dosage of 103mg/kg/h (IQR 100 to 10 As to classification by level of risk of NI, morphine

Table 1. Characteristics assessed (n = 286) and non assessed patients (202)

	N (%) Sample	N (%) Not assessed	*p-value*
Boys/Girls	174/112 (60.8 /39.2)	110/92 (54.5/45.5)	*0.16*
GA groups		N=197	
24.2 - 28.0 wk	58 (20.3)	9 (4.6)	
28.1 - 32.0 wk	86 (30.1)	50 (25.4)	*<0.0001*
32.1 - 37.0 wk	49 (17.1)	62 (31.5)	
37.1 - 42.6 wk	93 (32.5)	76 (38.6)	
Birth weight in grams		N=192	
ELBW < 1000 g	60 (21.0)	13 (6.8)	*<0.0001*
VLBW 1000 to 1499 g	57 (19.9)	33 (17.2)	
> 1500 g	169 (59.1)	146 (76.0)	
SGA	52 (18.2)	33 (16.5)	*0.63*
Primary diagnosis			
Respiratory insufficiency	153 (53.5)		
Prematurity and/or SGA	41 (14.3)		
Congenital cardio(vascular) defects	25 (8.7)		
Perinatal asphyxia	25 (8.7)		
Other[1]	42 (14.8)		
Neurological outcome[2]			
A=At high risk for NI	52 (18.2)		
B=At moderate risk for NI	25 (8.7)		
C=At low risk for NI	209 (73.1)		
Postnatal age in days, median (IQR)			
At first assessment	1 (0 to 3)		
At last assessment	7 (2 to 19)		
One month survival	88.5%	96.5	*0.001*
Mechanical ventilation during study period			
Ventilated all the time	68 (23.8)		
Part of the time	126 (44.0)		
not at all during the study period	92 (32.2)		
Total hospital stay in days			
Median (IQR)	11 (4 to 28)		
NICU stay	9 (4 to 23)	2 (1 to 6)	*<0.0001*
Patients receiving opioids, benzodiazepines or barbiturates	148 (51.7)	19 (9.4)	*<0.0001*
Patients receiving paracetamol	74 (25.9)	4 (2.0)	*<0.0001*

[1] Others: sepsis, cerebral abnormalities, metabolic problems and congenital anomalies

[2] *A;* perinatal asphyxia, IVH grade III or IV, syndrome or chromosomal anomaly, *B;* persistent pulmonary hypertension of the newborn, severe meconium aspiration, meningitis, necrotising enterocolitis *C;* respiratory distress requiring ventilation, sepsis (Stevens et al 2007)

abbreviations ELBW: extreme low birth weight; VLBW; very low birth weight SGA; small for gestational age; NI; neurological impairment IQR: interquartile range

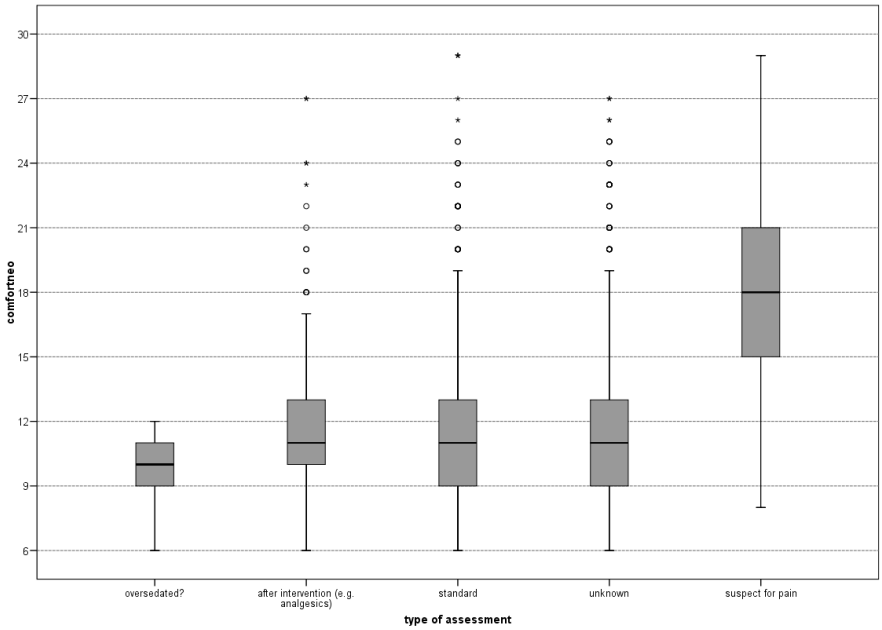


Figure 2. Boxplot of COMFORTneo scores for different types of assessment.

was significantly more often given to patients in the 'moderate risk for NI" group (Fisher exact test, p = 0.001) in higher dosages (Kruskal-Wallis, p=0.032). Midazolam was given to 50% of the "high risk for NI" group, to 72% of the moderate risk for NI group, and to 15,3% of the minor risk for NI group. These percentages were significantly different (Fisher exact test, p=0.001). the highest dosages were noted for the high risk group for NI (Kruskal-Wallis test, p=0.012)

Phenobarbital was needed in 41 patients (14.3%) with a median of 1 dosage (IQR 1 to 2). More than half of these patients were in the high-risk group. Patients in the "minor risk for NI" group required significantly less phenobarbital than the other groups (Fisher exact test, p≤0.001). There was no statistically significant effect of sex on prescribed analgesics or sedatives.[3]

Psychometric Properties

Reliability

In total 141 nurses and 3 researchers followed the training program. Linearly weighted κ's ranged from 0.65 to 0.97 with a median of 0.79. With Cronbach α of 0.88 in 1149 scores, internal consistency of the COMFORTneo items for the nonventilated neonates was good. Corrected item total correlations ranged from 0.52 for muscle tension to 0.80 for calmness. For mechanically ventilated neonates, 2447 scores yielded a Cronbach α of

0.84. Corrected item total correlations ranged from 0.49 for respiratory response to 0.72 for calmness.

Concurrent Validity

The Pearson product moment correlation coefficient between COMFORTneo and NRS-pain was 0.54 in 2684 paired assessments. The Pearson product moment correlation coefficient between mean COMFORTneo and mean NRS-pain per patient was r = 0.51 (95% CI 0.43-0.59, p <0.0001) in 253 neonates. The Pearson product moment correlation coefficient between COMFORTneo and NRS-distress was 0.83 in 2668 paired assessments. Using the mean values of COMFORTneo and NRS-distress per patient gives a Pearson product moment correlation coefficient of 0.75 (95% CI 0.70-0.79, p <0.0001) in 250 neonates.

Sensitivity to Change

For 110 paired assessments (in 76 neonates), we compared COMFORTneo scores before and after interventions to reduce pain or distress (Fig. 3). Mean scores before intervention (mean 19.8, SD 3.8) were significantly higher (paired *t* test, *t* = 14.99, df = 75, p ≤0.001) than after intervention (mean 12.0, SD 3.4). Interventions to reduce pain were administration of opioids (55 observations) and paracetamol (19 observations). Interventions to reduce distress were administration of sedatives (benzodiazepines, antiepileptics in 25 observations) and nonpharmacologic intervention (9 observations

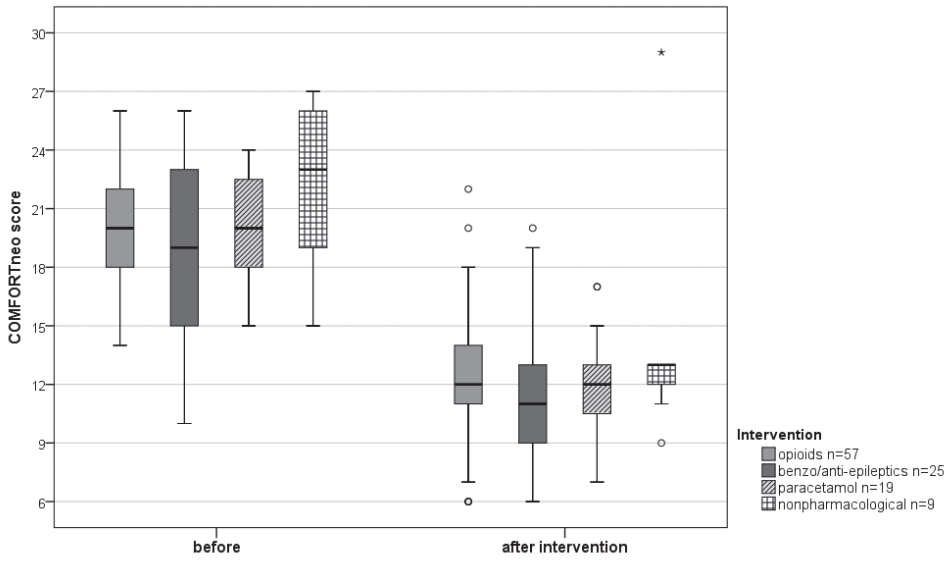


Figure 3. Boxplot of COMFORTneo before and after intervention, clustered by intervention type

including swaddling, pacifier, and consoling). Two observations resulted in administration of both opioids and sedatives.

Assessments both in standard and suspected for pain conditions were made for 102 patients. The mean COMFORTneo score was 11.4 (SD 2.6) for the standard condition and 18.4 (SD 3.8) for the suspected for pain condition. The mean difference of 6.9 points (95% CI 6.1-7.8) is highly significant (paired t test, t= 16.6, p ≤ 0.001) and indicates good sensitivity to change. For 66 patients mean COMFORTneo score during suspected oversedation was 9.7 (SD 1.7). This was significantly lower than the standard mean score of 10.9 (SD 1.8) for these patients (paired *t* test, t = 4.45, p ≤0.001).

Cut-off Scores for COMFORTneo

Cut-off scores for COMFORTneo were calculated against NRS-pain or NRS-distress (or both) of 4 or higher. Sensitivity and specificity for COMFORTneo cut-off scores of 14 or higher were 0.81 and 0.90, respectively.

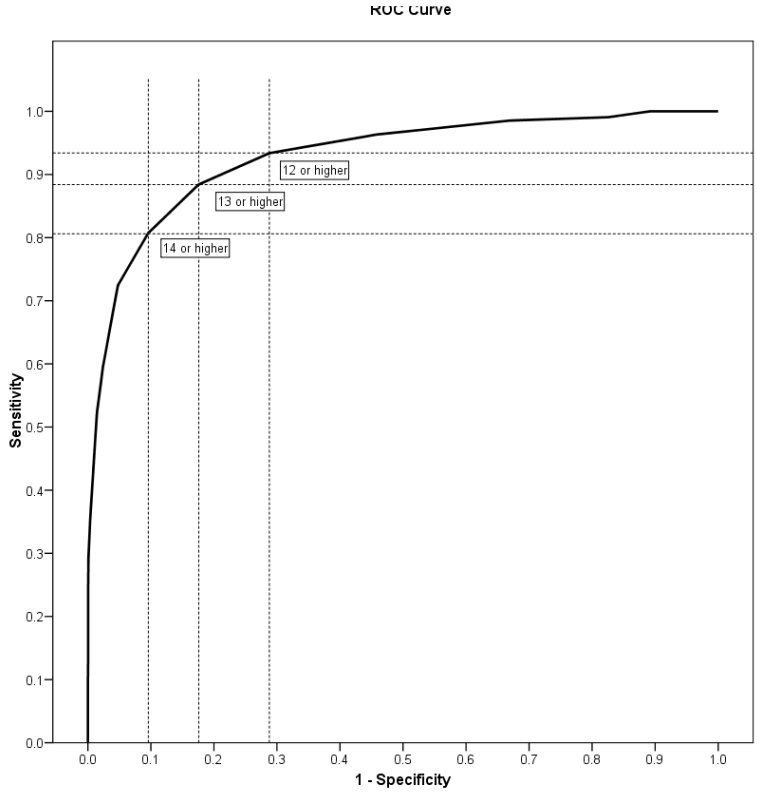


Figure 4. Receiver operator characteristic (ROC) curve for COMFORTneo.

Figure 4 gives the receiver operating characteristic curve showing sensitivity and specificity results for cut-off values close to the upper left-hand corner. Table 2 gives sensitivity and specificity at a cut-off score of 14 or higher for the different subgroups. Sensitivity ranged from 0.75 to 0.85 and specificity from 0.87 to 0.95 for the different subgroups. These findings do not support the need to apply different cut-off scores for these subgroups. Both sensitivity and specificity were comparable for the 3 groups at differing risk for NI. When using the NRS-pain of 4 or higher separately for these analyses, we would obtain 0.72 sensitivity and 0.80 specificity at COMFORTneo cut-off scores of 14 or higher. For the NRS-distress of 4 or higher, sensitivity would be 0.81 and specificity 0.90.

Table 2. Sensitivity and specificity for COMFORTneo cutoff scores of 14 or higher for different subgroups

Subgroups	N	Number of assessments	Sensitivity	Specificity
COMFORTneo combined with NRS	256	2751	0.81	0.90
Gestational age				
24 - 28 wks	49	935	0.76	0.91
28.1 - 32 wks	75	531	0.83	0.91
32.1 - 37 wks	46	436	0.78	0.89
>37 wks	86	849	0.84	0.90
Birth weight				
< 1000 grams	53	915	0.78	0.90
1000-1499 grams	47	548	0.77	0.94
1500 or more	156	1288	0.83	0.89
Small for Gestational Age	46	703	0.76	0.90
Neurological outcome				
A=At high risk for NI	45	508	0.85	0.95
B=At moderate risk for NI	25	443	0.75	0.90
C=At low risk for NI	186	1800	0.80	0.87
Gender				
Girls	100	963	0.77	0.92
Boys	156	1788	0.82	0.89

Discussion

Psychometric Properties

This psychometric study of 286 neonates includes as many as almost 3600 assessments. The results indicate that COMFORTneo can be assessed reliably after a 2-hour training session, has good interrater reliability, concurrent validity, and sensitivity to change. The calculated cut-off scores facilitate the design and implementation of treatment algorithms, thus enhancing its clinical utility. To test concurrent validity, most psychometric

studies use other existing behavioral pain instruments to compare with a newly developed one. We did not choose this approach because we feel the large overlap in items in neonatal behavioral pain instruments results in an artificially inflated correlation. Other ways to test validity should be considered.

Recent near-infrared spectroscopy studies for example showed that painful stimuli activate the cortex of preterm neonates.[38-40] Future research protocols to validate clinically applicable tools could well include near-infrared spectroscopy or other neuroimaging methods, thus providing an objective, observer-independent evaluation of validity.[38-42]

Cut-off Score

We set a rather conservative cut-off score of 14, because we feel there is a thin line between harmful pain and drugs harmful to the developing brain. In other words, to prevent that potentially harmful drugs are prescribed too soon. For that matter, a number of neonatal studies have shown the limited effects of morphine to reduce acute pain.[12-43] A recent study in neonatal rats repeatedly exposed to morphine reported prolonged pain hypersensitivity, decreased morphine antinociception, and decreased stress-induced analgesia.[44] Earlier rat studies suggest that anesthetic agents such as midazolam enhance neuro-apoptosis.[45] In contrast, there is substantial evidence from clinical studies that pain and distress are harmful in the short and long run. We therefore advice to assess pain frequently during analgesic treatment to facilitate optimal dosing of, for instance, opioids.

As pain scores for neonates of different gestational ages or for SGA neonates did not differ greatly, we conclude tailored cut-off scores are not needed.

This does not imply, however, that gestational age is of no consequence.

Clinical practice, for that matter, suggests that more immature babies need more time to recover after interventions; they show brief behavioral responses because they are more easily exhausted than the term neonates. In contrast, term neonates admitted to the NICU typically will have serious problems, such as asphyxia and meconium aspiration syndrome, which may dampen their pain responses.[19]

No substantial differences were found between the COMFORTneo scores for the groups distinguished by risk of NI, with comparable sensitivity and specificity for cut-off scores of 14 or higher. However, patients at moderate to high risk for NI seem to require more sedation and analgesia46 as was confirmed in this study. They can show irritability, restless behavior, inconsolability, or convulsions, and may require benzodiazepines for sedation or for treating convulsions. Preterm neonates with severe NI may also have lower levels of consciousness. For daily practice, it is important to take all such information into account.

Pain and Distress

Pain and distress can occur simultaneously, may influence each other and present with comparable responses in neonates. It is difficult therefore to discriminate between the 2. The most sensible way to address this challenge is to carefully observe the effects of pain-reducing or distress-reducing interventions. In addition, caregivers should be open to the impact of other factors that affect the neonate's level of pain and distress.

There are environmental factors such as noise and light levels, circumstantial factors, for example, postoperative state, having undergone painful procedures shortly before assessment, or analgesic and sedative treatment just started, and patient related factors. The latter category includes temperament, illness severity, behavioral signs impeded by specific conditions (e.g. NEC or sepsis), or NI.[35]

Nurses more often rated 4 or higher for NRS-distress than for NRS-pain, 18% versus 8%. We hypothesize that nursing staff may consider distress a more frequent problem than pain.

The prevalence of pain of longer duration in the NICU is unknown and not many studies have defined its clinical manifestations in NICU patients. Pain of longer duration may result from surgery (extending beyond the acute postoperative period), from inflammation (caused by NEC, or meningitis, or thrombo-phlebitis), from skin burns (heated transcutaneous sensors or chemicals), birth trauma, or extended mechanical ventilation. Prolonged pain may be associated with the signs of low-grade distress because of the neonate's poor energy reserves. It would seem important to educate nurses on the relevance of contextual factors, the complexities of pain and distress, and situations when prolonged pain is likely to occur.

On the other end of the pain and distress continuum is the challenge of potential oversedation. Nurses suspected oversedation once or more in 66 neonates. However, it might very well be that the real incidence is higher because lack of behavioral signs is less alarming to nurses than the presence of behavioral signs suggesting distress. The NICU treatment algorithm dictates that dose reduction should be considered, when COMFORTneo scores is 8 or lower.

Remaining in deep sleep for a considerable time (>12 h) even during handling should in most instances be reason to decrease morphine in small steps. But comfortable sleep during nighttime may result in a low COMFORTneo score, which does not necessitate change of therapy.[30]

Methodologic Limitations

This study had limitations, largely related to the fact that we used data from clinical practice. First, the nurse involved assessed both the COMFORTneo scale and NRS distress and NRS pain. It would be ideal to have a separate caregiver score 1 of the 3 independently, provided this person has the same contextual insight as the caregiving nurse. Second,

another instrument to compare the COMFORTneo against was lacking at the time. The N-PASS was not yet published and the EDIN had some items which nurses found difficult to assess. For instance the item "quality of contact with nurses." Response categories for the items range from 0, "smiles, attentive to voice," to 3 "refuses to communicate with nurses, no interpersonal rapport". This item was judged not applicable to very preterm neonates. Another EDIN item focuses on quality of sleep in preceding hours, which was considered difficult to assess reliably with the current practice of incubator covers.

In future studies, should independent observers score the N-PASS or PIPP or even EDIN simultaneously with the nurses' scoring of the COMFORTneo. In this way a better estimate of concurrent validity could be obtained.

Ratings before and after pain relieving interventions were rare. A possible explanation is that nurses tend to put trust in the prescribed therapeutic interventions and forget to check its effectiveness or deem this unnecessary, when the child responds to the prescribed drug as expected. More attention to oversedation could have given more insight into the extent of this undesired state and ways to prevent it.

Finally, although interrater reliability was satisfactory for all nurses when they took up scoring, it would have been better to reassess interrater reliability after 6 months so as to ensure quality of scoring.

Conclusions

We report the initial validation of a modified measure, the COMFORTneo scale, designed to assess persistent or prolonged pain in the NICU. It shows clinically acceptable reliability, internal consistency, beginning concurrent validity, and sensitivity to change, and provides a uniform cut-off value applicable to different subpopulations (ELBW, very low birth weight, SGA, term neonates, different NI risk levels, or sex).

Staff found that the COMFORTneo scale to be a clinically useful pain instrument for the NICU environment, with cut-off scores introduced in a useful treatment algorithm.[30] Preferably, interventions should be based on repeated assessments performed routinely and on suspicion of (prolonged) pain, distress, or oversedation. Such an approach, perhaps coupled with independent neuroimaging techniques, may help refine the measurement of persistent pain, thus providing a scientific framework for optimal comfort care in the NICU.

References

1. Harrison D, Loughnan P, Johnston L. Pain assessment and procedural pain management practices in neonatal units in Australia. J Paediatr Child Health. 2006;42:6–9.
2. Debillon T, Bureau V, Savagner C, et al. Pain management in French neonatal intensive care units. Acta Paediatr. 2002;91:822–826.
3. Klosowski S, Morisot C, Truffert P, et al. Multicentric study on neonatal medical pain management in the Nord-Pas-de-Calais. Arch Pediatr. 2003;10:766–771.
4. Carbajal R, Rousset A, Danan C, et al. Epidemiology and treatment of painful procedures in neonates in intensive care units. JAMA. 2008;300:60–70.
5. Lago P, Guadagni A, Merazzi D, et al. Pain management in the neonatal intensive care unit: a national survey in Italy. Paediatr Anaesth. 2005;15:925–931.
6. Anand KJ. Consensus statement for the prevention and management of pain in the newborn. Arch Pediatr Adolesc Med. 2001;155:173–180.
7. Grunau RVE, Craig KD. Pain expression in neonates: facial action and cry. Pain. 1987;28:395–410.
8. Holsti L, Grunau RE, Oberlander TF, et al. Is it painful or not? Discriminant validity of the Behavioral Indicators of Infant Pain (BIIP) scale. Clin J Pain. 2008;24:83–88.
9. Stevens BJ, Johnston CC, Petryshen P, et al. Premature Infant Pain Profile: development and initial validation. Clin J Pain.1996;12:13–22.
10. Stevens B, Johnston C, Taddio A, et al. Management of pain from heel lance with lidocaine-prilocaine (EMLA) cream: is it safe and efficacious in preterm infants? J Dev Behav Pediatr.1999; 20:216–221.
11. Ward-Larson C, Horn RA, Gosnell F. The efficacy of facilitated tucking for relieving procedural pain of endotracheal suctioning in very low birth weight infants. MCN Am J Matern Child Nurs. 2004; 29:151–156; quiz 157–158.
12. Carbajal R, Lenclen R, Jugie M, et al. Morphine does not provide adequate analgesia for acute procedural pain among preterm neonates. Pediatrics. 2005;115:1494–1500.
13. Shah V, Ohlsson A. Venepuncture versus heel lance for blood sampling in term neonates. Cochrane Database Syst Rev.2007:CD001452.
14. Cignacco E, Mueller R, Hamers JP, et al. Pain assessment in the neonate using the Bernese Pain Scale for Neonates. Early Hum Dev. 2004;78:125–131.
15. Hummel P, Puchalski M, Creech SD, et al. Clinical reliability and validity of the N-PASS: neonatal pain, agitation and sedation scale with prolonged pain. J Perinatol. 2008;28:55–60.
16. Bellieni C, Maffei M, Ancora G, et al. Is the ABC pain scale reliable for premature babies? Acta Paediatr. 2007;96: 1008–1010.
17. Anand KJ, Aranda JV, Berde CB, et al. Summary proceedings from the neonatal pain-control group. Pediatrics. 2006;117: S9–S22.
18. Stevens BJ, Pillai Riddell R. Looking beyond acute pain in infancy. Pain 2006;124:11–12.
19. Anand KJ. Pain assessment in preterm neonates. Pediatrics 2007;119:605–607.
20. Boyle EM, Freer Y, Wong CM, et al. Assessment of persistent pain or distress and adequacy of analgesia in preterm ventilated infants. Pain. 2006;124:87–91.
21. Debillon T, Zupan V, Ravault N, et al. 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.
22. Ambuel B, Hamlett KW, Marx CM, et al. Assessing distress in pediatric intensive care environments: the COMFORT scale. J Pediatr Psychol. 1992;17:95–109.

23. Simons SH, van Dijk M, van Lingen RA, et al. Routine morphine infusion in preterm newborns who received ventilatory support: a randomized controlled trial. JAMA. 2003;290:2419–2427.
24. van Dijk M, Guldemond F, de Jager Y, et al. The COMFORTneo for daily pain assessment on the Neonatal Intensive Care Unit. Pain Res Manag J. 2006;11:85B.
25. van Dijk M, de Boer JB, Koot HM, et al. The reliability and validity of the COMFORT scale as a postoperative pain instrument in 0 to 3-year-old infants. Pain. 2000;84:367–377.
26. Ista E, van Dijk M, Tibboel D, et al. Assessment of sedation levels in pediatric intensive care patients can be improved by using the COMFORT "behavior" scale. Pediatr Crit Care Med. 2005;6:58–63.
27. Prechtl HF. The behavioural states of the newborn infant (a review). Brain Res. 1974;76:185–212.
28. Holsti L, Grunau RE, Oberlander TF, et al. Body movements: an important additional factor in discriminating pain from stress in preterm infants. Clin J Pain. 2005;21:491–498.
29. Grunau RE, Holsti L, Whitfield MF, et al. Are twitches, startles, and body movements pain indicators in extremely low birth weight infants? Clin J Pain. 2000;16:37–45.
30. Hummel P, van Dijk M. Pain assessment: current status and challenges. Semin Fetal Neonatal Med. 2006;11:237–245.
31. McCaffery M, Pasero C. Pain Clinical Manual. 2nd ed. St Louis, MO: Mosby; 1999.
32. Cohen J. Weighted kappa: nominal scale agreement provision for scaled disagreement or partial credit. Psychol Bull. 1968;70:213–220.
33. Fleiss JL. Statistical methods for rates and proportions. 2nd ed. New York: Wiley; 1981.
34. Bland JM, Altman DG. Correlation, regression, and repeated data. BMJ. 1994;308:896.
35. Stevens B, McGrath P, Gibbins S, et al. Determining behavioural and physiological responses to pain in infants at risk for neurological impairment. Pain. 2007;127:94–102.
36. Guinsburg R, de Araujo Peres C, Branco de Almeida MF, et al. Differences in pain expression between male and female newborn infants. Pain. 2000;85:127–133.
37. Fuller BF. Infant gender differences regarding acute established pain. Clin Nurs Res. 2002;11: 190–203.
38. Bartocci M, Bergqvist LL, Lagercrantz H, et al. Pain activates cortical areas in the preterm newborn brain. Pain. 2006;122:109–117.
39. Matthews JNS, Altman DG, Campbell MJ, et al. Analysis of serial measurements in medical research. BMJ. 1990;300:230–235.
40. Slater R, Cantarella A, Gallella S, et al. Cortical pain responses in human infants. J Neurosci. 2006; 26:3662–3666.
41. Slater R, Fitzgerald M, Meek J. Can cortical responses following noxious stimulation inform us about pain processing in neonates? Semin Perinatol. 2007;31:298–302.
42. Slater R, Cantarella A, Franck L, et al. How well do clinical pain assessment tools reflect pain in infants? PLoS Med. 2008;5:e129.
43. Anand KJ, Hall RW, Desai N, et al. Effects of morphine analgesia in ventilated preterm neonates: primary outcomes from the NEOPAIN randomised trial. Lancet. 2004;363:1673–1682.
44. Zhang GH, Sweitzer SM. Neonatal morphine enhances nociception and decreases analgesia in young rats. Brain Res. 2008;1199:82–90.
45. Jevtovic-Todorovic V, Hartman RE, Izumi Y, et al. Early exposure to common anesthetic agents causes widespread neurodegeneration in the developing rat brain and persistent learning deficits. J Neurosci. 2003;23:876–882.
46. Hall RW, Kronsberg SS, Barton BA, et al. Morphine, hypotension, and adverse outcomes among preterm neonates: who's to blame? Secondary results from the NEOPAIN trial. Pediatrics. 2005; 115:1351–1359.

Chapter 3

Pain management in Neonatal Intensive Care: evaluation of the compliance with guidelines

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Abstract

Background

A pain management protocol was implemented in our Neonatal Intensive Care Unit in 2005, including individual pain assessments and pain treatment guidelines with a decision tree.

Objectives

To prospectively evaluate the degree of compliance of medical and nursing staff with the pain protocol.

Methods

Prospectively recorded pain scores (COMFORTneo score) and all prescribed analgesics and sedatives for the calendar year 2011 were retrieved. The primary outcome is the degree of compliance to the protocol with respect to pain assessments and treatment; the secondary outcome consists of reasons for non-compliance.

Results

Of the 732 included patients, 660 (90%) received fewer than the stipulated 3 assessments per day. Eighty-six per cent of all assessments yielded a score between 9 and 14, suggesting a comfortable patient. In cases of high pain scores (≥14), reassessment within 60 minutes took place in 31% of cases and in 40% treatment was started or adjusted. In cases of low pain scores (≤8) during treatment, 13% of the 457 assessments were reassessed within 120 minutes and in 17% a dose reduction was performed.

Conclusions

Although the majority of pain assessments suggested comfortable patients, there is room for improvement with respect to reassessments after adjustment of analgesic/sedative treatment. Some protocol violations such as oversedation in palliative patients are acceptable but should be well documented.

Introduction

Exposure to pain in early life may alter the central pain pathways, neuronal responses to noxious stimulation, and neuroendocrine and physiological stress responses [1-4]. This, in turn, may affect the neonate's pain processing and long-term development [1, 5]. Pain prevention and adequate pain treatment should therefore be part of daily care in the Neonatal Intensive Care Unit (NICU).

Preterm infants in the NICU undergo around ten to fourteen painful or stressful procedures per day[6-8]. Current pain management in most NICUs consists of pharmacological and non-pharmacological treatment. Non-pharmacological interventions to treat procedural pain consist of swaddling, containment by parent or care giver, non-nutritive sucking with or without a pacifier, facilitated tucking, decreased stimulation, and administration of sucrose [9, 10]. Most frequently used systemic analgesics are opioids (e.g. morphine and fentanyl) and paracetamol. However, standard use of opioids has not been proven effective during mechanical ventilation [11] or to reduce procedural pain [12]. Also, concerns have been expressed about the safety of sedatives as midazolam and phenobarbital in preterm neonates[13]. To prevent neurotoxic effects of anaesthetic and analgesic agents[14, 15], these medications should be individually titrated, based on outcome of assessment with validated pain assessment scales [16].

Between 15% and 65% of European and Australian NICUs have written guidelines for pain treatment; i.e. for acute pain, prolonged pain or both [17-21]. From 6% to 70% of the units use a pain assessment tool. Deindl et al showed that the introduction of pain and sedation protocols in two NICUs resulted in increased use of opioids and other pharmacological interventions [22]. In 2005, our NICU implemented an individualized pain management protocol that includes pain assessment and pain treatment guided by a decision tree (Figure 1). So far, it was never studied if this protocol provides for adequate analgesia in children overall. Also compliance with the protocol of medical staff and nurses was never evaluated.

The aim of this study was to evaluate the degree to which the medical and nursing staff comply with the standardized pain management protocol. A secondary aim was to identify reasons for non-compliance.

Methods

Patients and setting

This study was conducted in the level 3 NICU of the Erasmus MC – Sophia Children's Hospital, Rotterdam, the Netherlands. All neonates (postnatal age < 28 days) admitted longer than 8 hours were eligible for inclusion. The performed study was purely obser-

vational; no additional tests or interventions were done. According to the institutional review board no informed consent was necessary in this observational trial. (MEC-2012-260).

Study design and procedure

We retrospectively determined to what extent nurses and physicians complied to the unit's neonatal pain protocol in calendar year 2011. Data on pain scores (COMFORTneo score and analgesic/sedative medication) prospectively recorded in the electronic patient's chart were retrieved from the Patient Data Management System® (PDMS). Patients' sex, gestational age, birth weight, date of death, ICU length of stay, any surgical procedures, and diagnosis were retrieved from the hospitals' data system. If medication should have been prescribed according to the protocol but was not recorded, the nursing notes in the PDMS were consulted for a possible reason or for information on non-pharmacological interventions.

COMFORTneo score

The COMFORTneo score is a modified version of the COMFORT behavioural scale validated for NICU patients and tested for its psychometric qualities in our level 3 NICU [23]. It consists of behavioural items: alertness, calmness/agitation, respiratory response (only in mechanically ventilated patients) or crying (only in spontaneously breathing patients), body movements, muscle tone and facial tension (observation only).
The six are rated from 1 to 5. Summating the six ratings leads to a total score ranging from 6 to 30. A COMFORTneo score between 9 and 13 suggests 'comfort', between 14 and 30 indicates 'pain or distress' and between 6 and 8 may suggest 'oversedation' during intravenous analgesics or sedatives. The COMFORTneo score can be assessed reliably after a 2-hour training session, has good interrater reliability, concurrent validity and sensitivity to change [23] All nurses were trained to perform COMFORTneo assessments before the introduction of the pain management protocol in 2005. The COMFORTneo item scores are entered in an electronic form (in the PDMS) at the bedside in the PDMS. The system then provides a total score.

Pain management protocol

In 2005 we introduced a pain management protocol dictating that nurses assess pain with the COMFORTneo score in all patients three times every 24 hour (once every 8-hour shift). Extra assessments with the COMFORTneo score are required in case of suspected pain or distress, after an acute painful procedure, in case of suspected oversedation and in case of prolonged use (≥ 5 days) of analgesics and/or sedatives. The final decision whether treatment should be started, maintained, increased or tapered off is made by the attending physician guided by a decision tree (Figure 1).

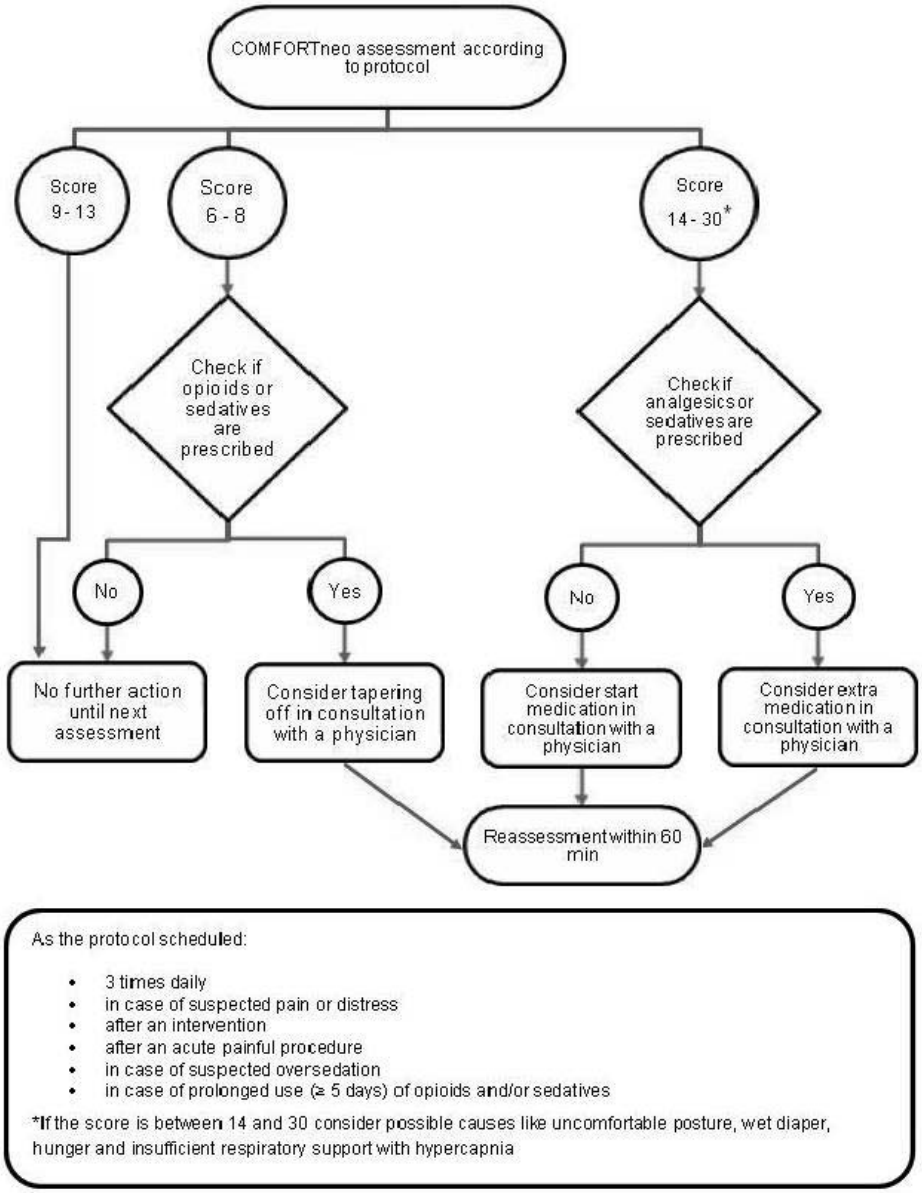

Figure 1. Decision tree NICU June 2012

If the COMFORTneo score is between 9 and 13, no further action is required until next assessment. If the score is between 6 and 8 no further action is required unless analgesics or sedatives are already prescribed. Then, medication should be tapered down. If the score is between 14 and 30, the nurse must consider if factors other than pain might be responsible, such as uncomfortable posture, wet diaper, hunger, and insufficient respiratory support with hypercapnia. The effect of adjusted treatment should be assessed within 60 minutes. However, as the PDMS displays the data on the whole hour only, it was decided to accept 120 minutes in the current study.

Table 1. Patient characteristics

	Patients with assessments (n = 648)	**Patients without assessments (n = 84)**	**p-value**
Boys, n (%)	350 (54.0)	47 (56.0)	0.74
Length of stay (in days), median (IQR)	5 (3 to 11)	1 (1 to 1)	< 0.001
Gestational age at birth (in weeks), n (%)*			< 0.001
23.6 – 26.6 weeks	76 (11.7)	3 (3.6)	
27.0 – 31.6 weeks	210 (32.5)	4 (4.8)	
32.0 – 36.6 weeks	128 (19.8)	17 (20.2)	
>=37 weeks	233 (36.0)	60 (71.4)	
Weight at birth (in g), mean (sd)	2061 (1039)	2769 (888)	< 0.001
Small for gestational age, n (%)	102 (15.7)	13 (15.5)	0.95
Mortality during NICU stay, n (%)	42 (6.5)	2 (2.4)	0.14
Surgical patients, n (%)	80 (12.3)		0.001
Patients receiving opioids, benzodiazepines or paracetamol, n (%)	224 (34.6)	5 (6.0)	<0.001
Diagnosis, n (%)			
Pre- and/or dysmaturity	297 (45.8)	25 (29.8)	
Respiratory insufficiency	79 (12.2)	9 (10.7)	
Asphyxia	51 (7.9)	2 (2.4)	
Congenital anomalies	46 (7.1)	7 (8.3)	
NEC	37 (5.7)		
Observation	36 (5.6)	5 (6.0)	
Suspect for infection	30 (4.6)	16 (19.0)	
PDA surgical closure	25 (3.9)		
Hyperbilirubinemia	10 (1.5)	9 (10.7)	
Convulsions	8 (1.2)	1 (1.2)	
Pneumothorax	7 (1.1)		
Miscellaneous	22 (3.4)	10 (11.9)	

* one missing value (n = 731)

abbreviations: IQR; interquartile range, NICU; neonatal intensive care unit, NEC; necrotizing enterocolitis, PDA; patent ductus arteriosus

Outcome measures

The primary outcome is the degree of compliance of medical and nursing staff with the pain management protocol. Compliance is understood to be at least 3 times daily pain assessments done by the nursing staff, reassessment within 120 minutes and performing a pharmacological intervention when indicated. First day of admission and discharge days were left out of consideration.
The secondary outcome consists of potential reasons for non-compliance; i.e. providing no treatment after a high COMFORTneo score or failure to reduce in case of low COMFORTneo scores.

Data analysis

In table 1 patient characteristics are presented as medians (interquartile ranges) in case of non-normally distributed variables and as means (standard deviations) in case of normally distributed variables. Two patient groups were distinguished: patients whose pain had indeed been assessed and patients who had not been assessed. Background characteristics were compared using the t-test or Mann-Whitney U-test in case of continuous variables. Chi square tests or Fisher exact tests were used in case of categorical variables. Data analyses were performed with Statistical Package for the Social Sciences (SPSS) version 20.0 (SPSS Inc.). A p-value of 0.05 was deemed statistically significant.

Results

Of the 778 patients admitted in 2011, n=46 were excluded because they had been admitted for less than 8 hours. Of the 732 included patients, 660 (90%) received fewer than the stipulated 3 assessments per day and in total 84 patients (11%) had not been assessed at all. Patients who were not assessed had a median length of stay of 1 day (IQR 1 to 1); assessed patients had a median of 5 days (IQR 3 to 11) ($p < 0.001$). The former were significantly more often born at term age ($p < 0.001$). All the surgical patients ($n = 80$) were assessed ($p < 0.001$).
Of all 732 included patients, one-quarter received opioids either as a bolus or continuously. A total of 159 (22%) patients received continuous morphine with a mean dose of 11.9 mcg/kg/hr. (SD 4.9). Main reasons for opioids use (more than one reason possible) were postoperative pain (n=59), distress or irritability (n=33), chest drain (n=22), palliative end-of-life care (n=14), distress (n=13), cooling due to asphyxia (n=11), conservative treatment of NEC ($n = 9$) and fractures (n=3). Hundred and four (14%) patients received sedatives, which consisted of midazolam in 78 (75%) patients.

Compliance to standard pain assessments

The total number of 24 hour admission days was 6858; full nursing staff compliance would therefore have required at least 3 x 6858 = 20,574 assessments. However, as only 12392 assessments were documented; the compliance rate was 60.2%. The assessments were evenly distributed among the shifts; 5409 (37%) during the day shift, 4827 (33%) during the evening shift and 4306 (30%) during the night shift.

Assessment and treatment compliance on high COMFORTneo scores

Eighty-six per cent of the pain assessments in 2011 suggested 'comfort' and required no further action (Figure 2). High COMFORTneo scores (between 14 and 30) were assigned in 1578 (11%) assessments and 31% were reassessed within 120 minutes. Pharmacological interventions were performed after 633 of these 1578 assessments (40%); analgesics in 77.4%, sedatives in 6.6% and a combination of the two in 16.0% assessments. In

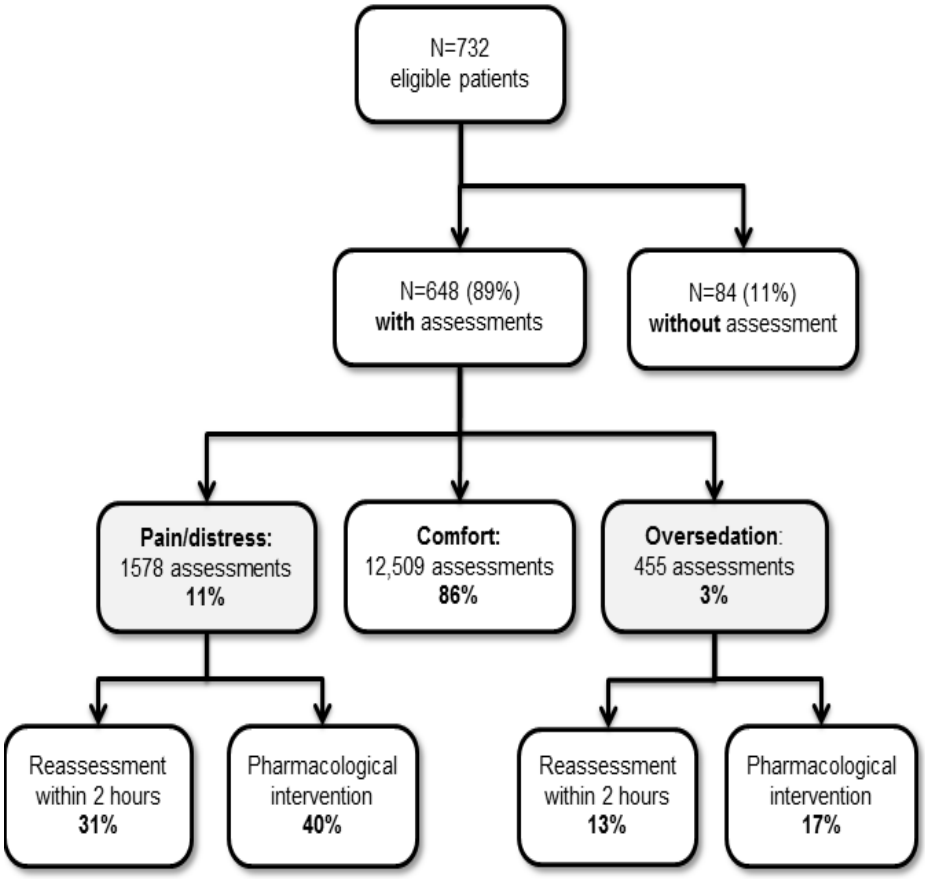

Figure 2. Flow Chart

27.5% of cases of non-compliance reasons were documented in the medical records; for example maximum analgesia reached, restlessness related to respiratory failure, hunger, obstipation, withdrawal syndrome or irritability.

Compliance after low COMFORTneo scores

Low COMFORTneo scores (between 6 and 8) in patients receiving continuous analgesics and/or sedatives were assigned in 3% of assessments and 13% were reassessed within 120 minutes. In 77 assessments (17%) medication was tapered according to protocol. If not, documented reasons were: sedatives used as anti-convulsant therapy, NEC or other painful disorder (e.g. skin lesions), chest tube, discomfort on the ventilator, end-of-life care and total body cooling in case of asphyxia.

Discussion

Overall, compliance with the pain management protocol was low but nevertheless some two thirds majority of the Comfort neoscores suggested comfortable patients despite a relatively limited use of morphine and sedatives. When morphine or midazolam was prescribed, a reason was well described in the medical records. Comparing this with years of routine continuous morphine infusions to ventilated neonates, we can be satisfied with this progress.

Daily pain assessments were introduced in 2003 in our unit because neonatal pain might be otherwise unrecognized and undertreated. In our study and in contrast to what we expected, 86% of all assessments suggested comfortable patients. The unassessed patients (11%) had a short length of stay in our NICU (median of 1 day) which indicates stable, not severely sick children who are most likely not in pain. This raises the question whether all patients should be strictly assessed three times a day. Perhaps an individualised strategy should be considered including a special focus on the high-risk patients. Patients at risk of pain that might need more intensive pain monitoring are extreme preterm infants enduring frequent painful procedures, postoperative patients, patients with necrotizing enterocolitis, patients with chest drains, and patients with rare painful skin diseases such as epidermolysis bullosa. A three times a day strategy would probably fit for septic instable or invasively ventilated newborns. This individualized approach could be less time consuming than standardised assessments in all patients and might enhance compliance to pain assessments. On the other hand, standard pain assessments may decrease the risk of missing patients who suffer pain. One way to stimulate better compliance to pain assessment could be introducing an alert to assess pain at a set time in each shift. Why standard pain assessment is so difficult to adhere to remains a question, already raised by Franck and Bruce in 2009[24] and others[25]. One reason we

could think of, is the fact that a gold standard for pain assessment is lacking in preverbal infants which is in contrast to vital signs such as 'heart rate' and 'blood pressure'. Nurses might feel that therefore pain assessment is less important than temperature taking for instance. In adults where self-reported pain is considered the gold standard it is easier to introduce pain assessment as the fifth vital sign as suggested by many international medical societies.

Articles on compliance and an acceptable level of compliance with pain protocols are largely lacking in literature. A percentage of 80% seems clinically feasible but compliance level of reassessment after interventions or readjustment of analgesics/sedative treatment should preferably be 100%. Ceelie et al also evaluated the compliance with a prescribed pain protocol [26]. This study looked at a post-operative pain protocol on the paediatric intensive care unit (PICU) in our institute. They found the same level of compliance, i.e. in approximately 15% the nurses and physicians followed the protocol of both treatment and reassessment. Deindl et al successfully implemented a Neonatal Pain and sedation Protocol and showed an increase in opiate prescription, pharmacologic interventions and staff satisfaction without affecting time on mechanical ventilation, length of stay on intensive care and adverse outcome [22]. However, one could question if increase in opiate prescription is necessarily an improvement bearing in mind the adverse long-term effects of opioids described in animal studies [27-31].

A limitation of our study is that nurses and physicians work in different electronic documentation systems for their clinical notes and there is no connection between these systems. Data might get lost or remain unread. Furthermore, medical and nursing staff do not always document the possible reasons for deviating from the treatment protocol. The retrospective nature of the study makes it more difficult to have complete access to these reasons.

Compliance with protocols and guidelines in health care is challenging in many settings [32, 33]. Dutch physicians considered the fact that protocols often do not match the individual patient as an important barrier to compliance. This also seems to apply in our patient group. Some patients are deeply sedated to provide optimal comfort in end-of-life care and therefore we accept low COMFORTneo scores. High COMFORTneo scores may also be accepted as maximum therapy is provided and further increase of medication will add more adverse effects e.g. on blood pressure, motility of the gut and bladder function. These exceptions may imply that 100% compliance is not feasible. A paradigm shift may be warranted here, in which working according to protocol also offers leeway to deviate from protocol in justifiable cases. A prerequisite is, however, that these justifiable violations are well communicated with co-workers and recorded in the patient data management system.

As this study was conducted in a single NICU, the generalizability of the results of our study is unclear. The fact the pain is a special point of interest in our center probably even

overestimates the pain and analgesia evaluation on other non-pain centers. Another matter of debate is the appropriateness of reassessment within 120 minutes after dose reduction, bearing in mind that drug clearance in neonates is slower than in adults and that clearance is altered in critically ill patients[34-38]. This suggests that reassessment after tapering off drugs should be performed more than once and preferably after 30 minutes of an alteration in medication.

Conclusion

This study reveals that the majority of pain scores suggested comfort, however pain assessments 3 times daily were not always performed. Compliance to pain assessment and treatment was far from perfect. Pain is insufficiently scored and decision making around treatment poorly reported.

Recommendations

All protocols should be routinely evaluated for their applicability. Justified and individualized adaptation might help increase compliance and get rid of useless and time-consuming procedures not resulting in adjustment of treatment. For neonatal pain protocols we recommend to tailor the number of pain assessments to the individual patient. Selecting critically ill patient groups for standardized pain assessment and leaving the stable patients aside is not commendable in NICU patients: a pitfall could be that stable non assessed patients are forgotten when their clinical situation deteriorates. We recommend that nurses and physicians should be better instructed to identify and document reasons for deviating from the pain protocol and emphasize the roll of reassessments. A recommendation for future research is studying the effect of monthly feedback about the compliance to reassessments as well as involving parents in pain assessment of their own child comparing to health care givers. For the current COMFORTneo score a 2 minutes observation is necessary. Future studies should also focus on the reliability of shorter, more efficient, pain observations. This would probably increase the compliance rate and would identify patients at 'risk' for pain.

References

1. Grunau R. Early pain in preterm infants. A model of long-term effects. Clin Perinatol 2002;29: 373-94, vii-viii.
2. Hermann C, Hohmeister J, Demirakca S, et al. Long-term alteration of pain sensitivity in school-aged children with early pain experiences. Pain 2006;125:278-85.
3. Hohmeister J, Demirakca S, Zohsel K, et al. Responses to pain in school-aged children with experience in a neonatal intensive care unit: cognitive aspects and maternal influences. Eur J Pain 2009; 13:94-101.
4. Walker SM, Franck LS, Fitzgerald M, et al. Long-term impact of neonatal intensive care and surgery on somatosensory perception in children born extremely preterm. Pain 2009;141:79-87.
5. Johnston C, Barrington KJ, Taddio A, et al. Pain in Canadian NICUs: have we improved over the past 12 years? Clin J Pain 2011;27:225-32.
6. Carbajal R, Rousset A, Danan C, et al. Epidemiology and treatment of painful procedures in neonates in intensive care units. JAMA 2008;300:60-70.
7. Simons SH, van Dijk M, Anand KS, et al. Do we still hurt newborn babies? A prospective study of procedural pain and analgesia in neonates. Arch Pediatr Adolesc Med 2003;157:1058-64.
8. Roofthooft DW, Simons SH, Anand KJ, et al. Eight years later, are we still hurting newborn infants? Neonatology 2014;105:218-26.
9. Cignacco E, Hamers JP, Stoffel L, et al. The efficacy of non-pharmacological interventions in the management of procedural pain in preterm and term neonates. A systematic literature review. Eur J Pain 2007;11:139-52.
10. Cignacco EL, Sellam G, Stoffel L, et al. Oral sucrose and "facilitated tucking" for repeated pain relief in preterms: a randomized controlled trial. Pediatrics 2012;129:299-308.
11. Holsti L and Grunau RE. Considerations for using sucrose to reduce procedural pain in preterm infants. Pediatrics 2010;125:1042-7.
12. Carbajal R, Lenclen R, Jugie M, et al. Morphine does not provide adequate analgesia for acute procedural pain among preterm neonates. Pediatrics 2005;115:1494-500.
13. Ng E, Taddio A and Ohlsson A Intravenous midazolam infusion for sedation of infants in the neonatal intensive care unit. Cochrane Database of Systematic Reviews. John Wiley & Sons, Ltd, 2012.
14. Loepke AW and Soriano SG. An assessment of the effects of general anesthetics on developing brain structure and neurocognitive function. Anesth Analg 2008;106:1681-707.
15. Mellon RD, Simone AF and Rappaport BA. Use of anesthetic agents in neonates and young children. Anesth Analg 2007;104:509-20.
16. Spence K, Henderson-Smart D, New K, et al. Evidenced-based clinical practice guideline for management of newborn pain. J Paediatr Child Health 2010;46:184-92.
17. Debillon T, Bureau V, Savagner C, et al. Pain management in French neonatal intensive care units. Acta Paediatr 2002;91:822-6.
18. Gharavi B, Schott C, Nelle M, et al. Pain management and the effect of guidelines in neonatal units in Austria, Germany and Switzerland. Pediatr Int 2007;49:652-8.
19. Harrison D, Loughnan P and Johnston L. Pain assessment and procedural pain management practices in neonatal units in Australia. J Paediatr Child Health 2006;42:6-9.
20. Lago P, Guadagni A, Merazzi D, et al. Pain management in the neonatal intensive care unit: a national survey in Italy. Paediatr Anaesth 2005;15:925-31.

21. Latimer MA, Johnston CC, Ritchie JA, et al. Factors affecting delivery of evidence-based procedural pain care in hospitalized neonates. J Obstet Gynecol Neonatal Nurs 2009;38:182-94.
22. Deindl P, Unterasinger L, Kappler G, et al. Successful implementation of a neonatal pain and sedation protocol at 2 NICUs. Pediatrics 2013;132:e211-8.
23. van Dijk M, Roofthooft DW, Anand KJ, et al. Taking up the challenge of measuring prolonged pain in (premature) neonates: the COMFORTneo scale seems promising. Clin J Pain 2009;25:607-16.
24. Franck LS and Bruce E. Putting pain assessment into practice: why is it so painful? Pain Res Manag 2009;14:13-20.
25. Lee GY, Yamada J, Kyololo O, et al. Pediatric clinical practice guidelines for acute procedural pain: a systematic review. Pediatrics 2014;133:500-15.
26. Ceelie I, de Wildt SN, de Jong M, et al. Protocolized post-operative pain management in infants; do we stick to it? Eur J Pain 2012;16:760-6.
27. Atici S, Cinel L, Cinel I, et al. Opioid neurotoxicity: comparison of morphine and tramadol in an experimental rat model. Int J Neurosci 2004;114:1001-11.
28. Bhutta AT, Rovnaghi C, Simpson PM, et al. Interactions of inflammatory pain and morphine in infant rats: long-term behavioral effects. Physiol Behav 2001;73:51-8.
29. Laprairie JL, Johns ME and Murphy AZ. Preemptive morphine analgesia attenuates the long-term consequences of neonatal inflammation in male and female rats. Pediatr Res 2008;64:625-30.
30. Traudt CM, Tkac I, Ennis KM, et al. Postnatal morphine administration alters hippocampal development in rats. J Neurosci Res 2012;90:307-14.
31. Zhang GH and Sweitzer SM. Neonatal morphine enhances nociception and decreases analgesia in young rats. Brain Res 2008.
32. Lugtenberg M, Burgers JS, Besters CF, et al. Perceived barriers to guideline adherence: a survey among general practitioners. BMC Fam Pract 2011;12:98.
33. Lugtenberg M, Burgers JS, Clancy C, et al. Current guidelines have limited applicability to patients with comorbid conditions: a systematic analysis of evidence-based guidelines. PLoS One 2011;6: e25987.
34. Scott CS, Riggs KW, Ling EW, et al. Morphine pharmacokinetics and pain assessment in premature newborns. J Pediatr 1999;135:423-9.
35. de Wildt SN, Kearns GL, Hop WC, et al. Pharmacokinetics and metabolism of intravenous midazolam in preterm infants. Clin Pharmacol Ther 2001;70:525-31.
36. Bouwmeester NJ, Anderson BJ, Tibboel D, et al. Developmental pharmacokinetics of morphine and its metabolites in neonates, infants and young children. Br J Anaesth 2004;92:208-17.
37. Jacqz-Aigrain E and Burtin P. Clinical pharmacokinetics of sedatives in neonates. Clin Pharmacokinet 1996;31:423-43.
38. Vet NJ, de Hoog M, Tibboel D, et al. The effect of critical illness and inflammation on midazolam therapy in children. Pediatr Crit Care Med 2012;13:e48-50.

Chapter 4

Eight years later, are we still hurting newborn infants?

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Abstract

Objective

To study whether new pharmacological and non-pharmacological guidelines lowered numbers of painful procedures in neonates and changed the amount and frequency of analgesic therapy as compared to the results of our previous study in 2001.

Design

A prospective observational study.

Setting

Level III NICU of the Erasmus MC-Sophia Children's Hospital, Rotterdam.

Participants

Neonates admitted at postnatal ages less than 3 days with length of stay at least 72 hours.

Main Outcome Measures

Number of all potentially painful procedures and analgesic therapy recorded at the bedside during the first 14 days of NICU stay.

Results

A total number of 21076 procedures were performed in the 175 neonates studied during 1730 patient-days (mean 12.2). The mean number of painful procedures per neonate per day was 11.4 (SD 5.7), significantly lower than the number of 14.3 (SD 4.0) in 2001 ($p<0.001$). The use of analgesics was 36.6% compared to 60.3% in 2001. Sixty-three percent of all peripheral arterial line insertions failed vs. 37.5% in 2001 and 9.1% venipuncture's failed vs. 21% in 2001.

Conclusions

The mean number of painful procedures per NICU patient per day declined. Non-pharmacological pain- or stress reducing strategies like NIDCAP and sucrose were fully embedded in our pain management. As further reduction of the number of painful procedures is unlikely we should apply more non-pharmacological interventions and explore newer pharmacological agents.

Introduction

In 2001 in a prospective study on procedural pain and analgesia in our level 3 NICU, neonates underwent a mean of 14.3 painful procedures a day [1]. In 2008, the EPIPPAIN study reported a median of 115 procedures during a study period of 14 days; in almost 80% of cases analgesics were not given [2]. Johnston et al. in 2010 reported a drop in the number of tissue damaging procedures in Canadian NICUs over a 12-year period, still half of the procedures were performed without analgesics [3]. In our previous study 60.3% of patients were given analgesics [1]. Care givers may be reluctant to prescribe analgesics to neonates for fear of adverse effects, drug tolerance and dependence. Moreover, dosing guidelines and pharmacokinetic data on common drugs for neonates of different gestational ages and birth weights are often lacking [4].

Neonates can feel pain from 23-24 weeks gestation[5] and early exposure to repetitive untreated pain portends immediate and long-term consequences on behavioural and neurological outcome [6] [7, 8]. Animal and human studies have shown significant risk for neurological impairment, besides learning, cognitive and behavioral effects [8, 9]If this holds true for the longer term is not known because longitudinal data are largely lacking. De Graaf et al. showed negative effects on cognitive functioning in 5-year old children who as a neonate received morphine [10]. In 2001 we introduced NIDCAP on our ward, placing a focus on non-pharmacological pain management [11, 12]. Several guidelines for procedural painmanagement in neonates have been published since our previous studies [1]. As a result of this we routinely administer sucrose 24% orally before painful procedures such as heel lancing, line insertion, and retinopathy screening since 2005. Sucrose has been proven to alleviate pain in mildly to moderately painful procedures in neonates [13-16].

Based on the results of our former study, current pain management includes repeated pain assessments with the COMFORTneo scale (Appendix 1), analgesic treatment according to a decision tree (Appendix 2), NIDCAP care, and other non-pharmacological pain relieving interventions.

In the current study we evaluated whether these policy changes lowered the number of notably painful procedures and investigated what types of procedures often failed. Finally, we quantified analgesic therapy and compared findings with those from the previous study.

Methods

Data collection

The study design was prospective and observational. From February 6, 2009 to August 5, 2009, nurses and medical staff collected bedside data on all procedures (successful

and failed) children underwent during the first 14 days of admission to the level III NICU of the Erasmus MC-Sophia Children's Hospital Rotterdam, the Netherlands. Patients older than 3 days at admission and those transferred or discharged within 72 hours after admission were excluded. Data collection for children discharged from the unit between 4 and 14 days was stopped on the day of discharge.

A procedure was defined as any medical, nursing, surgical, diagnostic or therapeutic intervention. Invasive or painful procedures were defined as interventions that cause mucosal or skin injury from removal or introduction of foreign material [2]. Pain guidelines of our department prescribe sucrose for minimal invasive or mild to moderate painful procedures and opioids for invasive and severe painful procedures (chest tube insertion). Intubations are performed with propofol. All procedures are conducted with parental or caregivers containment (NIDCAP).

Per calendar day data were recorded on a case record form and combined with the electronic patient's chart data in the Patient Data Management System® (PDMS). Data included background characteristics, type and duration of respiratory support and CRIB® (Clinical Risk Index for Babies) scores [17], as well as administration of pain medication.

Data on analgesics and pain scores during the study days were retrieved from the PDMS. Nurses assessed the neonates pain with the Numeric Rating Scale (NRS from 1-10) and the COMFORTneo scale at least once during every 8 hour shift [18, 19]. The COMFORTneo scale has been validated and its cutoff value for pain is a score of 14 and higher combined with an NRS score of 4 and higher. A COMFORTneo score of 14 and higher combined with an NRS score below 4 is considered a sign of distress and not pain. Extra assessments are performed after administration of sedatives or analgesics, or if pain, or over- or under-sedation are suspected.

The performed study was purely observational. No additional tests or interventions were done. According to the Dutch law no ethical approval was necessary in this purely observational trial.

Statistical analysis

Numbers of procedures were counted per calendar day and corrected for the actual length of stay on the first and last study days. Data are presented as mean (SD) for normally distributed variables and as median interquartile range for non-normally distributed variables. Numbers of painful procedures and background characteristics were compared between four gestational age groups (24-28 wks, 29-32 wks, 33-36 wks, and 37-42 weeks) using ANOVA with Bonferroni correction. Findings from 2001 are compared with those of 2009 using the independent t-test for continuous variables and the chi-square test for proportions. Analyses were performed with the SPSS statistical program (version 17.0).

Results

Patients

Table 1 lists the background characteristics of the 175 enrolled neonates. Mean gestational age was 31.6 weeks (range 24 1/7 to 41 6/7 weeks); 37.1% had a gestational age less than 29 weeks; Median birth weight was 1770 gram and 26.3% patients were small for gestational age.

Table 1. Background characteristics

	Total population N=175	**24-29 wks N=65 37.1%**	**30-32 wks N=49 28.0%**	**33-36 wks N=31 17.7%**	**37-42 wks N=30 17.1**
	N (%)	N (%)	N (%)	N (%)	N (%)
Male: N(%)	89(50.9)	28(43.1)	23(46.9)	19(61.3)	19(63.3)
Birth weight (grams):	1775	935	1405	2200	3545
Median (IQR)	(1080-1380)	(710-1110)	(1188-1705)	(1725-2620)	(3277-3962)
Small for gestational age (SGA): N (%)	46 (26.3)	20(30.8)	18(36.7)	7(22.6)	1(3.3)
CRIB Score: Mean(SD)	2.7 (2.6)	4.5(3.0)	1.3(1.7)	1.8(1.9)	2.2(1.6)
Maximum respiratory support:					
Mech ventilation	105(60.0)	58(89.2)	18(36.7)	14(45.2)	15(50.0)
CPAP/Non Invasive ventilation	36 (51.4)	7(10.8)	21(67.7)	6(35.3)	2(13.3)
Nasal prongs	16 (47.1)	-	5(50.0)	6(54.5)	5(38.5)
No respiratory support	18 (10.3)	-	5(10.2)	5(16.1)	8(26.7)
Duration maximum Respiratory support, median(IQR)					
Days on mechanical ventilation	4 (1-9)	5(1-13)	2(1-4)	3 (1-4)	4(3-5)
Days on CPAP	4 (2-7)	9 (3-14)	3(4-5)	3 (2-4)	1 day both
Days on Nasal Prongs	3 (1-5)	-	5 (2-10)	2 (2-3)	1 (1-8)
IRDS, n (%)	76 (43.4)	53 (81.5)	14 (28.6)	8 (25.8)	1 (3.3)
Asphyxia, n (%)	14 (8.0)	3 (4.6)	1 (2.0)	2 (6.5)	8 (26.7)
Intraventriculair haemorrhage, n (%)	20 (11.4)	17(26.2)	1(2.0)	1(3.2)	1(3.3)
Patent Ductus arteriosus, n (%)	42 (24.0)	32(49.2)	4(8.2)	2(6.5)	4(13.3)
Surgery during study period, n (%)	8(4.6)	6(9.2)	-	1(3.2)	1(3.3)
Died during study period, n (%)	9 (5.1)	5 (7.7)	1(2.0)	1(3.2)	2(6.7)

Abbreviations: CPAP, Continuous positive pressure ventilation; IRDS, Infant respiratory distress syndrome
ANOVA, because of significant difference between the youngest GA group and the other groups
† Fisher exact test, Percentages are column percentages

The overall CRIB score was 2.7 (SD ±2.6) on a 0 to 10 scale. Sixty percent of all patients received conventional ventilation or high frequency oscillation/ventilation as maximum ventilatory support. The incidences of IRDS, intraventriculair hemorrhage and patent ductus arteriosus were highest (81.5%, 26.2% and 49.2% respectively) in infants with a gestational age less than 29 weeks. Asphyxia was diagnosed in 8 patients (26.7%) in the age group of 37-42 weeks and in 6 patients (13.1%) with a gestational age less than 37 weeks. Nine patients (5.1%) died during the study period.

Compared to the study population of 2001 the present population included significantly more preterm infants (145 /175 versus 104/151 patients in 2001, p = 0.003).

Incidences of procedures

Table 2 shows the incidences of procedures in this study and in the 2001 study, ranked in order of frequency established in the 2001 study. The total number of procedures in the current study was 21076 during 1730 patient-days; or mean 12.2 per patient-day. The mean number of painful procedures per neonate per day equaled 11.4 (SD 5.7) versus14.3 (SD 4.0) in 2001 (p<0.001). Broken down for age groups, it was highest in age

Table 2. Incidences of procedures in 2001 and 2009, with frequencies per infant per day and p-values comparing frequencies

	% of Total procedures		Frequency per infant per day, Mean (SD)		SDM	p-value
Procedure	2001 N = 151	2009 N = 175	2001	2009		
Nasal suctioning	31.2	31.6	4.5 (2.3)	3.4 2.2)	0.49	p<0.001
Endotracheal suctioning	23.0	23.0	3.3 (4.0)	2.5 3.5)	0.21	p=0.06
NPT suctioning	9.4	5.7	1.3 (2.4)	0.6 (1.2)	0.37	p<0.001
Heel lancing	7.1	10.7	1.0 (1.6)	1.5 (1.1)	0.36	p=0.001
IV cannula insertion	3.8	3.2	0.5 (0.6)	0.4 (0.3)	0.21	p=0.06
Nasogastric tube insertion	3.8	1.9	0.5 (0.6)	0.2 (0.1)	0.70	p<0.001
IV cannula removal	3.2	2.2	0.5 (0.7)	0.3 (0.2)	0.39	p<0.001
Nasogastric tube removal	3.1	1.0	0.4 (0.5)	0.1 (0.1)	0.83	p<0.001
X-ray	2.9	2.8	0.4 (0.9)	0.3 (0.3)	0.15	p=0.17
NPT insertion	2.4	3.3	0.3 (0.6)	0.4 (0.4)	0.20	p=0.08
Failed IV cannula insertion	1.7	2.0	0.2 (0.9)	0.2 (0.3)	0	p=1.0
Laxative or enema	1.2	1.1	0.2 (0.5)	0.1 (0.1)	0.28	p=0.01
Nasal oxygen cannula insertion	1.0	1.0	0.2 (0.4)	0.1 (0.2)	0.32	p=0.004
Intubation	0.9	0.6	0.1 (0.4)	0.08 (0.08)	0.07	p=0.52
Peripheral arterial line insertion	0.8	0.4	0.1 (0.3)	0.05 (0.07)	0.23	p=0.04
Extubation	0.7	0.5	0.1 (0.3)	0.06 (0.08)	0.18	p=0.09
Peripheral arterial line removal	0.6	0.3	<0.1 (0.3)	<0.1 (0.06)		
Failed peripheral arterial line insertion	0.5	0.8	<0.1 (0.5)	<0.1 (0.06)		
Venipuncture	0.4	0.2	<0.1 (0.3)	<0.1 (0.1)		
Insertion umbilical line	0.4	0.3	<0.1 (0.2)	<0.1 (0.05)		
Removal umbilical line	0.3	0.4	<0.1 (0.2)	0.1 (0.1)		
Failed umbilical line insertion	0.2	0.2	<0.1 (0.2)	<0.1 (0.06)		
Insertion central line	0.2	0.4	<0.1 (0.2)	<0.1 (0.05)		
		0.2	<0.1 (0.2)	<0.1 (0.1)		
		0.2	<0.1 (0.2)	<0.1 (0.09)		
		0.02	<0.1 (0.2)	<0.1 (0.03)		
		0.2	<0.1 (0.1)	<0.1(0.05)		
		0.07	<0.1 (0.1)	<0.1 (0.04)		

Abbreviations: IV, intravenous; NPT, nasopharyngeal tube, SDM=standardized mean difference

group 24-29 weeks at 14.1 (SD 5.2); followed by age group 30-32 weeks at 9.9 (SD 3.8) and age group 33-36 weeks at 9.1 (SD 5.7).

Suctioning (endotracheal, nasopharyngeal and nasal) was the most frequent painful procedure, comprising 60.3% of all procedures vs.63.6% in 2001.

Second most commonly performed procedure was heel lancing, comprising 10.7% of all procedures vs. 7.1% in 2001. Nasal pharyngeal tube insertion accounted for 3.3% of all procedures in 2009 vs. 2.4% in 2001; intubation for 0.6% of all procedures vs. 0.9% in 2001. Sixty-three percent of all peripheral arterial line insertions failed vs. 37.5% in 2001. Umbilical arterial line insertions failed in 49.5% of cases; umbilical venous line insertions in 36.6% of cases (34.6% in 2001. Intravenous cannula insertions failed in 38% of cases vs. 30.9% in 2001, venipuncture's in 9.1% vs. 21% in 2001 (Figure 1). Of all intubations 22.5% needed more than 1 attempt.

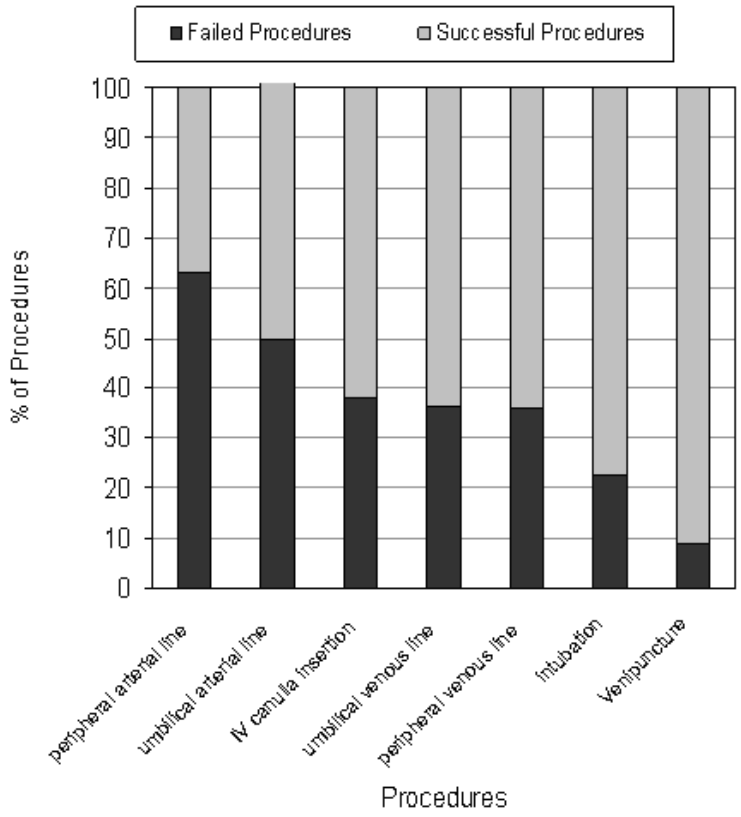


Figure 1. Percentages of failed and successful procedures

Although the present population included significantly more preterm infants, the mean number of painful procedures was lower in the age groups of 30-32.6 weeks (12.0 in 2001 versus 9.9 in 2009) and 33-36.6 weeks (12.3 versus 9.1 in 2009) but not in the more premature infants of 24-29.6 weeks (15.2 in 2001 versus 14.0 in 2009).

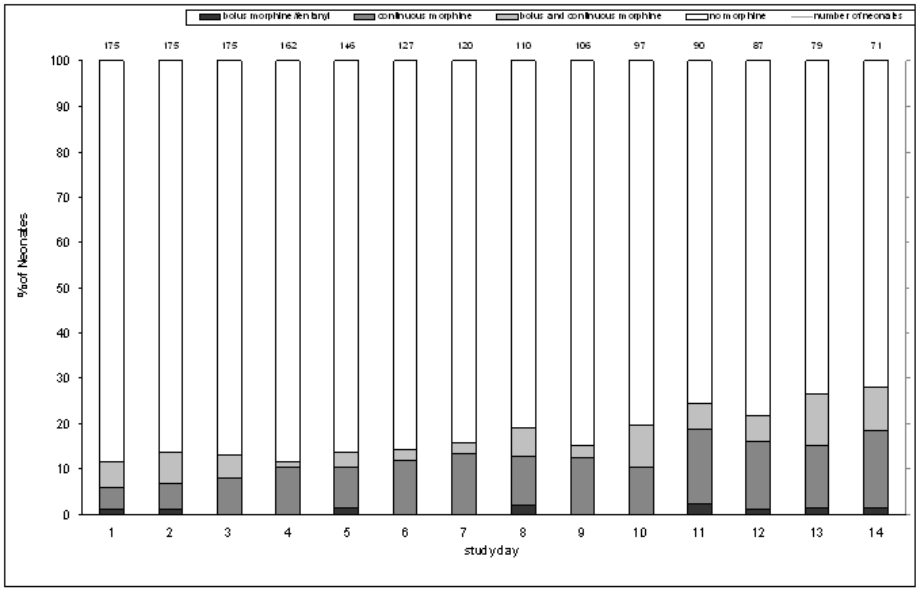


Figure 2. Pharmacological analgesic treatment

Analgesics

Sixty-four neonates (36.6%) received one or more doses of analgesics. Twenty-one of those (12%) received rectal paracetamol during a median of two days (range1 to 11 days). Forty-eight neonates (27.4%) received continuous morphine, whether or not combined bolus morphine for a median of 3 days (range 1 to 12 days); eight of those underwent an operation during the study period. Three neonates received fentanyl as bolus medication from 1 to 3 times (Figure 2).

Pain assessments

Almost all patients (169; (96.6%) underwent pain assessment during the study period i.e. a median 16 assessments (range 1 to 61) per patient. The number of COMFORTneo assessments was 2962 during 1690 patient-days, corresponding to 1.7 per patient-day. Out of 2962 assessments 2901 involved complete assessments meaning COMFORTneo, NRS pain and NRS distress scores. In 88.9% of the cases all three scores were low or at least acceptable. In 4.8% of assessments the COMFORTneo was 14 or higher but both

NRS were not > 3. In 3.7 of cases both COMFORTneo and NRS distress were high but NRS pain<3. In 1% of the assessments all three scores were too high. Of our complete cases of 2901 assessments, 89.2% included standard pain assessments, in 7.1% the reason for assessment was suspected pain or distress. 2.8% of assessments were performed after a pain or distress reducing intervention. In 0.6% (n=17) the reason to assess was suspected oversedation. Finally in only 8 assessments it was after an acute painful procedure. N=90 infants out of the 169 (53.2%) had at least once a COMFORTneo score of 14 or higher.
Possible reasons for high scores were NEC (n=7), skin-related (n=5), delivery-related (n=3, breech and vacuum extraction), thorax drainage (n=2), postoperative (n=2) and others (n=8).

Comment

Our analysis revealed that the mean number of painful procedures per patient per day had statistically significantly declined from 14.3 in 2001 to 12.2 in 2009. Studies from NICUs in different countries likewise have shown a similar trend with a range from 6 to 17.3 procedures per patient per day [1-3, 19-21]. Suctioning, venous catheter placement and heel lancing are most frequent across all settings. In our NICU endotracheal suctioning still accounts for 23.0% of all procedures (same in 2001) and is comparable with the 23.3% reported in the EPIPPAIN study, even though we stopped routine suctioning of mechanically ventilated neonates after 2003[2].
The nasopharyngeal tube insertion rate increased from 2.4% in 2001 to 3.3% in the current study and the number of intubations dropped from 0.9% (2001) to 0.6% of all procedures in 2009. This may be due to the introduction of non invasive ventilation in 2008. Introduction of propofol as premedication for intubation explains the lesser number of failed intubations in 2009. Compared to the 2001 cohort we included more preterm infants in the current study but the more preterm infants (24-29.6 weeks) unalterably need the highest number of painful procedures due to their unstable condition in the first period of life. It remains a source of concern that adequate care for our patients still involves on average 11 painful events per day.

In 2001, 60.3% of neonates received analgesics, especially opioids versus 36.6% in 2009. This significant drop is the result of study findings showing that routinely administration of morphine in ventilated neonates had no beneficial effects on pain expression and neurologic outcome [22-24]. A follow up study described negative effects of neonatal morphine administration on cognitive functioning at age 5 years [10]. Pre-emptive use of opioids in preterm newborns has not been properly studied. Morphine might be beneficial when pain is present, but could be neurotoxic in the absence of pain as suggested

in animal studies [9]. Intravenous paracetamol administration might be an alternative but is used off label and may be unsafe unless future PK/PD studies prove otherwise. In the meantime we should focus on expanding the range of (non)pharmacological and non-pharmacological pain treatment. As to the latter, we have already experience with sucrose and NIDCAP interventions such as positioning, swaddling, non-nutritive sucking, and kangaroo care. Furthermore, our pain management guidelines enabled caregivers in the current study to respond immediately when painful procedures were carried out or pain was suspected. All above measures together might explain the high percentage (89%) of low COMFORTneo scores (<14). In only 2.4% of the pain assessments the child was perceived to be in pain, attributable to pain conditions such as NEC and severe skin lesions. We have every reason to believe, therefore, that we did not undertreat or misdiagnose neonatal pain.

Two study strengths can be identified. First, the second study was conducted in the same level 3 NICU. Second, nursing and medical staff participated in both studies using the same case record forms.

Strikingly, however, the frequency of failed procedures did not decline over the years. An explanation might be that since 2008 more extremely premature infants (gestational age < 27 weeks) were admitted and survived on our ward. Nowadays, the critically ill patients and extremely premature infants stay on our ward and undergo the highest number of painful procedures during the first 14 days of life. The high frequency of failed procedures might also be explained by the reduction of the training period for residents on the NICU so they have less opportunity to gain experience.

Several limitations of this study should be addressed. First, to compare our results with our former study, we did not distinguish between painful and stressful procedures, like Carbajal et al. did[2]. A further distinction between skin breaking and non-skin breaking procedures would also have been interesting as the first were recently related to white matter changes and brain development on MRI scans at term equivalent age of ex premature born neonates [25]. Second, during painful procedures we used sucrose and containment according to our pain guidelines but no other non-pharmacological interventions such as skin-to-skin care or breastfeeding. Thirdly we found that routine administration of sucrose was not always documented in the patient's chart. A detailed sucrose prescription is now added in the electronic patient chart safeguarding more accurate report of the daily used doses of sucrose. Furthermore, this study was a single center study and not a multiple center study like in 2001 which could give limited generalizability of this data. And finally, we can't exclude that (failed) interventions or administration of analgesics went unrecorded.

Conclusions

Our new (non)pharmacological pain management has only minimally but statistically significant ($p<0.001$) reduced the number of daily painful procedures. It might be hard to achieve further reduction. The findings from the present study were reason to change our policy: only experienced senior neonatal nurses, nurse practitioners and neonatologists/fellows are allowed to perform procedures on extremely preterm infants in the first postnatal period. We also set up an intravenous access team, started a training program and introduced new support devices to identify reliable venous and arterial access.
Pain and distress management has become a key issue for the daily care in our NICU. In future we should focus on individualized pain management and collect data on pharmacokinetics and pharmacodynamics of analgesics in (extremely) preterm neonates.

References

1. Simons, SH, et al., Do we still hurt newborn babies? A prospective study of procedural pain and analgesia in neonates. Arch Pediatr Adolesc Med 2003;157:1058-1064.
2. Carbajal, R, et al., Epidemiology and treatment of painful procedures in neonates in intensive care units. JAMA 2008;300:60-70.
3. Johnston, C, et al., Pain in Canadian NICUs: have we improved over the past 12 years? Clin J Pain 2011;27:225-232.
4. Bellu, R, et al., Opioids for neonates receiving mechanical ventilation. Cochrane Database Syst Rev 2008:CD004212.
5. Anand, KJS and PR Hickey, Pain and its effects in the human neonate and fetus. N Engl J Med 1987; 317:1321-1329.
6. Weisman, SJ, et al., Consequences of inadequate analgesia during painful procedures in children. Arch Ped Adol Med 1998;152:147-149.
7. Beggs, S and M Fitzgerald, Development of peripheral and spinal nociceptive systems., in Pain in neonates and infants (third edition), K.J.S. Anand, et al., Editors. 2007, Elsevier: Amsterdam. p. 11-24.
8. Grunau, R, Early pain in preterm infants. A model of long-term effects. Clin Perinatol 2002;29: 373-394.
9. Duhrsen, L, et al., Effects of Repetitive Exposure to Pain and Morphine Treatment on the Neonatal Rat Brain. Neonatology 2012;103:35-43.
10. de Graaf, J, et al., Long-term effects of routine morphine infusion in mechanically ventilated neonates on children's functioning: Five-year follow-up of a randomized controlled trial. Pain 2011.
11. Anand, KJ, Consensus statement for the prevention and management of pain in the newborn. Arch Pediatr Adolesc Med 2001;155:173-180.
12. Holsti, L, et al., Prior pain induces heightened motor responses during clustered care in preterm infants in the NICU. Early Hum Dev 2005;81:293-302
13. Holsti, L and RE Grunau, Considerations for using sucrose to reduce procedural pain in preterm infants. Pediatrics 2010;125:1042-1047.
14. Stevens, B, et al., Sucrose for analgesia in newborn infants undergoing painful procedures. Cochrane Database Syst Rev 2010:CD001069.
15. Taddio, A, et al., Variability in clinical practice guidelines for sweetening agents in newborn infants undergoing painful procedures. Clin J Pain 2009;25:153-155.
16. Stevens, B, et al., Sucrose for analgesia in newborn infants undergoing painful procedures. Cochrane Database Syst Rev 2013;1:CD001069.
17. The International Neonatal Network, The CRIB (clinical risk index for babies) score: a tool for assessing initial neonatal risk and comparing performance of neonatal intensive care units. Lancet 1993;342:193-198.
18. van Dijk, M, et al., The COMFORT$_{neo}$ for daily pain assessment on the Neonatal Intensive Care Unit. Pain Research & Management Journal 2006;11:85B.
19. van Dijk, M, et al., Taking up the challenge of measuring prolonged pain in (premature) neonates: the COMFORTneo scale seems promising. Clin J Pain 2009;25:607-616.
20. Cignacco, E, et al., Neonatal procedural pain exposure and pain management in ventilated preterm infants during the first 14 days of life. Swiss Med Wkly 2009;139:226-232.
21. Lago, P, et al., Guidelines for procedural pain in the newborn. Acta Paediatr 2009;98:932-939.

22. Anand, KJ, et al., Effects of morphine analgesia in ventilated preterm neonates: primary outcomes from the NEOPAIN randomised trial. Lancet 2004;363:1673-1682.
23. Bellu, R, et al., Opioids for neonates receiving mechanical ventilation. Cochrane Database Syst Rev 2005:CD004212.
24. Simons, SH, et al., Routine morphine infusion in preterm newborns who received ventilatory support: a randomized controlled trial. JAMA 2003;290:2419-2427.
25. Brummelte, S, et al., Procedural pain and brain development in premature newborns. Ann Neurol 2012;71:385-396.

Please tick the appropriate response

COMFORTneo scale

Date :

Time :

Observer :

patient sticker

alertness

1 ☐ quiet sleep (eyes closed, no facial movement)
2 ☐ active sleep (eyes closed, facial movement)
3 ☐ quietly awake (eyes open, no facial movement)
4 ☐ actively awake (eyes open, facial movement)
5 ☐ awake and hyperalert

calmness / agitation

1 ☐ calm (appears lucid and serene)
2 ☐ slightly anxious (shows slight anxiety)
3 ☐ anxious (appears agitated but remains in control)
4 ☐ very anxious (appears very agitated, just able to control)
5 ☐ panicky (severe distress with loss of control)

respiratory response (only in mechanically ventilated children)

1 ☐ no spontaneous respiration
2 ☐ spontaneous respiration on ventilator
3 ☐ unrest or resistance to ventilator
4 ☐ actively breathes against ventilator or coughs regularly
5 ☐ fights ventilator

crying (only in spontaneously breathing children)

1 ☐ no crying
2 ☐ faint crying
3 ☐ soft crying or moaning
4 ☐ hard crying
5 ☐ intense crying or screaming

body movement

1 ☐ no or minimal movement
2 ☐ up to three slight arm and / or leg movements
3 ☐ more than three slight arm and / or leg movements
4 ☐ up to three vigorous arm and / or leg movements
5 ☐ more than three vigorous arm and / or leg movements, or whole body

facial tension

1 ☐ facial muscles fully relaxed, relaxed open mouth
2 ☐ normal facial tension
3 ☐ intermittent eye squeeze and brow furrow
4 ☐ continuous eye squeeze and brow furrow
5 ☐ facial muscles contorted and grimacing (eye squeeze, brow furrow, open mouth, nasal-labial lines)

(body) muscle tone (observation only)

1 ☐ muscles fully relaxed (open hands, dribbling, open mouth)
2 ☐ reduced muscle tone; less resistance than normal
3 ☐ normal muscle tone
4 ☐ increased muscle tone (clenched hands and/or clenched, bent toes)
5 ☐ extreme muscle tone (rigidity and flexion of fingers and/or toes)

total score ☐

Details medication/treatment .....

.....

Details child's condition .....

.....

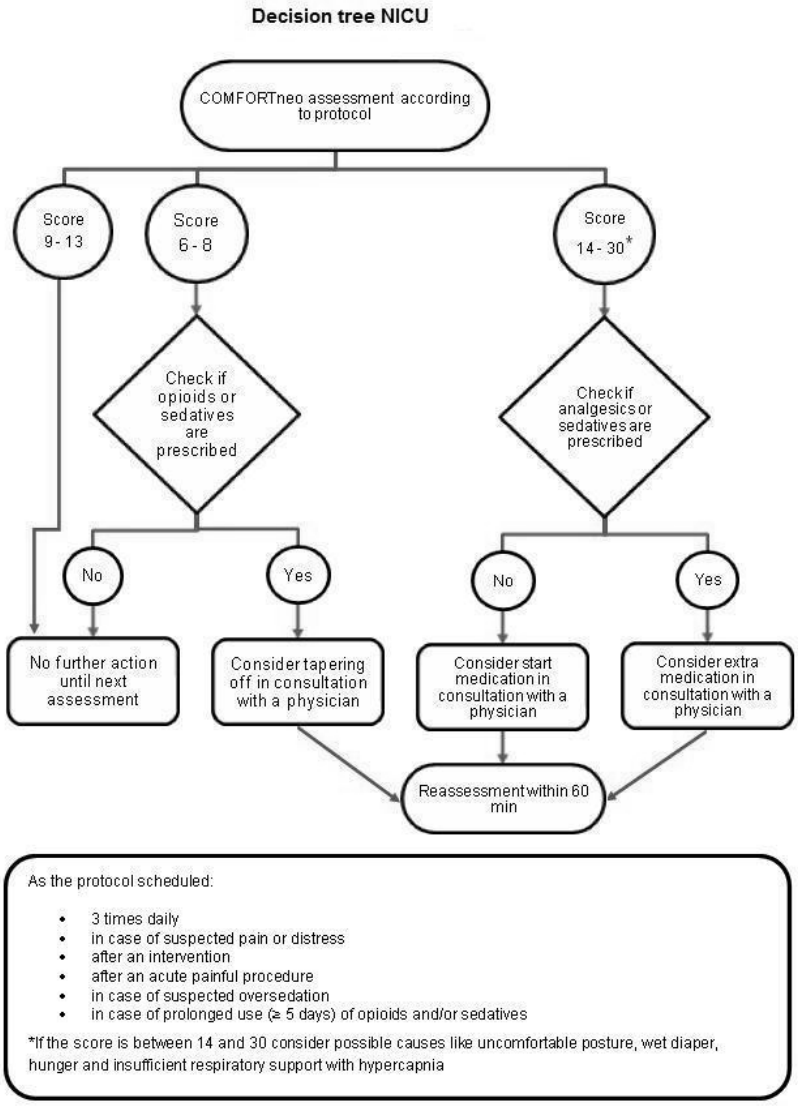
Type of assessment .....

Estimate of pain (0=no pain to 10= worst possible pain) ☐

Estimate of distress (0=no distress tot 10=worst possible distress) ☐

COMFORTneo Scale version 4, April 2005

Appendix 1. COMFORT neo Scale



Appendix 2. Decision tree

Chapter 5

Metabolism of intravenous paracetamol in very preterm infants: first analyses of plasma levels of paracetamol and its metabolites.

Introduction

Preterm infants are at high risk for repetitive and prolonged pain due to indispensable intensive care treatment [28]. Yet, the use of analgesics to alleviate their pain and to prevent negative short and long term consequences of pain is still very limited [13]. Opioids and NSAIDs have been studied in newborns, and were found associated with harmful side effects. Furthermore, in animal models of pain the use of opioids has been linked with an increase in neuroapoptosis [8, 9, 14, 20].

Paracetamol (N-acetyl-p-amino-phenol; Acetaminophen; APAP) is a well-known drug given to children and adults worldwide to relieve fever and pain. So far it has been little used in very preterm infants because rectal and oral administration often was not feasible due to the small body size and frequent occurrence of feeding intolerance. Moreover, the absorption of paracetamol is unpredictable under these conditions. With the introduction of paracetamol for intravenous use it has now become available for infants of all gestational ages.

Consequently, little data are available on dosing, efficacy, safety, and the ontogeny of paracetamol metabolism in very preterm neonates (gestational age < 32 weeks), especially in extremely preterm infants (gestational age < 28 weeks) [1, 7, 10]. A number of studies documented the metabolism of paracetamol administered by oral or rectal route in preterm infants with a gestational age > 28 weeks to generate age-appropriate dosing recommendations [18, 23, 30]. There is still no literature on the optimal loading dose of paracetamol in extremely preterm infants with a gestational age ≤28 weeks.

In neonates paracetamol is primarily metabolized into APAP-Sulphate (APAP-Sulph) and to a lesser extent into APAP-Glucuronide (APAP-Gluc) due to an immature glucuronide conjugation system [22, 24, 30]. An even smaller part of APAP is oxidized mainly by cytochrome P450 iso-enzyme CYP2E1 into the toxic N-acetyl-p-benzoquinone imine (NAPQI). NAPQIs play a role in the paracetamol induced hepatotoxicity and are directly transformed into the metabolites APAP-Glutathione (APAP-Glut), APAP-Cysteine (APAP-Cys) and APAP-N-acetyl-Cysteine (APAP-NAC) (See Figure 1).

Paracetamol dosages that result in increased, potentially toxic, NAPQI levels have never been reported in neonates, and the activity of CYP2E1 is still not quantified. NAPQIs are formed after oxidation of paracetamol and are directly converted by glutathione S transferase into APAP-glutathione; direct measurements of NAPQI levels are difficult, therefore. Whether polymorphisms of different CYP-iso-enzymes play a role in APAP-induced hepatotoxicity is not known for any population.

The primary objective of this study was to describe paracetamol metabolism and to detect dose and age dependent changes in paracetamol metabolism after intravenous administration in very preterm infants with a gestational age <32 weeks.

The secondary objective of this study was to determine the relation between CYP2E1 polymorphisms and the formation of metabolites formed after conversion of NAPQI, as an indirect measurement for NAPQI formation.

Methods

Patients

From October 2010 until October 2013 this randomized, two-center trial was performed at the level 3 Neonatal Intensive Care Units (NICUs) of the Erasmus MC-Sophia Children's Hospital in Rotterdam and Isala Clinics in Zwolle, both in the Netherlands. The study was conducted according to European Good Clinical Practice regulations.
Approval of the Ethics Review Committees of both hospitals and written informed consent from parents/legal guardians were obtained prior to study initiation (MEC-2009-250, National Trial Register 2290).
Eligible for inclusion were all preterm neonates with a gestational age ≤ 32 weeks with an indwelling arterial catheter already in place for clinical purposes, and undergoing central venous catheter (CVC) placement in the first 7 days of life. Exclusion criteria were: major congenital anomalies, intraventricular haemorrhage ≥ grade 3, use of neuromuscular blockers, and maintenance dose of analgesics or more than one loading dose of morphine or midazolam any time prior to this study.
Patients were randomly allocated to one of three different single doses of paracetamol (Perfalgan©; Bristol-Meyers Squibb): 10, 15 and 20 mg/kg bodyweight, respectively. Paracetamol was administered before peripheral CVC placement, which procedure served as the standardized painful event.

Interventions

Subjects received a single dose (10, 15, or 20 mg/kg) of paracetamol via a 15-minute infusion through a peripheral venous cannula. At scheduled times (see below) five blood samples (maximum of 200 µl/sample) were taken for pharmacokinetic analyses from an indwelling arterial line after the infusion of paracetamol was fully completed and the venous line was flushed with a 2 cc saline solution (0.9%). One additional blood sample (250 microliters EDTA) was taken for DNA analyses. Sparse blood sampling was necessary because of the low circulating blood volume. We therefore used two different sample schedules: samples were taken either on T = 0 (before start of paracetamol administration), 20, 60, 240 and 540 minutes schedule 1) or on T= 15 (after the paracetamol infusion was completed), 30, 120, 360 and 720 minutes (schedule 2).

Outcome measures

The primary outcome of this study included plasma concentrations of paracetamol and its metabolites (APAP, APAP-Sulphate, APAP-Glucuronide, APAP-Glutathione, APAP-Cysteine and APAP-N-acetyl–Cysteine).

The secondary outcome of this study is the occurrence of CYP2E1 1* polymorphisms.

Randomization

Stratified randomization was performed for two gestational age groups (24-28 weeks and $28^{1/7}$-32 weeks) by using sequentially numbered, sealed, opaque envelopes, containing a note with the prescribed dose of paracetamol and the sampling schedule. The envelopes for the two different age groups were kept apart (marked A and B, respectively and both numbered from 1-30). For each included patient a nurse or nurse practitioner not involved in the care of the included patient opened the lowest numbered envelope for the age group in question, and prepared the indicated paracetamol dose. Patients could be included only once.

Blinding

The researchers as well as all nursing and medical staff taking care of included patients were blinded for the administered dose of paracetamol. The medication was prepared by a nurse or nurse practitioner from another NICU-ward. The note stating the paracetamol dose was then put back in the envelope, which was sealed again and locked away, inaccessible for the researchers. Unblinding of the study medication took place after all patients were included.

Analysis of APAP and Metabolites in Human Plasma

Blood samples were centrifuged directly after collection and plasma was isolated and stored at –80 degrees Celsius until further analyses. Paracetamol and metabolites analyses were performed in the laboratory of the Department of Pharmacology & Toxicology, University of Utah, Salt Lake City, USA.

Reference standards, chemicals, and reagents

The following reference standards and deuterated internal standards were obtained from Toronto Research Chemicals Inc. (Toronto, ON, Canada). Calibration standards and quality control (QC) samples were prepared concurrently with study samples by fortification of 10 µL of analyte-free, heparinized human plasma with reference standard working solutions. The calibration curve ranged from 0.05 to 50 µg/mL for paracetamol (APAP), and its metabolites: APAP-Gluc, APAP-Sulph and 0.01 to 5 µg/mL for APAP-glut, APAP-Cys and APAP-NAC (See figure 1); QC samples within the quantitative ranges were

included for assessment of accuracy. If necessary, patient samples were diluted for quantification within the curve range.

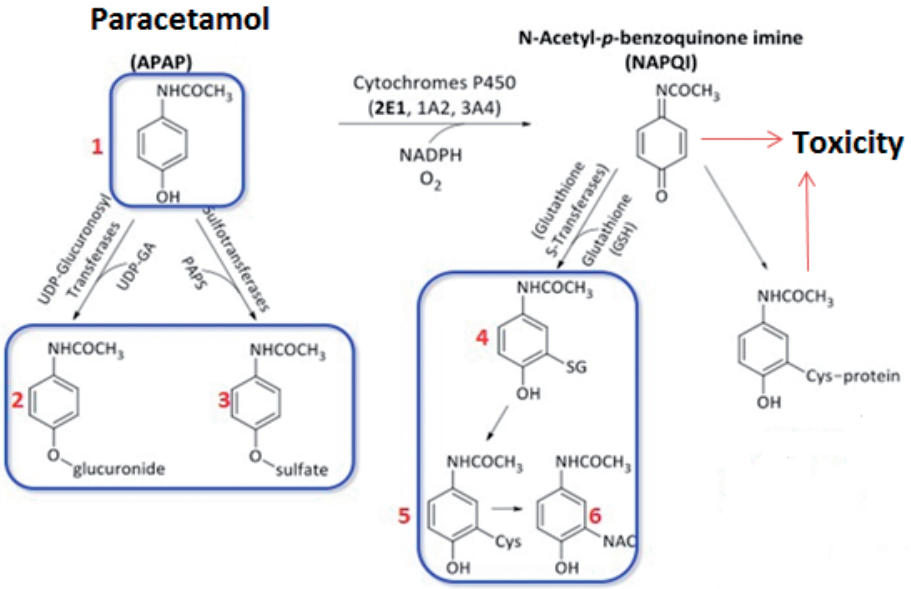


Figure 1. Metabolic pathway of Paracetamol.
Metabolites: 1: APAP; 2: APAP-Sulphate; 3: APAP-Glucuronide; 4: APAP-Glutathione; 5: APAP-Cysteine; 6: APAP-N-Acetyl-Cysteine

Sample preparation

To maintain equivalence in preparation, all study samples (10-µL aliquots) and fortified calibration and QC samples were brought to a total volume of 110 µL with methanol-water (1:1, v/v). Next, they were fortified with 10 µL of internal standard working solution containing 0.2 and 25 µg/mL of APAP-d4 and APAP-d3-sulf in water, respectively. Samples were analyzed on an Agilent 1200 Infinity Series HPLC system equipped with an Agilent Poroshell 120 EC-C18 column (2.1 x 100 mm ID, 2.7 µm particle size), which was interfaced with an Agilent 6460 triple quadrupole mass spectrometer (Agilent Technologies, Santa Clara, California, USA. Less than lower limit of quantitation (LLOQ) was defined as less than 10 (for APAP-Cys and APAP-NAC), 25 (for APAP-Glut), or 50 (for APAP, APAP-Sulph and APAP-Gluc) ng/mL.

Genotyping method

DNA was isolated from 250 µl EDTA blood samples according to the '*MagNA Pure Compact Nucleic Acid Isolation Kit I* (Roche®)'on the MagNA Pure Compact System (Roche®).

The *CYP2E1**1A, *1C an d*1D repeats were analyzed with the PCR method, with the use of forward primer(F)5'–TGGTACATTGTGAGACAGTC–'3 and reverse primer(R)5'-ATACGGGAACACCTCGTTTG – '3. The PCR program started with an initial denaturation at 94°C during 7 minutes, followed by denaturation at the same temperature during 1 minute. After denaturation primers were annealed at 55°C during 1 minute followed by elongation at 72°C during 1 minute. These steps were repeated 34 times, ending with an elongation at 72°C during 7 minutes. The alleles consisted respectively of 563 base pairs (bp), 611 bp and 707 bp products visualized with the Gel Doc™ XR+ System (Biorad®) [19].
CYP2E1-wild type was defined as CYP2E1*1C/*1C and CYP2E1 mutation as CYP2E1*1D/*1D (homozygote) or heterozygotes CYP2E1*1C/*1D (heterozygote).

Statistical analysis
Patient characteristics are presented as median (IQR: interquartile range) in case of non-normally distributed variables and as mean (standard deviation) in case of normally distributed variables. Non-normally distributed continuous variables were compared between the treatment groups using the Kruskal-Wallis tests. Areas under the plasma concentration-time curves from time zero to time t (AUC0-t) were calculated for paracetamol, APAP-Sulph, APAP-Gluc, APAP-Cyst and APAP-NAC. Plasma concentrations of patients in sampling schedule B at T=360 and T=720 were averaged. In this way the AUCs were calculated for t0-t540 and t0-t550 for patients in schedule A and B, respectively, and judged to be comparable.
Linear regression analyses with AUCs of APAP and its metabolites as outcome variables were applied using 2 dummy variables for the 3 treatment conditions) and gestational age group (with 0=24-28 weeks and 1=$28^{1/7}$ weeks to 32 weeks) and CYP2E1 polymorphism (0= wildtype and 1= homozygote or heterozygote) as predictor variables. Statistical significance was set at $p < 0.05$ (two-sided). Statistical data analysis was performed with SPSS software, version 21.0 (SPSS Inc., Chicago, USA). Data were analysed using the intention-to-treat-principle.

Sample size calculation
Based on the pharmacodynamics outcome, including 20 infants per treatment group would result in a power of 83%. This number of 20 patients per treatment group (10 patients with the same dose in each age category) was also deemed sufficient for the planned pharmacokinetics. Thus, the total sample size was 60.

Results

Sixty very preterm infants (N=20 per dose group) were included with an overall median gestational age of 27.9 weeks (IQR 3.4 weeks) and a median birth weight of 953 grams (IQR 398 grams). Central venous catheter (CVC) placement took place on a median post menstrual age of 28.6 weeks (IQR 3.2 weeks). Background characteristics for the different treatment groups are listed in Table 1.
Paracetamol is metabolized into APAP-Sulphate and APAP-Glucuronide and the APAP metabolites are formed over time. Figure 2A gives the plasma concentrations of APAP over time; figures 2B and 2C the plasma concentrations of APAP-Sulph and APAP-Gluc.

Table 1. Background characteristics

	10 mg/kg APAP N = 20	15 mg/kg APAP N = 20	20mg/kg APAP N = 20
Gestational age (weeks)			
Median	27.8	27.6	27.8
IQR	3.9	3.2	2.9
Birth weight (grams)			
Median	970	988	885
IQR	349	430	360
SGA, n (%)	4 (20)	5 (25)	7 (35)
Sex, n (%)			
Boy	10 (50)	10 (50)	8 (40)
Girl	10 (50)	10 (50)	12 (60)
Antenatal steroids, n (%)	19 (95)	18 (90)	18 (90)
PIH, n (%)	4 (20)	7 (35)	3 (15)
PPROM, n (%)	3 (15)	3 (15)	2 (10)
Apgar 1' median	6	6	6
IQR	3.5	3.0	3.0
Apgar 5'median	7.5	8	8
IQR	2.7	2.0	2.5
PNA(days) line placement			
median (IQR)	4.5 (4)	6 (1)	6 (5.7)

Abbreviations: APAP: paracetamol; SGA: Small for gestational age;
PIH: pregnancy induced hypertension; PPROM: prolonged premature rupture of membranes

AUCs of APAP and its metabolites in relation to the intravenous paracetamol dose and gestational age are shown in Tables 2 and 3. All AUCs of APAP and its metabolites increased statistically significantly with increasing paracetamol doses (p-values ranged from $p<0.001$ for AUCs of APAP and APAP-Sulph to $p=0.04$ for AUCs of APAP-NAC

(Table 2). The AUC of APAP-Gluc for the older gestational age group was statistically significantly larger than that for the younger age group (Table 3). The AUC of APAP-Glut could not be calculated in 27 patients because APAP-Gluthation plasma levels were not detectable. In another 5 patients all APAP-Glut levels were measured but were below the threshold value of 0.00001 mg/l. In 28 patients 1 to 4 samples were obtained with a plasma concentration between 0.0005-0.007 mg/l.

Table 2. AUCs of APAP and APAP metabolites

Paracetamol dose AUC (mg/l*min) median (IQR)	10mg/kg	15 mg/kg	20 mg/kg	*p-value*[1]
APAP	5614 (1151)	8574 (3688)	10811 (3007)	*< 0.001*
APAP-Sulph	9933 (5511)	15480 (4564)	21330 (8569)	*<0.001*
APAP-Gluc	409 (327)	767 (896)	865 (1372)	*0.001*
APAP-Cys	1021 (821)	1549 (869)	1659 (1372)	*0.02*
APAP-NAC	338 (245)	500 (339)	483 (505)	*0.04*

APAP: paracetamol; APAP-Sulph: paracetamol-sulphate; APAP-Gluc: paracetamol-glucuronide
APAP-Cys: paracetamol-cysteine; APAP-NAC: paracetamol-N-acetyl-cysteine
[1]Kruskal-Wallis tests were applied as appropriate

Table 3. AUCs of APAP and metabolites in relation with gestational age

Gestational age (weeks) AUC (mg/l*min) median (IQR	24-28 weeks	$28^{1/7}$-32 weeks	*p-value*[1]
APAP	8277 (4789)	8954 (5482)	*0.14*
APAP-Sulph	14783 (8137)	15005 (9197)	*0.78*
APAP-Gluc	586 (386)	950 (1021)	***0.02***
APAP-Cys	1287 (1271)	1504 (11423)	*0.6*
APAP-NAC	446 (402)	370 (349)	*0.2*

APAP: paracetamol; APAP-Sulph: paracetamol-sulphate; APAP-Gluc: paracetamol-glucuronide
APAP-Cys: paracetamol-cysteine; APAP-NAC: paracetamol-N-acetyl-cysteine
[1] Mann Whitney tests were applied appropriate

In regression analysis with gestational age and paracetamol dose as predictor variables the AUCs-APAP were higher for the older gestational age group ($28^{1/7}$-32 weeks) (p=0.001) and for the 15mg/kg and 20mg/kg treatment groups. The AUCs-Sulph were not predicted by gestational age group (p=0.78) but increased with an increasing dose of paracetamol (p<0.001).

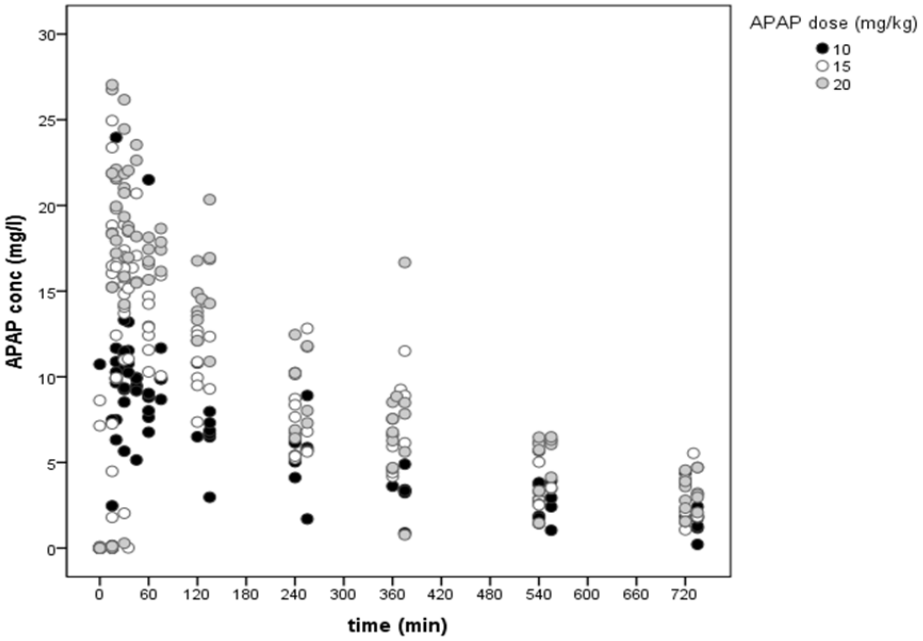
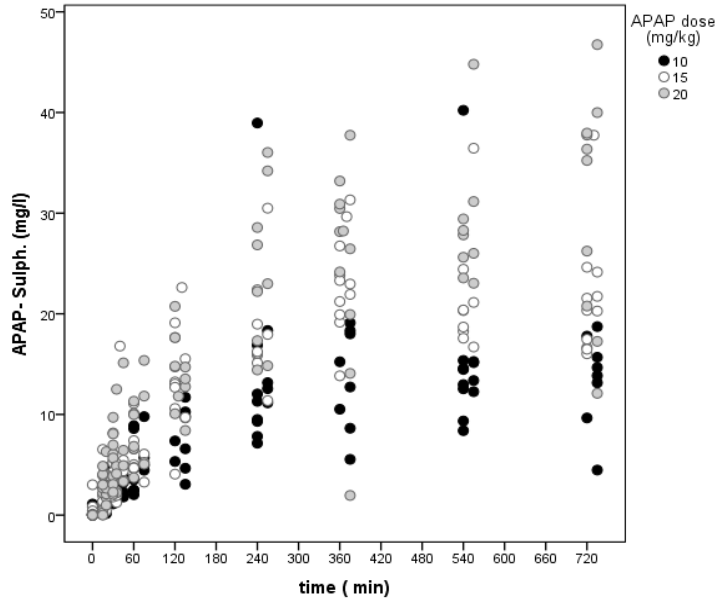


Figure 2. A Paracetamol plasma concentrations for the 3 treatment groups

After a single dose of 10, 15 and 20 mg/kg acetaminophen median plasma peak Concentrations were 10.6 mg/l (IQR 2.1), 16.5 mg/l (IQR 5.0) and 21.3 mg/l (IQR 3.6) ($p < 0.001$, Kruskal-Wallis test).



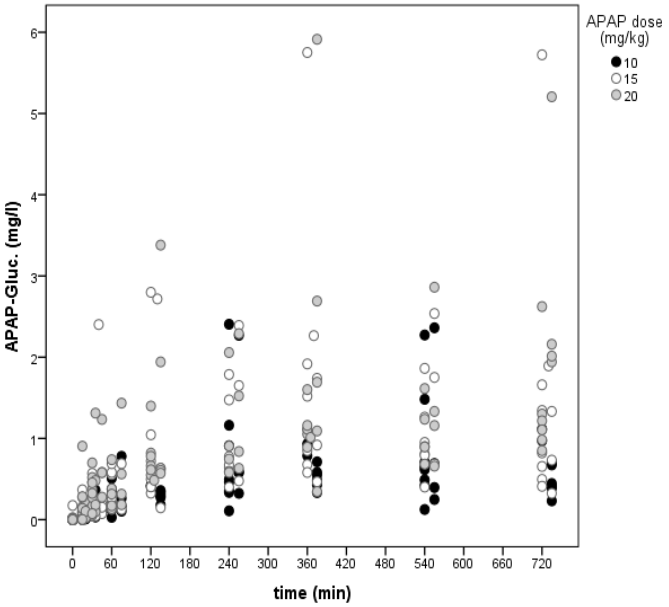


Figure 2. B and 2 C APAP-Sulphate and APAP-Glucuronide plasma concentrations for the 3 treatment groups

The AUCs of APAP-Gluc for the youngest gestational age group were significantly lower than those for the older gestational age group (Figure 3). Furthermore Figure 3 shows two outlying very high AUC of APAP-Gluc in the older gestational age group. The infants in question had not received co-medication capable of inducing or increasing glucuronidation, such as rifampicin, phenytoin or phenobarbital. AUCs of APAP-Gluc for the 10 mg/kg dose group were significantly lower than for the 15- and 20 mg/kg groups (p=0.02). Gestational age did not predict the AUCs of APAP-Cys and APAP-NAC, whereas the 10 mg/kg paracetamol dose led to lower AUCs of APAP-Cys (p=0.01) and of APAP-NAC (p=0.04) than those for the other two doses in regression analyses.

NAPQIs are considered to be related to hepatotoxicity. CYP2E1 genotyping was performed in samples of 58 of the 60 included patients (n=1 sample was lost and n=1 no specific informed consent was given for genotyping). CYP2E1-wild type (CYP2E1*1C/*1C) was found in 45 patients (77.6%) and CYP2E1 mutation in 13 patients (22.4%); two homozygotes (CYP2E1*1D/*1D) and 11 heterozygotes (CYP2E1*1C/*1D). As shown in Figure 4 the patients with relatively high APAP-Cys and APAP-NAC AUCs all had CYP2E1 wild type (CYP2E1*1C/*1C) genotypes. In a multiple regression analysis with CYP2E1 polymorphism as a predictor and controlling for gestational age and paracetamol dose, there was a trend towards lower AUCs of APAP-Cys for patients with the CYP2E1 polymorphisms (p=0.07). This trend was not found for APAP-NAC (p=0.15).

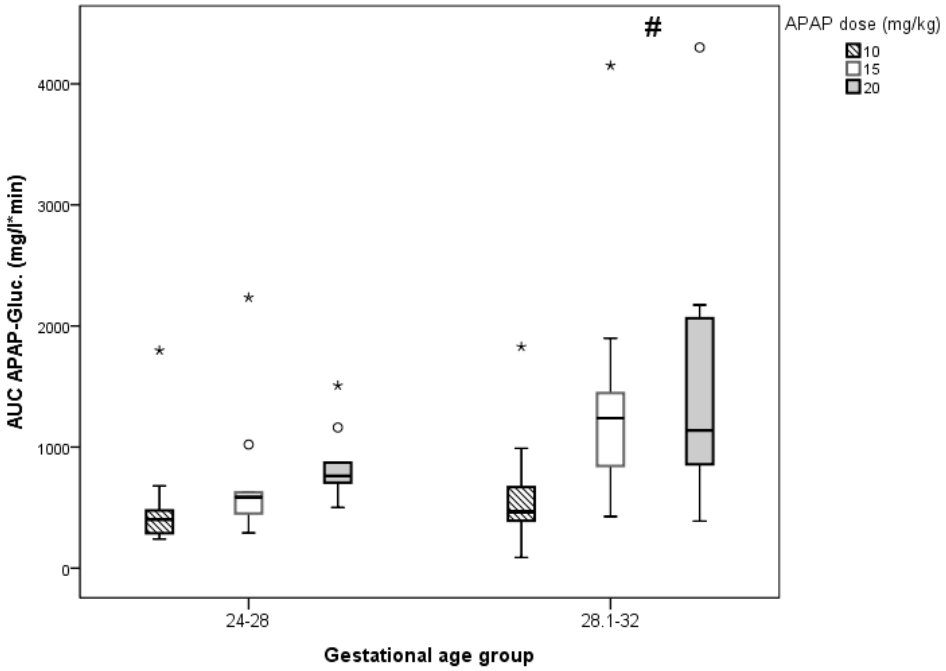


Figure 3. Boxplots for AUC-APAP-Glucuronide for infants of 24-28 weeks gestational age versus 28.1-32 gestational age

[#] The two patients with high AUCs of APAP-Glucuronide in the $28^{1/7}$-32 weeks gestation group did not receive co-medication e.g. phenobarbital that could explain increased glucuronidation.

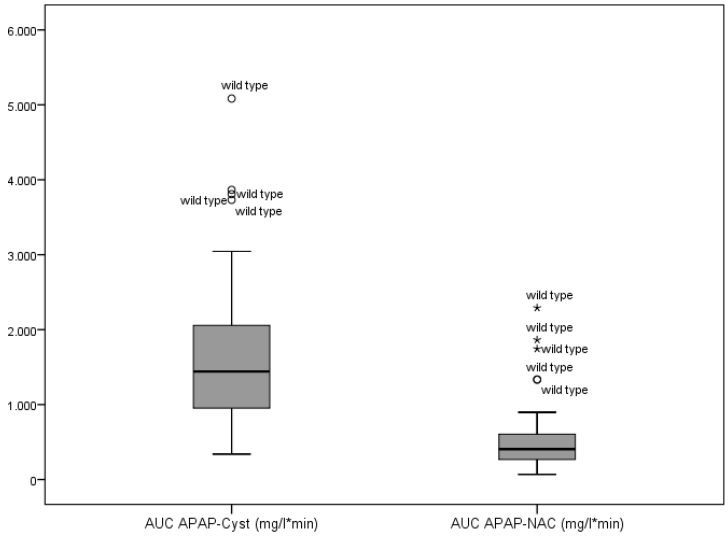


Figure 4. AUCs for APAP-Cysteine and APAP-NAC in all patients. Outliers are marked for their CYP2E1 genotype.

Discussion

In this study we affirmed that very premature born neonates are able to metabolize paracetamol by different pathways of sulphation, glucuronidation and oxidation. Furthermore we showed that with the three used dosages these dosages the plasma levels of the paracetamol metabolites derived from the toxic metabolite NAPQI are rather low. This was as expected, as only up to 10% of paracetamol is known to be metabolized by oxidation.

More than 10 years ago, when intravenous paracetamol was not even on the market in our country, Allegaert et al. already studied the pharmacokinetics of single dose of intravenous propacetamol (a pro-drug of paracetamol that equals half of amount of paracetamol: 2 mg propacetamol = 1 mg paracetamol) [7]. They showed comparable volumes of distribution in preterm neonates (mean gestational age 31.4 weeks) and term born neonates (mean gestational age 38.5 weeks), i.e. 0.61 l/kg and 0.64l/kg, respectively, and with peak plasma levels above 6 mg/l and 10 mg/l for the 10 mg/kg and 20 mg/kg paracetamol (= 20 and 40 mg/kg propacetamol), respectively. When corrected for paracetamol, those peak plasma levels correspond with the peak levels of intravenous paracetamol in our study, suggesting that the volume of distribution of paracetamol in the very preterm and the term infants is also comparable. In our study, peak plasma levels in all patients, except 3 newborns in the 10 mg/kg group, were above the commonly accepted target analgesic threshold level of 9 mg/l. Recently Calvier et al (unpublished data) compared dosing recommendations for children weighing less than 10 kg provided in the Dutch Kinderformularium with recommendations published by Palmer [25]. The latter led to paracetamol concentrations between 10 and 25mg/l. Thereupon Calvier et al developed new dosing recommendations leading to the concentration of 9 mg/l (+/-1) for infants weighing less than 1500 grams. The loading dose of intravenous paracetamol was predicted to be 12.5 mg/kg and 13mg/kg for infants weighing between 500-750 and 750-1500 grams, respectively. These new recommendations for intravenous paracetamol might explain the plasma concentrations < 9 mg/l we found in three patients in the 10mg/kg-dosing group in our study and confirm that higher doses than 10 mg/kg paracetamol are needed to reach the target concentration of > 9 mg/l in very preterm infants. Prospective PK/PD studies are needed to evaluate the population PK model based on these new dosing recommendations of intravenous paracetamol for infants weighing less than 1500 grams.

Allegaert et al also found much increased paracetamol half-lives as well as lower clearance in preterm born neonates (mean gestational age 31.2 weeks) than in term born neonates (mean gestational age 38.5 weeks) [7]. This suggests that preterm born neo-

nates would need lower paracetamol maintenance doses. Using a population PK model the same research group indeed showed that maintenance dosages of 20 up to 30 mg/kg every 6 hours were needed from 28 to 36 weeks post menstrual ages, respectively [2]. The population PK model for intravenous paracetamol has been further expanded towards models that encompasses the whole human life span [5, 33]. These models showed that body weight is the major covariate of intravenous clearance variance in neonates and older patients.

Only few data about paracetamol metabolism and metabolite levels in preterm neonates are available in the literature. Van Lingen et al reported urinary glucuronide/sulphate ratios of 0.12 and 0.28 in 28-32 weeks and 32-36 weeks old preterm neonates, respectively, after rectal paracetamol [31]. Allegaert et al showed increasing paracetamol glucuronidation with increasing postconceptional and postnatal age [3]; this is in accordance with the results of our study as we also measured higher AUCs of paracetamol-Glucuronide for infants born between $28^{1/7}$-32 weeks' gestation compared with younger infants. The AUCs of APAP-Sulphate, APAP-Cysteine and APAP-NAC for the youngest infants were not higher than the corresponding AUCS for the older neonates, suggesting that lower paracetamol glucuronidation did not stimulate sulphation or oxidation with the formation of higher levels of metabolites derived from NAPQIs. Possibly the sulphation and glucuronidation pathways of paracetamol become saturated, on account of which a larger amount of APAP is excreted unchanged in the urine [27].
Based on recovered metabolite ratios in the urine, it is thought that glucuronidation may be upregulated upon multiple dosing [4]. Krekels et al. showed that increased glucuronidation in neonates and infants is more determined by developmental changes rather than by repeated administration of paracetamol. In our study we showed that next to paracetamol plasma concentrations, paracetamol-sulphate and glucuronide plasma levels were also higher after an increase of a single paracetamol dose, indicating that with the currently used single paracetamol dosages no saturation of these metabolic pathways occurs. As we did not study the metabolism of multiple paracetamol doses in the current study, further research is needed to evaluate the effect of multiple paracetamol dosing regimen on the different metabolic pathways.

To the best of our knowledge, this is the first study that measured the metabolites of paracetamol derived from the toxic metabolite NAPQI in plasma of very preterm infants. Data on these metabolites in adults are predominantly derived from urine samples after administration of relatively high doses of paracetamol [16]. Zuppa et al published a population based PK study in which neonates, infants, children and adolescents were included receiving either 12.5 mg/kg/4 hours intravenous paracetamol (neonates as an exception 12.5 mg/kg/6hours) or 15 mg/kg/6 hours (neonates 15 mg/kg/8hours). Both

regimens were well tolerated and achieved adequate paracetamol plasma concentrations[34].

Although paracetamol is considered to be safe and efficacious in adults, children and preterm infants > 32 weeks of gestation, accidental [12, 15, 29] or deliberate overdose can result in acute liver failure in adults as well as in infants and neonates. The maximum therapeutic dosages of paracetamol are 4 grams daily for adults and 50-75 mg/kg for children. A single acute ingestion exceeding 7.5 gram in adults or 150 mg/kg in children has been considered toxic [17]. Liver damage has been reported even after approved doses, and this was found associated with both genetic and epigenetic factors [11, 21]. Paracetamol-induced hepatotoxicity is dependent on the balance of the formation rate of NAPQI by CYP2E1, the elimination rate of unmetabolized paracetamol, sulphation and glucuronidation conjugation pathways, and the glutathione stores in the liver[6]. CYP2E1 levels are low at neonatal age and increase gradually during the first year of life [32]. It appears that young children are more resistant than are adults to paracetamol-induced hepatotoxicity due to reduced rates of oxidation by CYP2E1 and the ability to replete glutathione. In our study we also found very low plasma and peak concentrations of metabolites of paracetamol formed by oxidation [26]. APAP protein metabolites have a long elimination half-life that markedly exceeds that of the parent compound APAP, permitting detection of APAP toxicity long after the parent compound has been cleared from the blood. In adults the mean elimination half-life of APAP-metabolites with APAP-induced acute liver failure was 41.3 hours compared to 5.4 to 18.4 hours for APAP (depending on severity of acute liver failure en presence of encephalopathy) [20]. However, in neonates, metabolic clearance of paracetamol through sulphation matures more rapidly than glucuronidation, which at neonatal age is still poor. This results in lower clearance and slower decrease after the peak paracetamol concentration has been reached. Because of this lower clearance, accumulation is more likely [6]. This effect needs to be taken into account when prescribing a maintenance dose of intravenous paracetamol to very preterm infants. Moreover Calvier et al (unpublished data) gave dose recommendations for maintenance doses for intravenous paracetamol: 5.5 mg/kg/6hours for preterms with a body weight of 500 to 750 grams and 6 mg/kg/6 hours for preterm infants weighing 750-1500 grams. Given the ongoing debate on the optimal dosing of intravenous paracetamol for closure of a hemodynamic relevant ductus arteriosus (currently set at 15 mg/kg/6hours) prospective PK studies should evaluate the dose regimens used for the treatment of PDA as well as the new dose recommendations of paracetamol as analgesic treatment of Calvier et al.

CYP2E1 is important in the oxidation of paracetamol into NAPQIs, which are responsible for paracetamol-induced hepatotoxicity; these toxic metabolites are converted into the metabolites APAP-Cys and APAP-NAC. In the present study there is a trend of lower AUCs

of APAP-Cys and APAP-NAC in the presence of a polymorphism of the CYP2E1 compared to the wildtype of CYP2E1. These "premature" data might suggest a protective role of the CYP2E1 polymorphism in the formation of reactive metabolites. Our sample size was too small and our study underpowered, however, to draw firm conclusions but it is definitely a field worth exploring in future studies.

In conclusion, we showed that the AUCs of APAP and all its metabolites increased statistically significantly with increasing paracetamol doses in very preterm neonates. Gestational age was found an important determinant for glucuronidation, as the AUCs of APAP-Gluc in the preterm infants younger than 28 weeks were significantly lower than those in the older infants.
The results of this study can validly be included in a population based modelling study to calculate new dose recommendations for intravenous paracetamol use in infants with a gestational age ≥ 24 weeks.

References

1. Allegaert K, Anderson BJ, Naulaers G, de Hoon J, Verbesselt R, Debeer A, Devlieger H, Tibboel D (2004) Intravenous paracetamol (propacetamol) pharmacokinetics in term and preterm neonates. Eur J Clin Pharmacol 60: 191-197
2. Allegaert K, Anderson BJ, Naulaers G, de Hoon J, Verbesselt R, Debeer A, Devlieger H, Tibboel D (2004) Intravenous paracetamol (propacetamol) pharmacokinetics in term and preterm neonates. European Journal of Clinical Pharmacology 60: 191-197
3. Allegaert K, De Hoon J, Verbesselt R, Vanhole C, Devlieger H, Tibboel D (2005) Intra- and inter-individual variability of glucuronidation of paracetamol during repeated administration of propacetamol in neonates. Acta Paediatrica 94: 1273-1279
4. Allegaert K, de Hoon J, Verbesselt R, Vanhole C, Devlieger H, Tibboel D (2005) Intra- and inter-individual variability of glucuronidation of paracetamol during repeated administration of propacetamol in neonates. Acta Paediatr 94: 1273-1279
5. Allegaert K, Palmer GM, Anderson BJ (2011) The pharmacokinetics of intravenous paracetamol in neonates: size matters most. Arch Dis Child 96: 575-580
6. Allegaert K, van de Velde M, van den Anker J (2014) Neonatal clinical pharmacology. Paediatr Anaesth 24: 30-38
7. Allegaert K, Van der Marel CD, Debeer A, Pluim MA, Van Lingen RA, Vanhole C, Tibboel D, Devlieger H (2004) Pharmacokinetics of single dose intravenous propacetamol in neonates: effect of gestational age. Arch Dis Child Fetal Neonatal Ed 89: F25-28
8. Anand KJ, Hall RW, Desai N, Shephard B, Bergqvist LL, Young TE, Boyle EM, Carbajal R, Bhutani VK, Moore MB, Kronsberg SS, Barton BA, Group NTI (2004) Effects of morphine analgesia in ventilated preterm neonates: primary outcomes from the NEOPAIN randomised trial. Lancet 363: 1673-1682
9. Aranda JV, Thomas R (2006) Systematic review: intravenous Ibuprofen in preterm newborns. Semin Perinatol 30: 114-120
10. Autret E, Dutertre JP, Breteau M, Jonville AP, Furet Y, Laugier J (1993) Pharmacokinetics of paracetamol in the neonate and infant after administration of propacetamol chlorhydrate. Dev Pharmacol Ther 20: 129-134
11. Bondoc FY, Bao Z, Hu WY, Gonzalez FJ, Wang Y, Yang CS, Hong JY (1999) Acetone catabolism by cytochrome P450 2E1: studies with CYP2E1-null mice. Biochem Pharmacol 58: 461-463
12. Bucaretchi F, Fernandes CB, Branco MM, De Capitani EM, Hyslop S, Caldas JP, Moreno CA, Porta G (2014) Acute liver failure in a term neonate after repeated paracetamol administration. Rev Paul Pediatr 32: 144-148
13. Carbajal R, Eriksson M, Courtois E, Avila-Alvarez A, Berger A, Lago P, Van Overmeire B, Papadouri T, Ilmoja M, Pölkki T, Schroth M, Sarafidis K, Tameliene R, Attard Montalto S, Simons S, Andersen R, Dobrzanska A, Matos C, Boyle E, Europain E, Lagercrantz H, Bergqvist L, Anand K (2014) O-103 Sedation And Analgesia For Neonates In Nicus Across Europe: The Europain Survey. Archives of Disease in Childhood 99: A64
14. Cook SF, King AD, Chang Y, Murray GJ, Norris HR, Dart RC, Green JL, Curry SC, Rollins DE, Wilkins DG (2015) Quantification of a biomarker of acetaminophen protein adducts in human serum by high-performance liquid chromatography-electrospray ionization-tandem mass spectrometry: Clinical and animal model applications. J Chromatogr B Analyt Technol Biomed Life Sci 985: 131-141
15. Cuzzolin L, Antonucci R, Fanos V (2013) Paracetamol (acetaminophen) efficacy and safety in the newborn. Curr Drug Metab 14: 178-185

16. Fannin RD, Russo M, O'Connell TM, Gerrish K, Winnike JH, Macdonald J, Newton J, Malik S, Sieber SO, Parker J, Shah R, Zhou T, Watkins PB, Paules RS (2010) Acetaminophen dosing of humans results in blood transcriptome and metabolome changes consistent with impaired oxidative phosphorylation. Hepatology 51: 227-236
17. Forrest JA, Clements JA, Prescott LF (1982) Clinical pharmacokinetics of paracetamol. Clin Pharmacokinet 7: 93-107
18. Hansen TG, O'Brien K, Morton NS, Rasmussen SN (1999) Plasma paracetamol concentrations and pharmacokinetics following rectal administration in neonates and young infants. Acta Anaesthesiol Scand 43: 855-859
19. Hu Y, Hakkola J, Oscarson M, Ingelman-Sundberg M (1999) Structural and functional characterization of the 5'-flanking region of the rat and human cytochrome P450 2E1 genes: identification of a polymorphic repeat in the human gene. Biochem Biophys Res Commun 263: 286-293
20. James LP, Chiew A, Abdel-Rahman SM, Letzig L, Graudins A, Day P, Roberts D (2013) Acetaminophen protein adduct formation following low-dose acetaminophen exposure: comparison of immediate-release vs extended-release formulations. Eur J Clin Pharmacol 69: 851-857
21. Lavonas EJ, Reynolds KM, Dart RC (2010) Therapeutic acetaminophen is not associated with liver injury in children: a systematic review. Pediatrics 126: e1430-1444
22. Levy G, Khanna NN, Soda DM, Tsuzuki O, Stern L (1975) Pharmacokinetics of acetaminophen in the human neonate: formation of acetaminophen glucuronide and sulfate in relation to plasma bilirubin concentration and D-glucaric acid excretion. Pediatrics 55: 818-825
23. Lin YC (1997) Plasma concentration after rectal administration of acetaminophen in preterm infants. Paediatric Anaesthesia 7: 457-459
24. Miller RP, Roberts RJ, Fischer LJ (1976) Acetaminophen elimination kinetics in neonates, children, and adults. Clin Pharmacol Ther 19: 284-294
25. Palmer GM, Atkins M, Anderson BJ, Smith KR, Culnane TJ, McNally CM, Perkins EJ, Chalkiadis GA, Hunt RW (2008) I.V. acetaminophen pharmacokinetics in neonates after multiple doses. Br J Anaesth 101: 523-530
26. Pineiro-Carrero VM, Pineiro EO (2004) Liver. Pediatrics 113: 1097-1106
27. Roberts I, Robinson MJ, Mughal MZ, Ratcliffe JG, Prescott LF (1984) Paracetamol metabolites in the neonate following maternal overdose. Br J Clin Pharmacol 18: 201-206
28. Roofthooft DW, Simons SH, Anand KJ, Tibboel D, van Dijk M (2014) Eight years later, are we still hurting newborn infants? Neonatology 105: 218-226
29. Van Eyken P, Nemolato S, Faa G, Ambu R (2012) Hepatic injury to the newborn liver due to drugs. Curr Pharm Des 18: 3050-3060
30. van Lingen RA, Deinum JT, Quak JM, Kuizenga AJ, van Dam JG, Anand KJ, Tibboel D, Okken A (1999) Pharmacokinetics and metabolism of rectally administered paracetamol in preterm neonates. Arch Dis Child Fetal Neonatal Ed 80: F59-63.
31. van Lingen RA, Deinum JT, Quak JM, Kuizenga AJ, van Dam JG, Anand KJS, Tibboel D, Okken A (1999) Pharmacokinetics and metabolism of rectally administered paracetamol in preterm neonates. Arch Dis Child Fetal Neonatal Ed 80: F59-63.
32. Vieira I, Sonnier M, Cresteil T (1996) Developmental expression of CYP2E1 in the human liver. Hypermethylation control of gene expression during the neonatal period. Eur J Biochem 238: 476-483
33. Wang CG, Allegaert K, Tibboel D, Danhof M, van der Marel CD, Mathot RAA, Knibbe CAJ (2014) Population Pharmacokinetics of Paracetamol Across the Human Age-Range From (Pre) term Neonates, Infants, Children to Adults. Journal of Clinical Pharmacology 54: 619-629

34. Zuppa AF, Hammer GB, Barrett JS, Kenney BF, Kassir N, Mouksassi S, Royal MA (2011) Safety and population pharmacokinetic analysis of intravenous acetaminophen in neonates, infants, children, and adolescents with pain or Fever. J Pediatr Pharmacol Ther 16: 246-261

Chapter 6

Intravenous acetaminophen is not effective for pain management during central venous catheter placement in very preterm infants.

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Abstract

Objectives

To determine the analgesic effects and safety aspects of different single doses of intravenous acetaminophen on pain from central venous catheter (CVC) placement in very preterm neonates.

Methods

A blinded randomized controlled trial was conducted at two level III NICU centers in the Netherlands. Neonates with gestational ages from 24.0 to 32.0 weeks were randomly allocated to one of three different doses of intravenous acetaminophen (10, 15, 20 mg/kg) before CVC placement in the first week of life. Age-matched neonates receiving sucrose served as a control group. Pain was assessed with the Premature Infant Pain Profile (PIPP) and the COMFORTneo scale. Peak concentrations of acetaminophen (aimed target concentration > 9mg/l), and kidney and liver function were monitored.

Results

No statistically significant differences in pain scores were found between the acetaminophen treatment groups and sucrose group. The median PIPP score was 7 (IQR 6-9) for the 15 mg/kg acetaminophen group and 8 (IQR 6-10.5) for all other groups, reflecting mild to moderate pain. COMFORTneo scores of 14 or higher, suggesting pain or distress, were assigned to 25% of infants who received sucrose and to up to 50% of infants in the 15 mg/kg and 20 mg/kg acetaminophen groups. All subjects, except for three who received 10 mg/kg acetaminophen, had peak plasma concentrations > 9mg/l. Kidney and liver function was normal in all infants.

Conclusions

We found no analgesic benefit from a single intravenous dose of acetaminophen over sucrose in CVC placement in very preterm neonates.

Introduction

Today, pain assessment is an essential component of the medical care for neonates. Its importance is illustrated by the finding that preterm infants on average undergo many painful procedures, estimated at 6 to 17 procedures per day[1-7]. Analgesia is necessary to protect against the negative short and long term consequences of pain from skin breaking procedures such as changes in brain development[8]. The analgesic properties of sucrose to reduce procedural pain have been questioned although its administration is still standard of care in many NICUs[9-11]. Long-acting opioids, such as morphine, have also been shown not to be effective in reducing pain from heel sticks in preterm infants[12] The reluctance to use strong analgesic drugs in newborns is triggered by studies in rodents showing accelerated neuronal apoptosis and other morphological changes in the developing brain after administration of commonly used opioids, benzodiazepines and general anaesthetics[13-15]. These findings warrant studies on the effectiveness and safety of other analgesics, such as acetaminophen.
Acetaminophen (acetyl-para-aminophenol, APAP) is the most used analgesic drug to relieve mild to moderate pain in children and adults, with proven safety and efficacy. Its safety and efficacy after intravenous administration have not yet been proven, however, in extreme preterm neonates. Besides, there are no dosing guidelines for preterm infants with gestational ages of less than 28 weeks[16,17]. A population pharmacokinetic study of acetaminophen including subjects older than gestational age of 27 weeks found that an acetaminophen concentration of > 9 mg/l should be aimed for[18].
Pain assessment is an indispensable component of pain management; acute pain in preterm infants is often assessed with the Premature Infant Pain Profile (PIPP) basis.

In our quest for 'new' pharmacological agents we performed a study on the effectiveness of acetaminophen administered before central venous catheter (CVC) placement in very preterm infants.
The objective of the study was to determine the analgesic effect of different doses of acetaminophen. A group of age-matched neonates who underwent the same procedure after administration of sucrose only, served as control group.

Material and Methods

Design, Patients and Setting

This multicenter, blinded and randomized controlled trial was performed from October 2010 until October 2013 at the level III NICUs of the Erasmus MC-Sophia Children's Hospital in Rotterdam and the Isala Clinics in Zwolle, the Netherlands. Subjects were

randomly allocated to one of three different single doses of acetaminophen (Perfalgan©; Bristol-Meyers Squibb) (10, 15, 20 mg/kg) given before peripheral CVC placement.
Inclusion criteria were the following: gestational age between 24 and 32 weeks, an indwelling arterial catheter already in place for clinical purposes, and CVC placement in the first 7 days of life. Exclusion criteria were the following: receiving a maintenance dose of analgesics, and/or receiving morphine or midazolam prior to the study intervention. NSAIDs used for patent ductus arteriosus closure were allowed and were accurately documented for each patient. The study was conducted according to European Good Clinical Practice regulations and registered in the Dutch Trial Registry (Trial number: 2290); Ethics Review Board approval from both hospitals and written informed consent from parents or legal guardians was obtained prior to study initiation (MEC-2009-250).
Because this study was a pharmacological dose finding trial, the Ethics Review Board did not allow including patients treated with oral sucrose as control group. Instead, after finishing this trial, and with approval of the Erasmus MC Ethics Review Board (MEC-2014-386), we applied the same pain assessment instruments to a group of age-matched patients who were given oral sucrose before CVC placement.

Pain assessment instruments
Pain during CVC placement was assessed with two assessment tools. First, the Premature Infant Pain Profile (PIPP), which is a multidimensional tool that has been well validated to assess procedural pain in preterm and term neonates[19,20]. The PIPP rates seven indicators (gestational age, behavioural state, heart rate and oxygen saturation changes, occurrence of brow bulge, eye squeeze and nasolabial furrow) on a 4-point scale from 0 to 3 (total score range 0 to 21). A total PIPP score of < 7 is taken to reflect no or minimal pain and a score ≥ 7-12 mild to moderate pain; scores > 12 reflect severe pain[20,19,21]. Because the recently revised PIPP[22] also assigns points to drops in heart rate as an indicator of pain after completion of the study we analysed how inclusion of heart rate change would have affected PIPP assessment.
PIPP scores were assigned by the researchers only, who had been trained to this purpose and all showed sufficient inter-rater reliability (assessed with a linearly weighted Cohen's kappa > 0.65) in 10 bedside assessments.
Second, the COMFORTneo scale, which was found to have good psychometric qualities[6]. It consists of 6 behavioural items: alertness, calmness, respiratory response (used in mechanically ventilated patients) or crying (used in spontaneously breathing patients), physical movements, muscle tone and facial tension (total score range 6-30). The COMFORTneo scale was applied by trained nurses with sufficient inter-rater reliability (a linearly weighted Cohen's kappa >0.65) as determined with 10 bedside assessments. A COMFORTneo score between 14 and 30 indicates 'pain or distress' (sensitivity of 0.81 and specificity of 0.90)[6].

Interventions

CVC placement was selected as intervention in this study because nurses and physicians of the NICUs in the two participating centers rated its painfulness 8 on a 0 to 10 scale,[1].

In the separate sucrose study, sucrose 24% (EPMC Pharma, Belgium) was administered in the buccal cavity together with the use of a pacifier 2 minutes before CVC placement; i.e. 0.5 cc if body weight was lower than1000 grams, and 1.0 cc if body weight was 1000 grams or more.

From subjects who received acetaminophen, five blood samples (maximum of 200 µl/sample) were taken from an indwelling arterial line at set times after infusion of the allocated acetaminophen single dose. Peak plasma levels were determined for the current study.

The attending ICU nurse applied the COMFORTneo scale 15 minutes before CVC placement while the researchers assessed the two PIPP baseline items 'behavioral state' and 'gestational age' simultaneously. Next, acetaminophen was administered for 15 minutes as a single intravenous dose through a peripheral venous cannula that was already in place. The total volume administered was 2 cc (acetaminophen supplemented with NaCl 0.9%) in the 24-28 weeks gestational age group and 4 cc in the group of $28^{1/7}$-32 weeks. While the attending physician or nurse practitioner inserted the CVC, one of the researchers assigned the PIPP score after 30 seconds observation. In addition, the attending ICU nurse assigned the COMFORTneo score during the intervention. Subjects in the control group receiving sucrose were assessed in exactly the same way.

Study Outcomes

PIPP score was the primary outcome; COMFORTneo score was the secondary outcome. Furthermore, changes in heart rate and oxygen saturation during CVC placement were analysed, as well as blood urea nitrogen (BUN), creatinine, ASAT, ALAT and total – and direct bilirubin levels before administration of the loading dose of acetaminophen and 12 – 24 hours after (if blood sampling was required as standard care). The following reference values were considered: BUN < 15 mmol/l, creatinine 27-88 µmol/l, ALAT < 52U/l, ASAT <120 U/l, total bilirubin according to curves[23,24] and conjugated bilirubin < 5% of total bilirubin.

Randomization

Stratified randomization was performed for two gestational age groups (24-28 weeks and $28^{1/7}$-32 weeks) by using sequentially numbered, sealed, opaque envelopes, containing a note with the prescribed dose of acetaminophen. The envelopes for the different age groups were kept apart (marked A and B, respectively and both numbered from 1-30). A nurse or nurse practitioner not involved in the care of the included patient opened the first envelope for the applicable age group, containing the allocated dose of acetaminophen. For each new patient the next subsequently numbered envelope was opened. Patients could be included only once.

Blinding

Both the researchers and all nursing and medical staff taking care of the subjects were blinded for the administered dose of acetaminophen. The medication was prepared by a nurse or nurse practitioner from another NICU-unit. The slip of paper stating the acetaminophen dose was then put back in the envelope, which was sealed again and locked away, inaccessible for the researchers. Unblinding of the study medication took place after all subjects were included.

Statistical analysis

Patient characteristics are presented using descriptive statistics. Categorical data were compared between the treatment groups using Fisher's exact tests.

Non-normally distributed continuous variables were compared between the treatment groups using the Kruskal-Wallis tests. Linear regression analysis with PIPP scores as outcome variable was applied to determine the effects of the treatment (using 3 dummy variables for the 4 treatment conditions) as predictor variable. In addition administration of ibuprofen or indomethacin (as patent ductus arteriosus (PDA) treatment) within 24 hours before the procedure was added as covariate. Statistical significance was set at $p < 0.05$ (two-sided). Data were analysed with SPSS software, version 21.0 (SPSS Inc., Chicago, USA) using the intention-to-treat-principle.

Sample size calculation

With 20 infants per treatment group the power to detect differences in the mean PIPP scores of 1 point equals 83%. This calculation is based on the reported SD of PIPP scores assigned for a skin breaking procedure, i.e. 1.3 points[20]. Thus the total sample size was 80 subjects including 20 in the sucrose control group.

Results

In total we assessed 266 patients for eligibility and included 60 patients in the acetaminophen trial (see Figure 1 flowchart); thirty patients in the gestational age group of 24-28 weeks and thirty patients in the 28[1/7]-32 weeks group. Twenty age-matched patients who received only sucrose as analgesic treatment formed the control group. Background characteristics of all subjects are shown in Table 1. Clinical outcome data are listed in Table 2.

Table 3 shows PIPP scores for the three different acetaminophen dose groups and control group treated with sucrose. Regression analysis showed that PIPP scores were not significantly different between the 4 treatment groups Treatment with indomethacin/ibuprofen added as covariate did not significantly affect the PIPP scores (p=0.60) either.

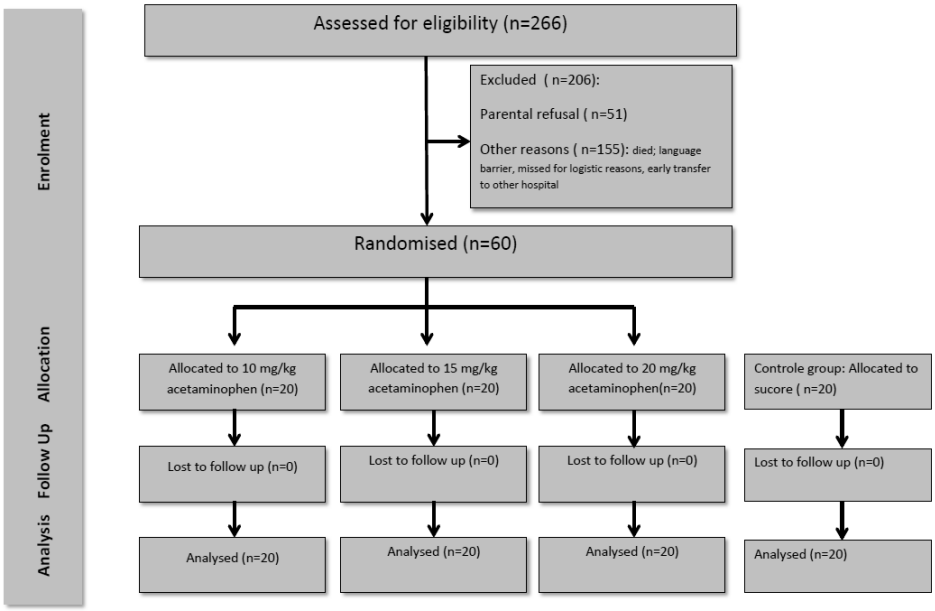


Figure 1. Participant flowchart

Table 1. Background characteristics

Characteristics	10 mg/kg APAP N = 20	15 mg/kg APAP N = 20	20mg/kg APAP N = 20	Sucrose N = 20
Gestational age (weeks)				
Median	27.8	27.9	28.8	27.9
Range	24.0 to 31.1	24.2 to 30.4	24.2 to 30.3	24.1to 31.4
Birth weight (grams)				
Median	970	988	885	898
Range	462 to 1550	475 to 1440	630 to 1380	520 to 1330
SGA, n (%)	4 (20)	5 (25)	7 (35)	8 (40)
Sex, n (%)				
Boy	10 (50)	10 (50)	8 (40)	10 (50)
Girl	10 (50)	10 (50)	12 (60)	10 (50)
Antenatal steroids, n (%)	19 (95)	18 (90)	18 (90)	18 (90)
PIH, n (%)	4 (20)	7 (35)	3 (15)	5 (25)
PPROM, n (%)	3 (15)	3 (15)	2 (10)	1 (5)
Apgar 1' median	6	6	6	7.5
IQR	4.3-7.8	5.0-8.0	5.0-8.0	4.3-9.0
Apgar 5'median	7.5	8	8	9
IQR	6.3-9.0	7.0-9.0	6.5-9.0	8.0-9.0
PNA(days) line placement median (IQR)	4.5(2-6)	6(5-6)	6 (1.3-7)	3 (1-7)

Abbreviations: APAP: acetaminophen; SGA: small for gestational age; PIH: pregnancy induced hypertension; PPROM: prolonged premature rupture of membranes

Table 2. Clinical outcome data

Characteristics	APAP 10mg/kg n = 20	APAP 15 mg/kg n = 20	APAP 20 mg/kg n = 20	Sucrose 24% n = 20
IRDS: n (%)	15 (75)	16 (80)	13 (65)	14 (70)
Mechanical				
Ventilation: n (%)	15 (75)	16 (80)	14 (70)	13 (65)
Surfactant: n (%)	14 (70)	15 (75)	14 (70)	13 (65)
PDA: n (%)	8 (40)	7 (35)	12 (60)	8 (40)
Treatment indomethacin	3 (15)	2 (10)	5 (25)	0
Treatment ibuprofen	2 (10)	3 (15)	4 (20)	8 (40)
Surgery: n (%)	5 (25)	2 (10)	4 (20)	1 (5)
Sepsis: n (%)	9 (45)	9 (45)	10 (50)	12 (60)
NEC: n (%)	1 (5)	1 (5)	2 (10)	1 (5)
FIP: n (%)	0	2 (10)	2 (10)	0
Surgery NEC: n (%)	0	1 (5)	3 (15)	1 (5)
IVH: n (%)	2 (10)	5 (25)	6 (30)	4 (20)
Dead: n (%)	0	4 (20)	2 (10)	2 (10)
Kidney function:				
BUN[1] before APAP (n)	18	17	16	
median (IQR)	8.3 (6.4-12.1)	7.7 (5.7-9.1)	7.4 (5.4-9.4)	
BUN[1] after APAP (n)	17	14	15	
median (IQR)	7.5 (5.9-10.2)	6.8 (5.6-9.2)	6.4 (5.2-8.8)	
Creatinine[2] before APAP (n)	17	18	13	
median (IQR)	63 (59-76)	55 (50-70)	67 (56-77)	
Creatinine[2] after APAP (n)	17	13	12	
median (IQR)	56 (49-68)	47 (40-62)	57 (46-69)	
Liver enzymes &bilirubin				
ASAT[3] before APAP (n)	3	5	5	
Median (IQR)	98 (15-98)	29 (18-38)	19 (14-50)	
ASAT[3] after APAP (n)	4	5	1	
Median (IQR)	46 (10-153)	38 (17-46)	32	
ALAT[3] before APAP (n)	2	4	5	
Median (IQR)	50 (16-50)	5 (5-8)	5 (5)	
ALAT[3] after APAP (n)	3	5	1	
Median (IQR)	36 (5-36)	5 (5-8)	10	
Tot. Bili[4]. before APAP (n)	19	18	18	
Median (IQR)	102 (81-131)	100 (76-128)	95 (79-125)	
Tot. Bili[4]. After APAP (n)	17	18	4	
median (IQR)	82 (72-141)	93 (65-110)	86 (68-104)	
Dir.Bili[4]. before APAP (n)	2	5	5	
median (IQR)	1 (1)	1 (1)	1 (1)	
Dir. Bili[4]. After APAP (n)	2	6	3	
median (IQR)	3 (2-3)	1 (1-15)	1 (1)	

Abbreviations: APAP: acetaminophen; PDA: patent ductus arteriosus; NEC: necrotizing enterocolitis; FIP: focal intestinal perforation; IVH: intraventriculair hemorrhage

[1] BUN levels in mmol/l; [2] creatinine levels in µmol/l; [3] ASAT/ALAT levels in U/l; [4] Total and Direct bilirubin values in µmol/l

Figure 2 shows boxplots of the PIPP scores broken down by acetaminophen dose and gestational age group.

After a single dose of 10, 15 and 20 mg/kg acetaminophen median plasma peak concentrations were 10.6 mg/l (IQR 9.5-11.6), 16.5 mg/l (IQR 14.9-19.6) and 21.3 mg/l (IQR

18.5-22.1), respectively (p< 0.001, Kruskal-Wallis test). All subjects, except for three who received 10 mg/kg acetaminophen, had a peak plasma concentration > 9mg/l.
The COMFORTneo scores are also shown in table 3. The median COMFORTneo scores were higher in the 15 and 20 mg/kg acetaminophen groups with a median of 14 (IQR 11-19.8 and 11-20) versus median 11 (IQR 10-13.8) and 12 (IQR 10-14.8), respectively, in the sucrose and 10 mg/kg group. This difference was not statistically significant (p=0.17, Kruskal-Wallis test).

Table 3. PIPP and COMFORTneo scores during CVC placement

Pain assessments during procedure	APAP 10mg/kg n = 20	APAP 15 mg/kg n = 20	APAP 20 mg/kg n = 20	Sucrose 24% n = 20	*p-value*[3]
PIPP					
Median	8	7	8	8	
IQR	6-10.5	6-9	6-10	6.3-10	0.97
PIPP Score < 7[1]					
n (%)	9 (45)	11 (55)	8 (40)	9 (45)	0.81
PIPP Score 7 – 12					
n (%)	10 (50)	8 (40)	10 (50)	11(55)	
PIPP Score > 12					
n (%)	1 (5)	1 (5)	2 (10)	-	
COMFORTneo					
Median	12	14	14	11	
IQR	10-14.8	11-19.8	11-20	10-13.8	0.17
COMFORTneo 14-30[2]					
n (%)	6 (30)	10 (50)	10 (50)	4 (25)	0.28

[1] PIPP < 7 no to mild pain; 7-12 moderate pain; [3]13 severe pain
[2] a COMFORTneo score between 14 and 30 suggests pain and/or distress
[3] Fisher exact tests and Kruskal-Wallis tests were applied as appropriate

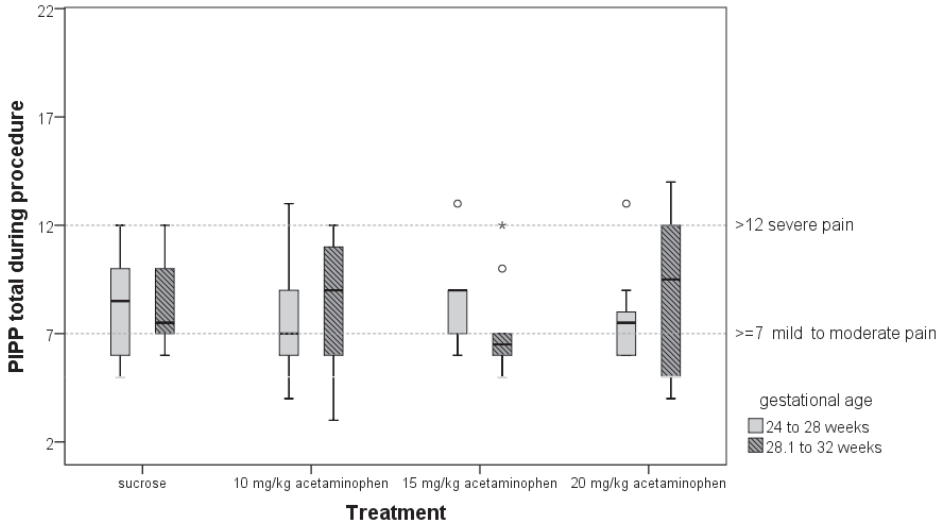


Figure 2. PIPP scores during CVC placement (n=10 in each box)

Figure 3 gives the boxplots for the peri-intervention changes in heart rate and oxygen saturation for the groups separately. In most subjects the heart rate increased (76%) or remained unchanged (4%) but it dropped in 16 subjects (20%): 2 in the sucrose group, 6 in the 10 mg/kg acetaminophen group and 4 in both the 15mg/kg and 20 mg/kg acetaminophen groups.

BUN, creatinine, aspartaat-aminotransferase (ASAT), alanine-aminotransferase (ALAT), total- and direct bilirubin values after acetaminophen administration were available for 46 (77%), 42 (70%), 10 (17%), 9 (15%), 39 (65%) and 11(18%) patients, respectively. BUN and creatinine values (reflecting kidney function) and transaminases and bilirubin values (reflecting liver function) were not statistically significantly different between the three acetaminophen treatment groups (p=0.54 for BUN, p=0.12 for creatinine, Kruskal-Wallis test) (see Table 2).

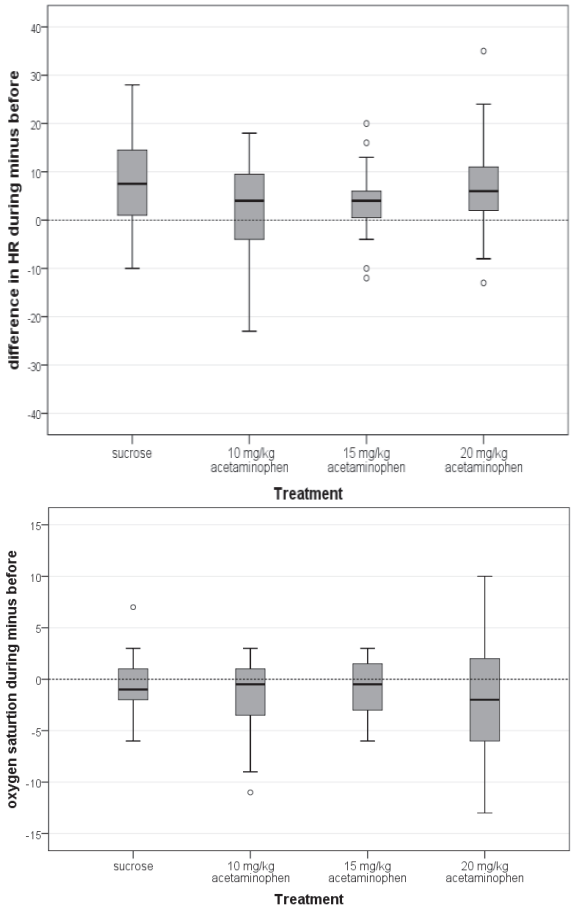


Figure 3. Peri-intervention changes in heart rate and oxygen saturation

Discussion

Pain scores assigned to preterm newborns during CVC placement did not significantly differ between the three groups receiving a different dose of iv. acetaminophen. This procedure is considered very painful, and in most cases the scores still reflected mild to moderate pain. Comparison with an oral sucrose control group showed that acetaminophen was not more effective than sucrose.

Comparative studies of different acetaminophen doses in infants are lacking. Allegaert et al showed a significant reduction of the Leuven Neonatal Pain Score after a single intravenous loading dose of 20 mg/kg acetaminophen in preterm and term neonates suffering from delivery trauma[25]. The available pharmacokinetic (PK) studies on intravenous acetaminophen in preterm infants also suggest that a loading dose of 20 mg/kg intravenous acetaminophen for neonates with postconceptional ages of 28-32 weeks and body weights above 1.5 kilograms is sufficient for pain relief[17,26]. Our study population was younger, both in gestational age and postnatal age. We compared outcome in terms of pain scores after a single dose of 20 mg/kg intravenous acetaminophen with that after 10 and 15 mg/kg doses to find the optimal dose to provide proper and safe analgesia.

While there are data from PK studies[16,17,27], the pharmacodynamics (PD) of acetaminophen in neonates are poorly described. Therefore it is not known if we can simply extrapolate the available PK observations (concentration/time profile) to dosing regimens, assuming that there is no age-dependent difference in PD (concentration/effect or level of analgesia). PK/PD data in older infants showed that an effect compartment concentration of >9 mg/l acetaminophen will achieve the target effect[18]. Our study showed median plasma peak concentrations of acetaminophen > 9 mg/l in 95% of all subjects across the three acetaminophen treatment groups. As a consequence, the lack of differences of PIPP scores between acetaminophen treatment groups cannot be explained by too low acetaminophen doses.

On the other hand, it is well possible that acetaminophen, which has been found suitable to relieve mild to moderate pain, does not relieve severe pain associated with acute skin breaking procedures. Thus, other medications, such as fentanyl and remifentanil, need to be considered[27]. Remifentanil, for example, is a short acting drug with highly suitable analgo-sedative qualities for acute procedural interventions. On the other hand, however, short acting opioids can cause adverse effects such as respiratory insufficiency and chest rigidity. Before repeated or continuous use can be advocated, further studies on hyperalgesia, tolerance and long term neurodevelopmental outcome are also needed.

Exposure to stressful procedures and procedural pain without analgesia is associated with alterations in brain development of very preterm infants. This is due to reduced maturation of white matter and subcortical grey matter shown on MRI scans, assessed at term equivalent age[8], and reduced brain size in the frontal and parietal regions[28]. The

necessity of pain relief in very preterm infants is beyond any doubt, but the best drug for that purpose has yet to be found.

Pain assessment in very preterm neonates is a challenge; numerous pain assessment instruments are available but only a few take gestational age into account, such as the PIPP used in our study. The PIPP includes changes in heart rate and oxygen saturation, which might better reflect acute pain. Other than the originally published PIPP, the revised PIPP[22] takes both a rise and a drop in heart rate into account. The fact that 20% of our patients showed a drop in heart rate proves the usefulness of the revised PIPP score in future studies.
Several limitations of this study should be addressed. First, numbers of patients in each dose group per gestational age group were relatively small. However, like in all studies in neonates it is very difficult to obtain larger sample sizes because participating in a trial is not the first priority for parents of these very young and generally severely ill infants. Second, seven patients received NSAIDs for PDA treatment, reflecting standard clinical care of very preterm neonates. These patients were not excluded, but the use of NSAIDs was added as covariate in our analyses. Third, to avoid infusion site discomfort the acetaminophen was administered over 15 minutes. This time period may not have been long enough to reach optimal analgesia. Still, the peak plasma levels seemed to be adequate if compared to studies in older infants. Fourth, we were not able to check liver and kidney function before and after administration of acetaminophen in all patients. It is not part of the routine care in both centers and we were not allowed to withdraw extra blood for sampling. Moreover the study had not enough power to determine the overall safety of acetaminophen in extreme preterm infants.

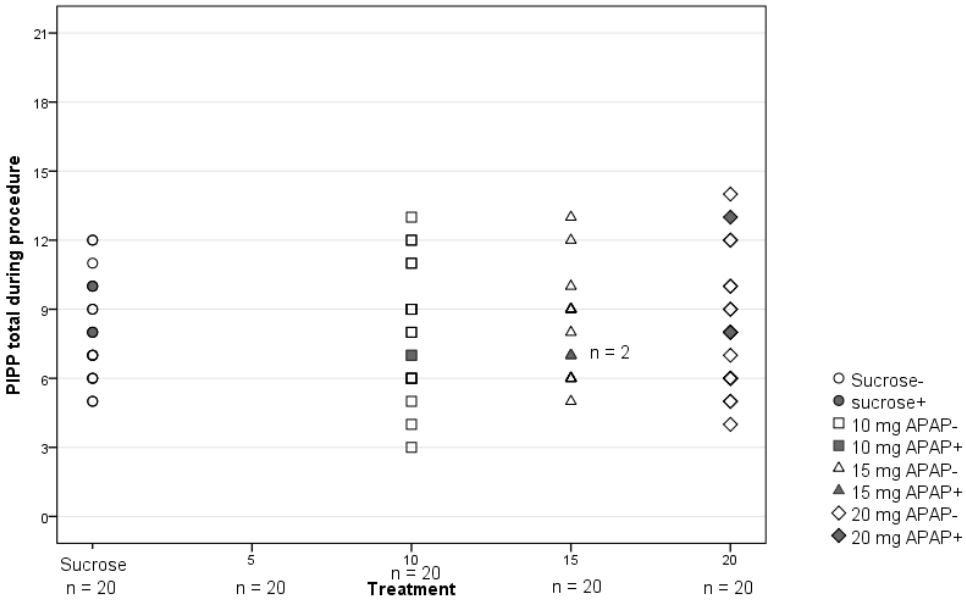
Conclusion

In our quest for efficient pain medication before acute painful procedures we performed a study on the use of acetaminophen. We did not show a benefit of doses higher than 10 mg/kg acetaminophen for pain relief during central venous catheter placement in preterm infants. Besides, acetaminophen was not more effective than sucrose. In view of our findings it is quite likely that acetaminophen is not the drug of choice for this or other skin breaking procedures in preterm infants; whether it could be suitable for mild or more prolonged pain needs to be further elucidated.

References

1. 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.* Nov 2003;157(11):1058-1064.
2. Carbajal R, Rousset A, Danan C, et al. Epidemiology and treatment of painful procedures in neonates in intensive care units. *JAMA.* Jul 2 2008;300(1):60-70.
3. Cignacco E, Hamers J, van Lingen RA, et al. Neonatal procedural pain exposure and pain management in ventilated preterm infants during the first 14 days of life. *Swiss Med Wkly.* Apr 18 2009; 139(15-16):226-232.
4. Johnston C, Barrington KJ, Taddio A, Carbajal R, Filion F. Pain in Canadian NICUs: have we improved over the past 12 years? *Clin J Pain.* Mar-Apr 2011;27(3):225-232.
5. Lago P, Garetti E, Merazzi D, et al. Guidelines for procedural pain in the newborn. *Acta Paediatr.* Jun 2009;98(6):932-939.
6. van Dijk M, Roofthooft DW, Anand KJ, et al. Taking up the challenge of measuring prolonged pain in (premature) neonates: the COMFORTneo scale seems promising. *Clin J Pain.* Sep 2009;25(7): 607-616.
7. Roofthooft DW, Simons SH, Anand KJ, Tibboel D, van Dijk M. Eight Years Later, Are We Still Hurting Newborn Infants? *Neonatology.* Feb 4 2014;105(3):218-226.
8. Brummelte S, Grunau RE, Chau V, et al. Procedural pain and brain development in premature newborns. *Ann Neurol.* Mar 2012;71(3):385-396.
9. Wilkinson DJ, Savulescu J, Slater R. Sugaring the pill: ethics and uncertainties in the use of sucrose for newborn infants. *Arch Pediatr Adolesc Med.* Jul 1 2012;166(7):629-633.
10. Slater R, Cornelissen L, Fabrizi L, et al. Oral sucrose as an analgesic drug for procedural pain in newborn infants: a randomised controlled trial. *Lancet.* Oct 9 2010;376(9748):1225-1232.
11. Lasky RE, van Drongelen W. Is sucrose an effective analgesic for newborn babies? *Lancet.* Oct 9 2010;376(9748):1201-1203.
12. Carbajal R, Lenclen R, Jugie M, Paupe A, Barton BA, Anand KJ. Morphine does not provide adequate analgesia for acute procedural pain among preterm neonates. *Pediatrics.* Jun 2005;115(6): 1494-1500.
13. Jevtovic-Todorovic V, Hartman RE, Izumi Y, et al. Early exposure to common anesthetic agents causes widespread neurodegeneration in the developing rat brain and persistent learning deficits. *J Neurosci.* Feb 1 2003;23(3):876-882.
14. Fredriksson A, Ponten E, Gordh T, Eriksson P. Neonatal exposure to a combination of N-methyl-D-aspartate and gamma-aminobutyric acid type A receptor anesthetic agents potentiates apoptotic neurodegeneration and persistent behavioral deficits. *Anesthesiology.* Sep 2007;107(3): 427-436.
15. Duhrsen L, Simons SH, Dzietko M, et al. Effects of repetitive exposure to pain and morphine treatment on the neonatal rat brain. *Neonatology.* 2013;103(1):35-43.
16. Wang C, Allegaert K, Tibboel D, et al. Population pharmacokinetics of paracetamol across the human age-range from (pre)term neonates, infants, children to adults. *J Clin Pharmacol.* Jun 2014; 54(6):619-629.
17. Allegaert K, Van der Marel CD, Debeer A, et al. Pharmacokinetics of single dose intravenous propacetamol in neonates: effect of gestational age. *Arch Dis Child Fetal Neonatal Ed.* Jan 2004; 89(1):F25-28.

18. Wang C, Allegaert K, Tibboel D, et al. Population pharmacokinetics of paracetamol across the human age-range from (pre)term neonates, infants, children to adults. *J Clin Pharmacol.* Dec 30 2013.
19. Stevens B, Johnston C, Petryshen P, Taddio A. Premature Infant Pain Profile: development and initial validation. *Clin J Pain.* Mar 1996;12(1):13-22.
20. Ballantyne M, Stevens B, McAllister M, Dionne K, Jack A. Validation of the premature infant pain profile in the clinical setting. *Clin J Pain.* Dec 1999;15(4):297-303.
21. Pasero C. Pain assessment in infants and young children: Premature Infant Pain Profile. *Am J Nurs.* Sep 2002;102(9):105-106.
22. Stevens BJ, Gibbins S, Yamada J, et al. The premature infant pain profile-revised (PIPP-R): initial validation and feasibility. *Clin J Pain.* Mar 2014;30(3):238-243.
23. Ahlfors CE. Criteria for exchange transfusion in jaundiced newborns. *Pediatrics.* Mar 1994;93(3): 488-494.
24. Maisels MJ, Watchko JF. Treatment of jaundice in low birthweight infants. *Arch Dis Child Fetal Neonatal Ed.* Nov 2003;88(6):F459-463.
25. Allegaert K, Naulaers G, Vanhaesebrouck S, Anderson BJ. The paracetamol concentration-effect relation in neonates. *Paediatr Anaesth.* Jan 2013;23(1):45-50.
26. Anderson BJ, Allegaert K. Intravenous neonatal paracetamol dosing: the magic of 10 days. *Paediatr Anaesth.* Apr 2009;19(4):289-295.
27. Allegaert K, Thewissen L, van den Anker JN. Remifentanil in neonates: a promising compound in search of its indications? *Pediatr Neonatol.* Dec 2012;53(6):387-388.
28. Smith GC, Gutovich J, Smyser C, et al. Neonatal intensive care unit stress is associated with brain development in preterm infants. *Ann Neurol.* Oct 2011;70(4):541-549.



Appendix 1. Supplementary data on PIPP scores with NSAID use prior to acetaminophen/sucrose administration

Chapter 7

Limited effects of intravenous paracetamol on patent ductus arteriosus in very low birth weight infants with contra-indications for ibuprofen or after ibuprofen failure

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Paracetamol for ductus arteriosus closure: not always a success story. Concerning the article by M.Y. Oncel et al: intravenous paracetamol treatment in the management of patent ductus arteriosus in extremely low birth weight infants [Neonatology 2013;103:166-169].

Neonatology. 2013;104(3):170

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Abstract

Finding the optimal pharmacological treatment of a patent ductus arteriosus (PDA) in preterm neonates remains challenging. There is a growing interest in paracetamol as a new drug for PDA closure.

In this prospective observational cohort study we evaluated the effectiveness of intravenous paracetamol in closing a PDA in very low birth weight infants with a hemodynamically significant PDA who either did not respond to ibuprofen or had a contra-indication for ibuprofen. They received high dose paracetamol therapy (15mg/kg/6h intravenous) for 3-7 days. Cardiac ultrasounds were performed before and 3 and 7 days after treatment. Thirty-three patients were included with a median gestational age of $25^{1/7}$ weeks (IQR 1.66), a median birth weight of 750 grams (IQR 327) and a median post-natal age of 14 days (IQR 12). Paracetamol was ineffective in 27/33 patients (82%). Even more, after previous exposure to ibuprofen this was even 100%.

Conclusions

In this study paracetamol after ibuprofen treatment failure was not effective for PDA closure in VLBW infants. From the findings of this study, paracetamol treatment for PDA closure cannot be recommended for infants with a postnatal age >2 weeks. Earlier treatment with paracetamol for PDA might be more effective.

Introduction

The ductus arteriosus fails to close after birth in 30 to 60% of prematurely born infants [16]. This condition – patent ductus arteriosus (PDA) – is associated with a prolonged ventilation need and carries an increased risk of morbidity (i.e., necrotizing enterocolitis, chronic lung disease) and even mortality [10, 15, 18]. Pharmacological closure with non-steroidal anti-inflammatory drugs (NSAIDs), mainly ibuprofen and indomethacin, is currently the standard of care [27]. NSAIDs are not effective in around 30% of patients, however, and can have side-effects such as gastrointestinal bleeding and perforation, diminished platelet aggregation, hyperbilirubinemia and transient renal function impairment [22, 23]. Moreover, NSAIDs are contra-indicated in a considerable proportion of newborns, notably those with renal failure, intracerebral hemorrhage, gastrointestinal problems and thrombocytopenia. If NSAIDs fail or are contra-indicated, the only currently available solution is surgical ligation, which is associated with the risks of cardiothoracic surgery and impaired neurological outcome [17]. Therefore, alternative pharmacological interventions are needed.

Paracetamol has been suggested as an alternative drug for PDA closure [21]. More than 10 observational and retrospective studies have described oral or intravenous high-dose paracetamol treatment with varying effectiveness [1, 8, 9, 11, 12, 19, 21, 26, 29](see Table 1). Two recent prospective randomized controlled trials comparing oral paracetamol with ibuprofen both showed a slightly favorable effect of paracetamol (closure rate 81.2% versus 78.8% for ibuprofen) [5]. The other trial by Oncel et al. even showed a higher closure success rate in the paracetamol group (97.5% versus 95% in the ibuprofen group) after a maximum of two courses of ibuprofen or paracetamol [20]. After publication of the first studies on paracetamol and PDA closure we added intravenous paracetamol to our PDA treatment guideline. However, the results in the first patients did not match the high closure rates of other studies and only 20% of patients did not need further PDA treatment after paracetamol [24]. Based on the promising results of other published studies we decided to continue paracetamol treatment for PDA closure in preterm infants with ibuprofen contra-indications or ibuprofen treatment failure.

In the current study we describe the evaluation of the efficacy of intravenous paracetamol on PDA closure in very low birth weight (VLBW) infants admitted to our hospital.

Table 1. Literature review on PDA treatment with paracetamol

Author	Dose mg/kg/dose	Treatment Interval	Route	Treatment PCM(days)	Gestational age (weeks)	PN age start PCM(days)	ductal closure/no surgical ligation
1. Hammerman[2011]	15	6h	oral	max. 7	26-29$^{6/7}$	3-35	5/5 patients
2. Yurttutan[2013]	15	6h	oral	max. 6	26-30	3-7	5/6 patients
3. Oncel[2013]	15	6h	i.v.	3 - 6	24-29	2-15	10/10 patients
4. Alan[2013]	15	6h	i.v.	max.19	26$^{2/7}$-33$^{5/7}$	8-19	0/3 patients
5. Özmert[2013]	15	6h	oral	3 – 6	23-32	20-47	5/7 patients
6. Sinha[2013]	15	8h	oral	2	27-33	4-7	10/10 patients
7. Kessel[2013]	15	6h	oral	3 – 11	26-30	?	7/7 patients
8. Jasani[2013]	15	6h	oral	2.3 – 4.3	28.5–31.1	2.6-8.9	6/6 patients
9. Dang[2013] RCT	15	6h	oral	3	31.2+/- 1.8		65/80 patients
10. Oncel[2013]RCT	15	6h	oral	3-6	≤ 26	2-3	12/13 patients
	15	6h	oral	3-6	< 28	2-3	22/23 patients
11. Tekgunduz[2014]	15	6h	i.v.	1	29	3	0/1 patient*
	10	8h	i.v.	1-4	24-31	2-9	10/12 patients$^{\phi}$
12. Nadir[2014]	15	6h	oral	max. 7	24-27	2-22	4/7 patients
13. el-Khuffash[2014]	15	6h	oral	2	26-33	14-56	0/5 patients
	15	6h	oral	7	26-30	8-35	6/7 patients
	15	6h	i.v.	2 – 5	26-32	3-41	9/9 patients
14. Terrin[2014]	7,5-15 max. 60mg/kg/d	4-6h	i.v.	3	26 ± 2	2.8 ± 1.2	6/8 patients
15. Roofthooft[2014]	15	6h	i.v.	3 – 7,5	23$^{6/7}$-26$^{4/7}$	3-33	6/33 patients
16. el-Khuffash[2015]	15	6h	i.v.	max.6	24.6-27.9	16-39	24/30 patients

* transaminases elevated: paracetamol treatment stopped after 3 doses; ductal closure with oral ibuprofen

$^{\phi}$ ductal closure of 2 remaining PDA's with oral ibuprofen after paracetamol

Methods

In a prospective observational, single center study performed from December 2012 until September 2014 at the level III NICU of the Erasmus MC-Sophia Children's Hospital in Rotterdam, the Netherlands, we included all neonates with a gestational age of less than 28 weeks and a birth weight of less than 1500 grams diagnosed with a hemodynamically significant patent ductus arteriosus (hsPDA) using clinical and cardiac ultrasound evaluation. Findings in the first 9 patients in the current study were also presented in a preliminary report in 2014 [4].

The first drug of choice in our department for PDA treatment was intravenous ibuprofen (Neobrufen♥; Pedea ©), a single daily dose for 3 days (10 mg/kg on first day, 5 mg/kg on second and third days), and a repeated 3-day course if closure was not yet obtained. Paracetamol (Perfalgan©; Bristol-Meyers Squibb) was given if two courses of ibuprofen had no effect or if ibuprofen was contra-indicated. Intravenous paracetamol 15 mg/kg every 6 hours (60 mg/kg/day) was administered for a minimum of three days and a maximum of 7 days.

Based on the indication for paracetamol, we created three groups. Group A: ibuprofen contra-indicated and paracetamol as first drug of choice (primary contra-indication); Group B: development of a contraindication for ibuprofen during treatment with ibuprofen as first choice and switch to paracetamol (incomplete ibuprofen); and Group C: two complete courses of ibuprofen failed to achieve closure and switch to paracetamol (complete ibuprofen). (See figure 1).

Contraindications for ibuprofen treatment were active intracerebral hemorrhage, thrombocytopenia or other known clotting disorders, severe sepsis, suspected or confirmed necrotizing enterocolitis (NEC), intestinal perforation, liver and kidney function disorders (oliguria < 1.0 ml/kg/h, serum creatinine > 110 µmol/l) and severe hyperbilirubinemia.

Echocardiographic examination was performed by the echocardiosonographer or pediatric cardiologist before the start of paracetamol treatment, after three days and after 7 days. Measurements included ductus diameter, PDA diameter/LPA (left pulmonary artery) diameter, and LA/Ao (left atrial to aortic root) ratio. Two-dimensional color Doppler echocardiography was performed using a Vivid-S6 (GE Health Care) ultrasound system equipped with a 10MHz transducer.

Cardiac ultrasound studies were done at the bed side by different echocardiosonographers, and measurements were checked by one pediatric cardiologist (I.M. v B). HsPDA closure was defined as no flow through the duct. An open duct with diameter < 1.5 mm, without significant left-to-right shunt, PDA:LPA diameter < 0.5 and LA/Ao ratio smaller than < 1.4 was defined as small PDA and not hemodynamically significant. HsPDA was defined as a ductal diameter of > 2.0 mm, PDA:LPA diameter > 0.8 and/or LA/Ao ratio > 1.6.

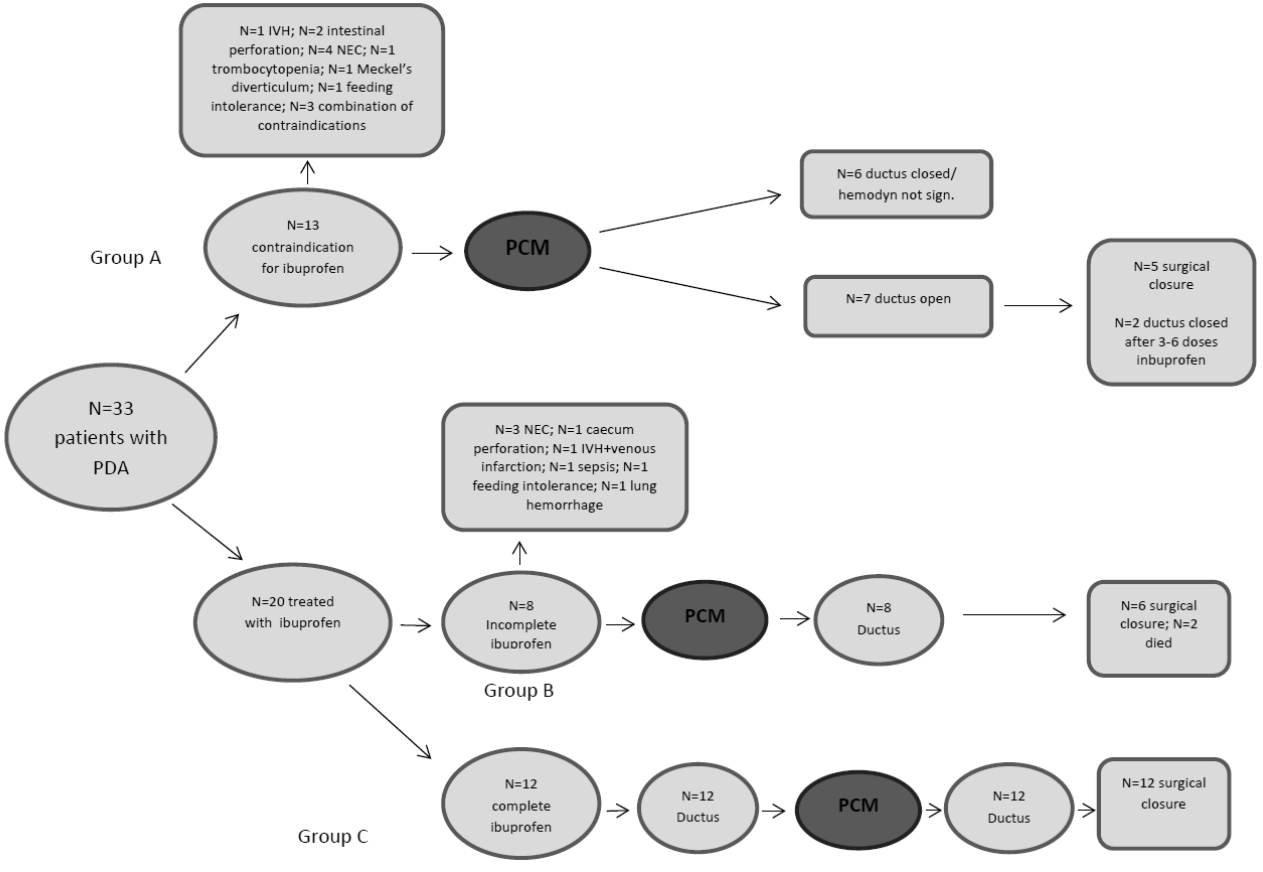

Figure 1. Flowchart included patients

Table 2. background characteristics

Characteristics	Group A N = 13	Group B N = 8	Group C N = 12
Gestational age (weeks); median	25.2	24.3	25.8
Range	24.0-26.4	24.0-26.3	23.6-26.6
IQR	0.8	0.7	1.0
Birth weight (gr): median	650	730	868
Range	400-1130	365-820	480-990
IQR	360.0	305.0	231.3
Small for gestational age: n (%)	5 (38.5)	3 (37.5)	2 (16.7)
Gender			
Male: n(%)	9 (69.2)	4 (50)	6 (50)
Female: n(%)	4 (30.8)	4 (50)	6 (50)
Died: n (%)	4 (30.8)	4 (50)	0 (0)
Post natal age: median/IQR	51/24.5	30/34.3	
Antenatal steroids: n (%)	11(84.6)	8 (100)	12(100)
PIH; n (%)	3 (23.1)	2 (25)	2 (16.7)
Cesarean section: n (%)	9 (69.2)	5 (62.5)	7 (58.3)
Mechanical ventilation: n (%)	12(92.3)	7 (87.5)	11(91.7)
Surfactant treatment	10(76.9)	7 (87.5)	11(91.7)
Diuretics	11(84.6)	4 (50)	11(91.7)
Fluid restriction	9 (69.2)	3 (37.5)	8 (66.7)
PNA start PCM:median (days)	12.0	12.5	16.5
IQR	11.5	14.75	10.75
Paracetamol treatment	6.0	6.5	5.5
days in total (median/IQR)	3	2.75	4
Surgical ligation: n(%)	5 (38.5)	6 (75.0)	12 (100)
PDA measurements			
PDA diam. before start PCM (mm): median/IQR	2.4/1.30	1.9/1.13	2.4/0.83
PDA diam. after 3 days PCM (mm): median/IQR	1.9/0.90	2.1/1.13	2.1/1.08
PDA diam. after 7 days PCM (mm): median/IQR	1.8/1.28	2.0/0.40	2.6/1.60
PDA:LPA ratio before start PCM median/IQR	0.85/0.55	0.85/0.45	0.90/0.30
PDA:LPA ratio after 3 days PCM median/IQR	0.90/0.60	0.80/0.15	0.95/0.50
PDA:LPA ratio after 7 days PCM median/IQR	0.75/0.58	0.80/0.30	0.85/0.28
LA/Ao ratio before start PCM median/IQR	1.6/0.30	1.7/0.53	1.75/0.30
LA/Ao ratio after 3 days PCM median/IQR	1.64/0.60	1.7/0.70	1.8/0.55
LA/Ao ratio after 7 days PCM median/IQR	1.4/0.55	1.9/0.40	1.7/0.65
NT-proBNP (pmol/l)¥: median	1097	2102	3078
IQR	3849.3	5832.0	4981.8

Pregnancy induced hypertension syndrome (PIH); Postnatal age (PNA); Paracetamol (PCM);
Left pulmonary artery (LPA); Left atrium-Aorta (LA-Ao)
N-terminal pro-Brain natriuretic Peptide (NT-proBNP); ¥: all NT-proBNP values were determined on day 3 PNA

Statistical analysis

Patient characteristics are presented as medians (IQR: Inter Quartile Range) in case of non-normally distributed variables and as means (standard deviations) in case of normally distributed variables. PDA diameters, PDA:LPA ratio and LA/Ao ratio before, during and after treatment with paracetamol were compared using paired t-tests. Fisher exact tests were used in case of categorical variables. Data analyses were performed with SPSS version 21.0 (SPSS Inc.). A p-value of 0.05 was set as statistically significant.

Results

A total of 33 VLBW infants with a median gestational age of $25^{1/7}$ weeks (range $23^{6/7}$-$26^{6/7}$, IQR 1.66) and a median birth weight of 750 grams (range 365-1130, IQR 327) were included. Intravenous paracetamol was started at a median postnatal age of 14 days (IQR 12). Background characteristics and clinical outcome are shown in Table 2.

Median duration of paracetamol treatment was 6 days (IQR 3); the median cumulative dose was 360 mg (IQR 180). Ductal closure or no hsPDA after treatment was achieved in 6 of the 33 patients (18%). In total 23 patients (76.7%) needed surgical ligation for a hsPDA with clinical symptoms. Findings in the three different groups (see Figure 1) are detailed below.

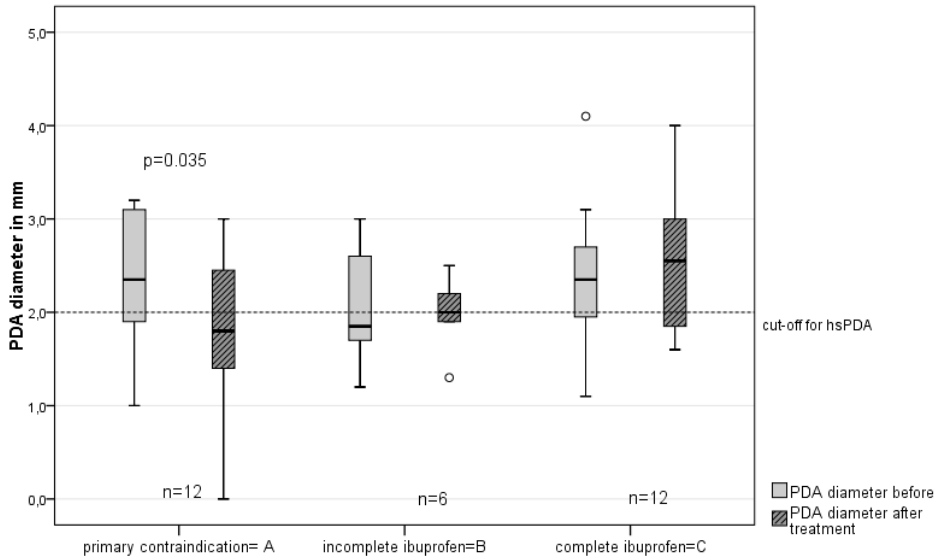

Figure 2. Change in ductus arteriosus diameter after 3 to maximum 7 days of intravenous PCM treatment for the 3 different groups (Group A: primary contraindication for ibuprofen; Group B: paracetamol after early stop of ibuprofen; Group C paracetamol after complete ibuprofen treatment).

Patients in **Group A** (=primary contra-indication for ibuprofen; N=13, 39.4%) received the first dose of paracetamol after a median of 12 (IQR 11.5) postnatal days and the median duration of the course was 6 (IQR 3) days. In six patients (46%) the ductus arteriosus was closed or not hemodynamically significant after paracetamol and further treatment was not indicated. Two of the seven patients who did not respond to paracetamol treatment were successfully treated with ibuprofen (one and two courses, respectively) afterwards because the contra-indication for ibuprofen had disappeared. Surgical ligation was performed in the other five (54%).
Patients in **group B** (= incomplete ibuprofen; N= 8, 24.2%) received the first dose of paracetamol after a median of 12.5 (IQR14.75) postnatal days and the median duration of the course was 6.5 (IQR 2.75) days. Two patients died on the 24th and 34th postnatal day (i.e., sepsis with extension of bilateral intraventricular hemorrhage with venous infarction, gram-negative bacterial infection) after paracetamol treatment (started on day 8 and 13 respectively) before a decision could be made to treat the persistent PDA with surgical closure. Paracetamol treatment was ineffective in the six remaining patients and all underwent surgical closure of the duct.
Patients in **group C** (= complete ibuprofen; N=12, 36.4%) received paracetamol for a median of 5.5 days (IQR 4) after two courses of ibuprofen. At start of paracetamol treatment their median postnatal age was 16.5 (IQR10.75) days. Paracetamol treatment was ineffective in all patients and consequently they all underwent surgical ligation.
Both the surgical ligation rate and the mortality rate differed between the three groups. Surgical ligation was performed in 5/13 (38.5%) in group A; 6/8 (75%) in group B; and 12/12 (100%) in group C (Fisher exact test for surgical ligation p=0.001). In total 8 patients died (Fisher exact test for death p=0.025), 4/13 (31%) in group A (median 51 days; IQR 24.5); 4/8 (50%) in group B (median 30 days; IQR 34.3); and none in group C. Two of the 4 non-survivors in group B died from the consequences of NEC before surgical closure of the PDA could be performed.

Cardiac ultrasound studies showed a statistically significant decrease in ductal diameter after paracetamol treatment only in group A, from median 2.4 mm to 1.8 mm after 7 days treatment (p=0.035, paired T-test). (Figure 2)
All pre- and post-treatment measurements of kidney function (urea and creatinine) and liver function (transaminases, conjugated bilirubin) were normal.

Discussion

In this study, high dose intravenous paracetamol for the treatment of hsPDA in VLBW infants was overall effective in only 18%. Looking at subgroups of patients, paracetamol

treatment was completely ineffective after previous ibuprofen treatment failure. However, it was effective in 46% of the newborns with primary contraindications for ibuprofen.

Variability in success rates this paracetamol therapy for PDA closure is hard to explain. Other studies reported success rates of 0% [7] up to 100% [12, 19]. Success rates in two RCTs comparing oral paracetamol versus oral ibuprofen were 81% [5] and 94% [20], respectively, thus much higher than in our study. Still, in all but one of those studies the same dosing regimen of 60 mg/kg/24h paracetamol was used. In the study by Tekgunduz et al. the dose was lowered to 30 mg/kg/day after a patient showed elevated transaminases [13]. The duration of paracetamol treatment in our study was 6.0 days compared to 4.1 days [29] and 3.9 days [19] in the studies with high success rates.

In previous studies, except the 2 RCTs [5, 20], the indication for paracetamol was the same as in our study (treatment failure or contraindication for ibuprofen). The interpretation of different studies on this subject seems to be hindered by the lack of an international guideline on the definition of hsPDA and cutoff values for a small, medium or large PDA (cardio ultrasound measurements).

It is remarkable that Oncel et al. reported a 100% success rate of PDA closure with intravenous paracetamol[19], the same administration route as in our study. Although unlikely, the route of administration could in part explain the variety in success rates: as long as data on bioavailability of the drug in the different routes and enterohepatic recirculation are lacking, we cannot tell which route of administration is superior. Still, ibuprofen studies also tend to find better results with oral administration [27]. Intravenous therapy probably leads to high peak plasma levels, but on the other hand also to a relatively fast decrease in levels. Oral therapy might result in lower, but more steady plasma levels. It can be hypothesized that PDA closure relies more on continuous prostaglandin inhibition than on intermittent high paracetamol or NSAIDs levels.

The relatively late start of paracetamol administration might be the most important explanation for our disappointing results compared to other studies. A second likely reason is the low gestational ages in our study. Other studies with better results [12, 19, 21, 29] included older infants with gestational ages > 28 weeks. PDAs in this age group are generally less hemodynamically significant and tend to close spontaneously and respond better to pharmacological treatment.

Ibuprofen therapy failure was previously found to be 17% in infants with gestational ages of 26-27 weeks versus 62% in 23 to 25-week-old infants [6]. PDAs in more preterm born infants are probably less responsive to cyclooxygenase inhibitors due to higher expression of prostaglandin receptors [3]. Next to gestational age and postnatal age, clinical factors such as the amount of fluids given, incidence of infections or sepsis, type of respiratory support, and use of co-medication might be influential factors for PDA closure success in extreme preterm infants.

Third, selection bias may have occurred in that we included patients in whom ibuprofen therapy had failed prior to paracetamol treatment. As NSAIDs are more potent prostaglandin synthesis inhibitors than paracetamol [8], resulting in lower peripheral PGE2 levels as is shown in orthodontic studies [25] the a priori probability of success of paracetamol in this group of patients was already relatively low.

Pharmacokinetics and pharmacodynamics of paracetamol for PDA closure have been hardly studied. Consequently, the effective plasma level of paracetamol to achieve PDA closure is unknown. The currently used dosages are already much higher than recommended for analgesia, and might be unsafe. Increasing the dose to improve closure rates is unadvisable. In a study by Kessel et al. plasma levels of paracetamol (15 mg/kg 6 hourly by nasogastric tube) did not exceed the recommended plasma levels of 10-20 mg/l for pain and fever control [14]. Based on predictive modeling, plasma levels will accumulate with the 15 mg/kg 6 hourly regimen, reaching peaks of nearly 25 mg/kg after four doses [1]. In very preterm infants and murine studies the effectiveness of paracetamol on PDA closure was suggested to depend on dosing, duration of treatment (>2 days course) and mode of administration [8].
Likewise, safety of paracetamol in very preterm infants (gestational age <28 weeks) has been little studied. Allegaert et al. showed no hemodynamic alterations during and following an intravenous loading dose of paracetamol and afebrile neonates maintained normothermia [2]. Paracetamol-induced hepatotoxicity is the most important concern; this is caused by the formation of a highly active metabolite N-acetyl-p-benzoquinone imine (NAPQ1) by the hepatic cytochrome P-450 dependent CYP 2E1 enzyme system [28]. The formation of NAPQ1 is suggested to be low due to the immaturity of the hepatic enzymes, although increased susceptibility to toxicity from supratherapeutic of paracetamol is described in infants and younger children with fever [13]. CYP 2E1 activity has not yet been quantified in neonates and the correlation between paracetamol concentration and increased NAPQ1 production is unknown.
Several limitations of this study should be addressed. First, this was an observational single center study with relatively few patients per group, without a matched control group to rule out spontaneous PDA closure. Firm statements are therefore difficult to make. Second, the echocardiosonographer and pediatric cardiologist were not blinded for the PDA treatment. Third, only patients were included in our study after ibuprofen failure or with a primary or secondary contraindication for ibuprofen; this selection bias might have contributed to our results.

Conclusions

In view of the findings from this study, we do not recommend the use of intravenous paracetamol for hsPDA closure in VLBW infants after ibuprofen failure. Still, as we did not rule out effectiveness of paracetamol as early PDA treatment, it might be recommended when started in the first week of life. On the other hand, as long as data on long term safety are lacking, high dosages of paracetamol should be used with caution. Better designed PK/PD studies are needed to shed a light on safety aspects and the optimal dose-concentration-effect relationship. PK modeling of available data on PDA treatment with paracetamol in different gestational age groups can lead to different dosing recommendations per age group; a trial with these dose recommendations of paracetamol for PDA is also an option for future research.

References

1. Alan S, Karadeniz C, Okulu E, Kilic A, Erdeve O, Ucar T, Atasay B, Atalay S, Arsan S (2013) Management of patent ductus arteriosus in preterm infants: clinical judgment might be a fair option. J Matern Fetal Neonatal Med 26: 1850-1854
2. Allegaert K, Naulaers G (2010) Haemodynamics of intravenous paracetamol in neonates. Eur J Clin Pharmacol 66: 855-858
3. Bouayad A, Kajino H, Waleh N, Fouron JC, Andelfinger G, Varma DR, Skoll A, Vazquez A, Gobeil F, Jr., Clyman RI, Chemtob S (2001) Characterization of PGE2 receptors in fetal and newborn lamb ductus arteriosus. Am J Physiol Heart Circ Physiol 280: H2342-2349
4. Bozdag SC, Tekgunduz E, Durgun G, Sarica A, Demiriz IS, Kocubaba S, Altuntas F (2013) Which regimen is better for stem cell mobilization of lymphoma patients? Transfus Apher Sci 48: 407-410
5. Dang D, Wang D, Zhang C, Zhou W, Zhou Q, Wu H (2013) Comparison of Oral Paracetamol versus Ibuprofen in Premature Infants with Patent Ductus Arteriosus: A Randomized Controlled Trial. PLoS One 8: e77888
6. Dani C, Bertini G, Corsini I, Elia S, Vangi V, Pratesi S, Rubaltelli FF (2008) The fate of ductus arteriosus in infants at 23-27 weeks of gestation: from spontaneous closure to ibuprofen resistance. Acta Paediatr 97: 1176-1180
7. Demiriz IS, Bozdag SC, Tekgunduz E, Ugur B, Durgun G, Kocubaba S, Altuntas F (2013) Predicting the successful peripheral blood stem cell harvesting. Transfus Apher Sci 48: 411-414
8. El-Khuffash A, Jain A, Corcoran D, Shah PS, Hooper CW, Brown N, Poole SD, Shelton EL, Milne GL, Reese J, McNamara PJ (2014) Efficacy of paracetamol on patent ductus arteriosus closure may be dose dependent: evidence from human and murine studies. Pediatr Res 76: 238-244
9. El-Khuffash A, James AT, Cleary A, Semberova J, Franklin O, Miletin J (2015) Late medical therapy of patent ductus arteriosus using intravenous paracetamol. Arch Dis Child Fetal Neonatal Ed:
10. Evans N, Kluckow M (1996) Early ductal shunting and intraventricular haemorrhage in ventilated preterm infants. Arch Dis Child Fetal Neonatal Ed 75: F183-186
11. Hammerman C, Bin-Nun A, Kaplan M (2012) Managing the patent ductus arteriosus in the premature neonate: a new look at what we thought we knew. Semin Perinatol 36: 130-138
12. Hammerman C, Bin-Nun A, Markovitch E, Schimmel MS, Kaplan M, Fink D (2011) Ductal closure with paracetamol: a surprising new approach to patent ductus arteriosus treatment. Pediatrics 128: e1618-1621
13. Heubi JE, Barbacci MB, Zimmerman HJ (1998) Therapeutic misadventures with acetaminophen: hepatoxicity after multiple doses in children. J Pediatr 132: 22-27
14. Kessel I, Waisman D, Lavie-Nevo K, Golzman M, Lorber A, Rotschild A (2014) Paracetamol effectiveness, safety and blood level monitoring during patent ductus arteriosus closure: a case series. J Matern Fetal Neonatal Med 27: 1719-1721
15. Kluckow M, Evans N (2000) Ductal shunting, high pulmonary blood flow, and pulmonary hemorrhage. J Pediatr 137: 68-72
16. Koch J, Hensley G, Roy L, Brown S, Ramaciotti C, Rosenfeld CR (2006) Prevalence of spontaneous closure of the ductus arteriosus in neonates at a birth weight of 1000 grams or less. Pediatrics 117: 1113-1121
17. Mandhan P, Brown S, Kukkady A, Samarakkody U (2009) Surgical closure of patent ductus arteriosus in preterm low birth weight infants. Congenit Heart Dis 4: 34-37
18. Noori S, McCoy M, Friedlich P, Bright B, Gottipati V, Seri I, Sekar K (2009) Failure of ductus arteriosus closure is associated with increased mortality in preterm infants. Pediatrics 123: e138-144

19. Oncel MY, Yurttutan S, Degirmencioglu H, Uras N, Altug N, Erdeve O, Dilmen U (2013) Intravenous paracetamol treatment in the management of patent ductus arteriosus in extremely low birth weight infants. Neonatology 103: 166-169
20. Oncel MY, Yurttutan S, Erdeve O, Uras N, Altug N, Oguz SS, Canpolat FE, Dilmen U (2014) Oral paracetamol versus oral ibuprofen in the management of patent ductus arteriosus in preterm infants: a randomized controlled trial. J Pediatr 164: 510-514 e511
21. Oncel MY, Yurttutan S, Uras N, Altug N, Ozdemir R, Ekmen S, Erdeve O, Dilmen U (2013) An alternative drug (paracetamol) in the management of patent ductus arteriosus in ibuprofen-resistant or contraindicated preterm infants. Arch Dis Child Fetal Neonatal Ed 98: F94
22. Patel J, Roberts I, Azzopardi D, Hamilton P, Edwards AD (2000) Randomized double-blind controlled trial comparing the effects of ibuprofen with indomethacin on cerebral hemodynamics in preterm infants with patent ductus arteriosus. Pediatr Res 47: 36-42
23. Pezzati M, Vangi V, Biagiotti R, Bertini G, Cianciulli D, Rubaltelli FF (1999) Effects of indomethacin and ibuprofen on mesenteric and renal blood flow in preterm infants with patent ductus arteriosus. J Pediatr 135: 733-738
24. Roofthooft DW, van Beynum IM, Helbing WA, Reiss IK, Simons SH (2013) Paracetamol for ductus arteriosus closure: not always a success story. Concerning the article by M.Y. Oncel et al: intravenous paracetamol treatment in the management of patent ductus arteriosus in extremely low birth weight infants [Neonatology 2013;103:166-169]. Neonatology 104: 170
25. Shetty N, Patil AK, Ganeshkar SV, Hegde S (2013) Comparison of the effects of ibuprofen and acetaminophen on PGE2 levels in the GCF during orthodontic tooth movement: a human study. Prog Orthod 14: 6
26. Sinha R, Negi V, Dalal SS (2013) An Interesting Observation of PDA Closure with Oral Paracetamol in Preterm Neonates. J Clin Neonatol 2: 30-32
27. Tekgunduz KS, Ceviz N, Demirelli Y, Olgun H, Caner I, Sahin IO, Yolcu C (2013) Intravenous paracetamol for patent ductus arteriosus in premature infants - a lower dose is also effective. Concerning the article by M.Y. Oncel et al: Intravenous paracetamol treatment in the management of patent ductus arteriosus in extremely low birth weight infants [Neonatology 2013;103: 166-169]. Neonatology 104: 6-7
28. Van Eyken P, Nemolato S, Faa G, Ambu R (2012) Hepatic injury to the newborn liver due to drugs. Curr Pharm Des 18: 3050-3060
29. Yurttutan S, Oncel MY, Arayici S, Uras N, Altug N, Erdeve O, Dilmen U (2013) A different first-choice drug in the medical management of patent ductus arteriosus: oral paracetamol. J Matern Fetal Neonatal Med 26: 825-827

Chapter 8

General discussion

Introduction

After revealing publications by Anand in the late eighties on the necessity of pain treatment in newborns, in particular in view of the preventive effect of peri-operative analgesia on mortality[1], we have now reached a new era in which pain is seen as the fifth vital sign, as suggested by the American Pain Society. It is generally accepted that prematurely born neonates are able to feel pain and that any pain must be adequately treated but, better still, prevented in the first place. The questions how and when to measure pain in these vulnerable newborns or how analgesics should be used with regards to dosage, timing and drug of first choice are still difficult to answer.

Worldwide 15 million premature neonates are born yearly. Advances in neonatal care and medical technology over the past two decades have led to a 31% increase in survival among infants born at 23 weeks' gestation[2,3], albeit at the expense of significant morbidity. In several countries the limit of viability is trending down and in the Netherlands the current limit for active treatment is 24 weeks' gestation. Other countries, such as the USA, Japan and Sweden, are already treating preterm infants with gestational ages of 22 weeks. As a consequence, the number of preterm infants who will be exposed to painful procedures in the NICU setting is rising.

Caution is needed as the growing pain awareness even led to overtreatment. In the 1990s, many centers started to use strong opioids such as morphine or fentanyl, sedatives like midazolam or even muscle relaxants, as standard of care during invasive ventilation in newborns. Then, Anand and colleagues showed in a large placebo controlled trial (Neopain study) that morphine had no effects on short term outcome. Even relatively high dosages of 20-30 mcg/kg/hour instead of the generally accepted 10 mcg/kg/hour[4] had inadequate analgesic effects in ventilated preterm neonates between 28 and 32 weeks of gestation. A trial from our own research group in which the neonates received 10 mcg/kg/hour morphine or placebo, irrespective of gestational age, was the first to conclude that routine administration of morphine in ventilated preterm infants is unnecessary[5]. A meta-analysis of the NEOPAIN study combined with other comparable, smaller trials confirmed this conclusion [6].

Furthermore, after a Cochrane review concluded that midazolam seemed associated with an increased risk of intraventricular hemorrhages, its use was almost completely abolished from neonatal intensive care units[7,8]. Data from animal studies suggesting that many analgesics and anesthetics[9] cause excessive cell death in the developing brain further increased clinicians' reluctance to use pharmacological agents to treat neonatal pain. Today, as is shown in this thesis (chapter 4), NICU stay is a stressful affair

for these vulnerable newborns and is associated with an enormous number of painful procedures[10]. The Europain Survey has shown wide variation in the use of analgesics and sedatives for neonates in NICUs across Europe – from 81.5% of ventilated infants, 17.8% of non-invasively ventilated infants to 9.3% of those with spontaneous ventilation[11].

Neonatal pain

Pain response

Although numerous questions concerning pain still remain unanswered, much has been learned about pain perception, pain response and pain assessment. Pain perception requires awareness of a noxious stimulus and development of pathophysiological pathways of pain[12].

Nociceptive cutaneous receptors start developing from 7.5 weeks gestational age[13] and nociceptive fibers from 22-23 weeks of gestation[14]. The nociceptive receptors in the skin are connected with specialized peripheral sensory neurons transmitting the pain signal to the cortex and vice versa. These pathways are fully developed at 30 weeks of gestation and the degree of myelinisation determines the speed of the conducted signal[15]. Reflexes mediated in the spinal cord as a response to mechanical skin stimulation are exaggerated in young infants compared with adults, with lower thresholds resulting in more synchronized, longer-lasting reflex muscle contractions[16]. This might be caused by immaturity of the inhibitory descending pain pathways. Slater et al showed that prematurely born infants who have experienced at least 40 days of intensive care, have an increased neuronal response to noxious stimuli compared with healthy newborns at the same corrected age[17]. This means that even the most extreme preterm neonates born at the limits of viability are capable to respond to painful stimuli with a pain response expressed as changes of physiologic parameters and behavior. These pain responses are comparable between preterm and term infants; even extremely low gestational age infants have similar, though dampened pain responses as older infants[18].

Some additional factors play a role in the extent and the way preterm newborns respond to pain. First, previous painful procedures can affect pain responses: in a cross-sectional comparative study, preterm infants who spent postconceptional weeks 28 through 32 in a NICU had a less mature pain response than premature infants born after 32 weeks' postconceptional age[19]. The infants exposed to frequent invasive procedures showed fewer behavioral manifestations of pain, but larger changes in heart rate during heel sticks than those of the same postconceptional age who were just born [19]. Furthermore, severity of illness may affect the way neonates express pain. For example, an infant with (suspected) necrotizing enterocolitis may show little behavioral pain expression, but the

estimated extensiveness of tissue inflammation and bowel ischemia may point at pain that should be treated.

Painful procedures

In 2003, the mean number of painful procedures per neonate per day on our NICU was 14 (SD 4), which figure compares agreeably with the 16 painful procedures per day of hospitalization in other international NICUs (EPIPPAIN study)[20]. After the introduction of a pain management protocol the mean number of painful procedures per neonate per day in our NICU went down to 11.2 (SD 5.7). A further reduction is unlikely as the NICU population has changed over the last years to include more preterm neonates with lower gestational ages and birth weights as well as more critically ill term infants. Insertions of lines, catheters and endotracheal tubes often need more than one attempt to succeed and are considered painful (chapter 4). With respect to line insertion there is room for improvement, however, which could be achieved by simulation training in skills labs with suitable manikins, by setting up an intravenous access team, training programs for senior neonatal nurses and residents and by introducing new support devices such as the translumination devices based on cold near-infrared LEDs.

Most of the suitable pain assessment instruments have been validated for acute pain, for example the pain experienced from heel lancing. Heel lancing is less time consuming and easier to perform than venous puncture, which is proven to be less painful[21,22]. Opting for venous puncture could obviously reduce the amount of pain. However, in some cases heel lancing is unavoidable, and then it is important to use an appropriately-sized heel lance and to perform the procedure in the correct way. To assess how correctly this is done in our unit, we observed how many squeezes of the heel (considered even more painful than the heel prick itself) were needed to obtain blood (unpublished study). In 150 newborns a median of 19 squeezes (IQR 9 to 32) were needed, which makes one think that there is room for improvement. Apart from pharmacological pain control, non-pharmacological interventions can help reduce pain. Weissman et al showed that any method of pain control is better than none. Of the non-pharmacological interventions they studied, nonnutritive sucking, skin-to-skin holding by the mother, oral glucose solution, and breastfeeding during heel lancing were found to be the most effective[23]. Ultimately, our goal will be to determine how multiple stressful and painful interventions in the NICU can be minimized, thereby improving clinical, behavioral and developmental outcomes of our patients.

Pain assessment

Routinely performed pain assessments have become quite common in the daily care for newborn infants although practices vary between European NICUs. There still seems to be room for improvement: the Europain Survey showed that pain assessments were performed in 58.5% of endotracheally ventilated and 45.0% of non-invasively ventilated neonates[24]. Still in many centers in the world pain assessment instruments are used as a research tool only and their use had not been integrated in pain management protocols. The gold standard of pain assessment is self-report but for obvious reasons this is not possible in nonverbal neonates. Pain assessment should go further than simply applying one of the many pain assessment instruments in the neonatal ICU. One important aspect is knowledge of the pain source or sources (including exposure duration and type of stimulus). For example, the insertion of a chest tube in case of a pneumothorax is extremely painful without the proper analgesic therapy and maintenance analgesic treatment is needed as long as drainage is necessary.
Another aspect should be taken into account is the infant's ability to respond to the noxious stimulus, level of consciousness, especially in the very preterm infants, and severity of illness.[25] Furthermore, current behavioral state and the amount of pain exposure prior in life are relevant: a study in preterm newborns showed that those who were more premature, asleep, and had undergone a painful event more recently, less likely demonstrated behavioral and physiologic indicators of pain during a heel stick[26]. Most research focuses primarily on acute pain, like in procedural interventions or surgery. In the clinical setting, assessing prolonged or persisting pain is also an important challenge, which so far has received only little attention because major methodological issues regarding scoring of chronic pain have not yet been solved.

Numerous pain assessment instruments have been developed since 1987 and there seems to be no limit to the introduction of new instruments (see Figure 1). This is unfortunate because the newer instruments show significant overlap with the older ones with respect to the indicators used. In the absence of a ´gold standard´ instrument, it remains hard to determine which is the best of the other instruments. Most pain assessment instruments are based on behavioral indicators such as facial expression, cry and body movements. This would explain why 'new' instruments are highly correlated with the existing ones and supposedly show good construct validity. However, this is rather a case of "old wine in new bottles". Some instruments, however, such as the N-PASS for prolonged pain and PIPP for acute pain, include physiological parameters, next to behavioral indicators, heart rate, oxygen saturation and blood pressure[27-29].

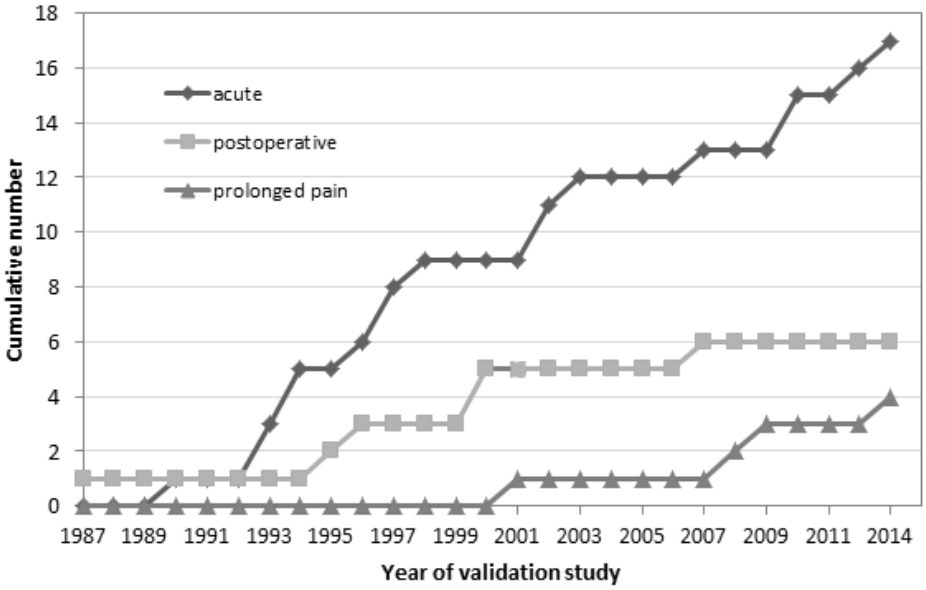

Figure 1. Development of neonatal pain instruments over time

Unfortunately most authors do not provide data about the correlation between these physiological indicators and the behavioral indicators but an average correlation of 0.3 between these two classes of measures has been reported. This leaves the question which of the two types of indicators is more valid for pain assessment.

In our department we have chosen for the COMFORT behavior scale, which we adapted from an instrument originally developed to assess the degree of sedation in ventilated infants between 0 and 18 years of age[30]. We further modified and validated it for use in neonates in a NICU[31] and named this modified version the COMFORTneo scale (chapter 2). It is intended to monitor prolonged pain and rather not to assess acute pain. One of its assets is that low scores can indicate oversedation and thus alert NICU staff to consider tapering of opioids or sedatives.

Validation is an ongoing process and it will be worthwhile to validate the COMFORTneo scale in other settings and relate it with physiological indicators and other instruments, which is especially relevant for the extreme premature neonates.

Possibly more objective indicators of pain have been explored, such as technology-based autonomic, brain-oriented, and bio hormonal indices, including heart rate variability[32-34], skin conductance[35-37], electroencephalogram (EEG)[38] and cerebral near-infrared spectroscopy (NIRS)[39,40].

Heart rate variability

Studies by the group of Lindh showed that heart rate increased and that the total heart rate variability – the variation in the interval between heart beats – decreased in term and preterm infants during lancing and squeezing of the heel[32,33]. Although heart rate variability seems a good indicator for acute pain, so far it has only been tested in heel lancing and mechanical ventilation, predominantly in term infants. The feasibility of its bedside use is still questionable. New advanced measurement devices are promising, however, as they can compare subtle heart rate variations measured multiple times per second.

Skin conductance

Skin conductance activity has been first validated as a physiological measure of the emotional state in full term infants, based on changes in the palmar and plantar sweat glands. These changes in conductance are due to the activity of the sympathetic nervous system, which responds to the emotional state by secreting acetylcholine in the postganglionic synapses[37,41]. The technique has been hardly tested in preterm infants and a study showed that even in children without evident pain, skin temperature was correlated with changes in skin conductance activity[41]. A study by Valkenburg et al suggested that sympathetic neural control of vital functions to maintain homeostasis (such as autoregulation of skin temperature) results in skin conductance peaks[42]. It has also been reported that oral glucose administration before heel lancing influences skin conductance peaks[43]. This finding impacts the specificity of the instrument because these peaks are seen in non-stressful or non-pain situations as well. As the majority of NICU patients are in incubators in which the temperature is continuously adjusted, and as they standardly are given oral sucrose before heel lancing, skin conductance measurements seem less suitable for the NICU population.

Electroencephalogram (EEG)

Painful stimuli in preterm infants elicit specific hemodynamic responses in the somatosensory cortex, caught on EEG, implying conscious sensory perception[38]. It may be difficult to distinguish increased cortical somatosensory activity from that related to motor activity, especially in the smallest infants. A study by Fabrizi et al was the first to map the maturation of tactile and nociceptive activity in the developing human brain from the extremely preterm stage (28 weeks) through full-term birth (>37 weeks). It was found that specific neural circuits necessary for discrimination between touch and nociception emerge from 35–37 weeks gestation in the human brain[44]. In a response to a painful stimulus, infants generally evoke parallel cortical and behavioral responses but pain may be processed at the cortical level without deflecting detectable behavioral changes. It is thus possible that a low score on a pain assessment instrument based

on behavioral indicators does not necessarily imply little or no pain[45]. Multi-modal pain assessment is needed, therefore, for example a behavioral pain assessment instrument combined with a newer technology-based instrument.

Near-Infrared Spectroscopy

Near-infrared spectroscopy (NIRS) works through the differential absorption of infrared light by hemoglobin and cytochrome aa3, with changes in hemoglobin absorption reflecting changes in cerebral blood flow. The hemodynamic changes caught by NIRS in the absence of visible behavioral changes in response to pain might support the idea that more conventional pain assessment instruments underestimate the pain response in infants[17]. Unfortunately NIRS also reacts on changes in blood pressure, drug treatment, respiratory changes and movement, which limits it clinical use as a pain assessment instrument. Still this technique might be interesting for research purposes. Evaluation of the frontal cortex oxygen consumption is measured with NIRS, and it is hard to see a relation with the projections of the nociceptive fibers in the specific "pain" areas of the brain. Further studies in which the oxygen consumption is measured at different places simultaneously are needed to determine the value of NIRS for pain assessment.

Multimodal pain assessment

Overall, little is known about threshold- and reference values, interfering factors and the applicability of these newer non-conventional markers for pain in (extreme) preterm infants. Furthermore, most have not been validated for pain assessment in neonates and the bedside utility is questionable at this moment. Still it could be worthwhile to explore their applicability as an adjunct to other such tools or to a validated pain scale. For example, multimodal pain assessment combining EEG, EMG with NIRS has been studied during heel lancing in 6 infants[46]. The combined measurements proved reliable and reproducible, but applicability in preterm and extreme preterm neonates needs to be elucidated. Besides, applying all these instruments simultaneously on the fragile skin of the extreme preterm infant is not only a technical but also a logistic challenge.

Stress hormones

Recently, salivary biomarkers such as salivary chromogranin and salivary amylase have been used to assess stress in adults[47,48] and older infants[49,50]. A study in healthy newborns enrolled 3-4 days after birth showed no change in salivary biomarkers before and after heel lancing and concluded that these biomarkers are not objective indices for assessing newborn pain[51]. Salivary and serum cortisol levels are determined to assess acute stress. Hair cortisol level is a possible biomarker for chronic stress in neonates, seeing that hospitalized term and preterm infants had significantly higher hair cortisol levels than healthy term infants (p=0.004). A subgroup analysis of term NICU infants showed a

statistically significant association between total number of ventilator days and hair cortisol levels. For every extra day on the ventilator, hair cortisol levels increased on average by 0.2 nmol/g ($p = 0.03$)[52]. The clinical relevance of this finding is limited as the need for ventilator support is determined by the infant's respiratory condition. Furthermore, it will take some time to receive test results of cortisol levels from the laboratory, which makes this method impractical for bedside real time pain assessment with subsequent acute therapeutic decisions. In addition we do not have the necessary knowledge at this moment to assure that stress hormone levels are really associated with repeated painful procedures.

Compliance with guidelines

Pain management by protocol has not always been the case. We know, for example, that some 30 years ago many neonates were not given analgesics during and after surgery[53]. The management of pain, agitation, and sedation had thus far been based on caregivers' subjective evaluations of the patient's condition. On the other hand, the many neonatal pain assessment instruments developed since then were not always effectively used in daily practice[54].

There is much to say for the idea that pain treatment should be guided by the pain scores. Treatment guidelines should therefore specify the pain scores that indicate the need to taper down or increase analgesic dosing. Achieving good compliance with new guidelines requires much more, however, than simply telling staff one day that we are going to work differently. Well planned implementation is needed, as well as a dedicated multidisciplinary pain team that keeps on propagating the importance of adequate pain management and assessment.

After implementing a pain management protocol in our NICU in 2005, in the year 2011 we prospectively recorded COMFORTneo scores and all prescribed analgesics and sedatives (chapter 3). We found that for 90% of patients fewer than the stipulated 3 assessments per day were done. Deindl et al reported their successful multilayer approach of developing and implementing a neonatal pain management and sedation protocol[55]. They concluded that the success was owed to medical staff education using interactive video-based tutorials, bedside teaching, questionnaires, regular assessments with defined responses, reassessments and feedback. From our experience we know that the electronic patient data management system is helpful in this respect, as it provides information about procedures performed, pain scores and subsequent administered analgesics. Still, we need to achieve better documentation of protocol violations and of reassessments performed after adjustment of treatment[56]

Long term consequences of untreated pain

To leave pain untreated is not an option from an ethical perspective and besides carries risks of short and long term sequelae. Studies in animals and humans have shown a significant risk of neurological impairment, as well as long term learning, cognitive and behavioral effects when neonatal pain was left untreated[57-59]. Furthermore, a study by Taddio et al found that pain scores for vaccination at the age of 4-6 months were highest for term neonates circumcised without pain treatment in the neonatal period compared with those for pretreated circumcised- and uncircumcised infants[60]. There is growing evidence that preterm infants whose clinical condition necessitates prolonged NICU stay undergo numerous painful procedures and not always receive analgesics[11]. As a consequence they may show altered brain development in terms of reduced maturation of white matter and subcortical grey matter shown on MRI scans at term equivalent age[61], and reduced brain size in the frontal and parietal regions[62]. Thus, we need to be aware that leaving painful interventions untreated can have far-reaching consequences for these neonates at later age.

Consequences of pharmacological treatment

Analgesic treatment of pain is also not without consequences: studies in rodents show accelerated neuronal apoptosis and other morphological changes in the developing brain after administration of commonly used opioids, benzodiazepines and of general anaesthetics[63-65]. Especially morphine administration in animals at neonatal age led to increased hypersensitivity[66] , impaired learning[67] and decreased cell division within the hippocampus, with possible neurobehavioral deficits in adulthood[68]. Other studies in rats have suggested, however, that morphine (at higher doses) may have a protective role as well[69,70]. Morphine can act as a modulator of cell proliferation and protect astrocytes against apoptosis[71]. In mice high doses of propofol and low doses of ketamine may provide a certain degree of neuroprotection but the underlying mechanism remains unclear[72]. Programmed cell death, apoptosis, has been described also in in vitro studies in human fetal brain cells exposed to morphine[73] .

Neonatal pain treatment

Non-pharmacological interventions

The Newborn Individualized Developmental Care and Assessment Program (NIDCAP) has become standard of care in many NICUs worldwide although a systematic review concluded that there is no evidence that NIDCAP improves long-term neurodevelopmental or short-term medical outcome[74]. NIDCAP embraces a range of interventions and behavioral changes, and levels of interventions have not been standardized, which

makes it difficult to study the effects. These interventions include skin-to-skin care, also during painful procedures[75], reduction of noise and light exposure, and providing containment of the infant by having procedures performed by two nurses. A positive effect on long-term medical outcome has been proven, as NIDCAP care was found associated with a better daily weight gain, a shorter hospitalization, and an increase in Bayley Scale of Infant Development scores at 9 months[74].The analgesic properties of sucrose to reduce procedural pain have also been questioned although it is still standard of care in many NICUs[76-78]. According to Slater et al[40], reduction in clinical observational scores should not be seen as pain relief as their data on EEG suggested that oral sucrose does not significantly affect activity in the neonatal brain and spinal cord nociceptive circuits. But the question remains if the lack of nociceptive-specific brain activity during a painful procedure for which patients are given sucrose reflects the absence of pain. Is it the nociception or the subsequent cortical pain response that leads to long term sequelae? New approaches to increase comfort are continually being introduced: for example, Mimo, a pillow system embedded with sensing and actuating functions providing comfort through mediation of a parent's physiological features (heartbeat) to the distressed neonate [79]. Again, the applicability of such approaches needs to be investigated as well as the practical use and possible effects on short term and long term outcomes for critically ill infants.

Pharmacological interventions

Opioids are the most commonly used drugs in the treatment of pain in adults and children. A follow-up study of 5-year-old children who as ventilated newborns participated in a morphine-placebo controlled trial, found no impairment of cognitive and behavioral development. Still it was found that morphine infusion in the first week of life [(10 mcg/kg/hour with extra median dosages of 3.0 mcg/kg/hour (IQR 1.30-6.8 mcg/kg/hour) open-label morphine on suspicion of pain or distress)] may negatively affect response inhibition, a domain of executive functions[80]. Studying these patients again at the age of 8-9 years demonstrated that the morphine given during the neonatal period had not harmed general functioning and may even have had a positive influence on executive functions at 8 to 9 years[81]. This positive result is in contrast with the results of a pilot study on preemptive morphine analgesia in preterm infants (a subset of subjects previously enrolled in the NEOPAIN trial) which reported a smaller head circumference, lower body weight, less social behavior and longer response latencies in early childhood in those children treated with morphine compared with the placebo group[82].

Paracetamol, a non-selective cyclooxygenase inhibitor, is a fairly old drug, known for its antipyretic and analgesic effect, and is sold over-the-counter worldwide. Although frequently used by people of all ages, this drug is also not without side effects. Hepato-

toxicity is described after intentional but also unintentional overdosing of paracetamol, but adverse effects are also described after proper dosing in children with myopathies[83]. The possible relationship between maternal use of paracetamol during pregnancy and wheezing or asthma in the offspring was first suggested by Shaheen et al[84]. So far, seven additional studies have been published on this subject but the evidence that any such association is causal is inconclusive. The Generation R study, a large cohort study involving 3184 pregnant women in the Netherlands, showed that intrauterine exposure to mild analgesics, primarily paracetamol, during the period in pregnancy when male sexual differentiation takes place, increases the risk of congenital cryptorchidism (odds ratio 2.12)[85]. In one animal study, paracetamol administration during neonatal brain development negatively affected cognitive function and altered the analgesic and anxiolytic response of these animals at adult age[86]. These findings are reasons for caution and suggest that simply extrapolating PK/PD data of paracetamol in older children and adults to neonates is not the way to go. Safety studies on paracetamol in fetal and preterm infants and even in term infants are therefore needed, although they are difficult to perform in these age groups. If the expected incidences of certain side effects are expected to be low, a large sample size is needed which is hard to achieve. Furthermore, many environmental factors might contribute to altered cognitive function on adult age after drug administration in the neonatal period of life[87].
Still, cautious interpretation and extrapolation of the results of animal studies towards humans is warranted. Not only are there important differences in pharmacokinetics, pharmacodynamics and pharmacogenetics between humans and animals, but also the used dosages of analgesics in human and animal studies are different.

Paracetamol and pain

Considering the limited PK/PD data available for adequate and safe dosing guidelines, pharmacological pain treatment for neonates is still in its infancy. Furthermore, in neonatology the use of unlicensed or off-label medications is quite common: these are given to up to 93% of neonates treated at NICUs and include analgesics and sedatives[88]. We are the first to provide single PK data of intravenously administered paracetamol and all its metabolites in very preterm infants (chapter 5). Three different doses of intravenous paracetamol were studied (10-, 15- and 20 mg/kg), administered as a single dose before placement of a central venous catheter, which is a painful procedure (chapter 6).

PK/PD data in older infants showed that an effect compartment concentration of >9 mg/l paracetamol will achieve the target effect[89]. Our study showed median plasma peak concentrations of paracetamol > 9 mg/l in 95% of all subjects across the three paracetamol treatment groups ($p < 0.001$)(chapter 5). Our PD data, expressed as PIPP- and COMFORTneo scores, showed no beneficial analgesic effect of the three different

doses of paracetamol over sucrose (chapter 6). What does this mean? Is paracetamol not effective in relieving acute pain or was the effect compartment concentration of >9 mg/l too low for very preterm neonates to reach the target effect, which was the case in older neonates? Alternatively, as also probably holds true for the use of morphine, we may have been measuring in the wrong body compartment and should have investigated the effect directly at receptor level – which probably is the way to go for the use of morphine. We do not know yet if this also holds true for the use of paracetamol because its mechanism of action is not fully understood. Endogenous binding sites or receptors for paracetamol are unknown, but it has been suggested that paracetamol stimulates activity of descending 5-HT pathways that inhibit nociceptive signal transmission in the spinal cord[90].

A maintenance dose of paracetamol – as a single analgesic – is probably more effective in relieving prolonged pain than acute pain. Paracetamol as adjuvant therapy in combination with other analgesics is another field to explore. In term infants after major non-cardiac surgery, postoperative use of intermittent intravenous paracetamol resulted in a lower cumulative morphine dose over 48 hours than did continuous morphine administration. [91] This observation is worth further investigation, as dose reduction of opioids is quite relevant in view of possible adverse effects of opioids administration.

Paracetamol and PDA

The association of paracetamol exposure and closure of the patent ductus arteriosus (PDA), a serendipity type of observation by Hammermann and colleagues[92], has led to widespread use of high doses of paracetamol in observational and intervention studies on PDA closure, without any dose finding study with pharmacokinetic data as evidence to support this finding. Placebo controlled trials are lacking.

More than 10 observational and retrospective studies on this subject have been published[92-100] (see chapter 7). In addition, two prospective randomized controlled trials compared oral paracetamol with ibuprofen in relation to PDA closure – both showing a slightly favorable effect of paracetamol (PDA closure rate 81.2% versus 78.8% for paracetamol and ibuprofen, respectively[101] and 97.5% versus 95%[102]). Our own study (chapter 7) could not confirm these high closure rates with paracetamol and the effect of paracetamol after ibuprofen failure treatment was even non-existent. This observation raises the question in what way paracetamol might add to PDA closure if ibuprofen and indomethacin, which are more potent non-selective cyclo-oxygenase (COX) inhibitors, are also not always successful? It has been suggested that paracetamol, like ibuprofen, exerts inhibitory activity on prostaglandin synthesis. There are no data on the effect of paracetamol and ibuprofen on prostaglandin synthesis in young infants and neonates

and the scarce data in older humans are often derived from orthodontic procedures as the classical test situation[103]. From these available data it was concluded that ibuprofen inhibits PGE2 synthesis more than does paracetamol. One study in term mice suggested a dose/'effect' (i.e. *in vitro* constriction), but paracetamol doses were up to 100 mg/l (10x higher than commonly used in humans), and constriction was more pronounced in the more mature ductus arteriosus samples, suggesting an age related effect [99]. Again, the pharmacokinetics of paracetamol in relation to PDA closure in extreme preterm neonates has not been comprehensively studied and data on target or effect concentrations of paracetamol needed for PDA closure are missing. The results of our study can partly be explained by the fact that we included only very low birth weight infants with a gestational age < 28 weeks. Studies from other groups included older infants in whom spontaneous PDA closure cannot be ruled out (chapter 7).

It might be worthwhile to also focus on dose regimens and route of administration of available drugs for PDA treatment. A population PK/PD study by Hirt et al in 2008 showed already that ibuprofen doses used worldwide are too low and should be adjusted to postnatal ages[104]. Furthermore, in that study oral administration of ibuprofen was found as effective as intravenous administration.

What have we learned so far?

- Neonates of all ages can feel pain, although the more preterm infants may show less explicit pain behavior[105].
- Neonates admitted to a NICU undergo many painful procedures per day (chapter 4). By introducing a pain management protocol integrated with a pain treatment decision-tree, we were able to reduce the number of painful procedures from 14 to 11.2 (SD 5.7) (chapter 4).
- Analgesic treatment decreased even more over the last decade when studies showed that routine administration of opioids to ventilated newborns was not beneficial. Further concerns arose with the adverse effects of certain analgesics and anesthetics on brain development in animals. Sinner et al reviewed the current literature of animal experiments, and nicely put together the possible transfer of experimental data to humans and the current clinical data[106]: the timing of anesthetic exposure as well as the frequency, duration and dose play a key role in the development of toxicity.
- Multiple pain assessment instruments have been developed, but a gold standard instrument useful for neonates of all ages and all types of pain (acute, prolonged or recurrent pain) is still lacking. We tested the COMFORTneo scale, a modified version of the COMFORT-behavioral scale tailored for use in premature neonates for its

psychometric qualities and its usefulness in measuring prolonged pain (chapter 2). Pain assessment is inseparable from pain treatment.
- Introducing new guidelines does not imply that medical and nursing staff will fully comply with these guidelines: we learned that documentation of protocol violations and reassessments after adjustment of analgesic/sedative treatment are points of improvement for the future (chapter 3).
- Intravenously administered paracetamol has become available in the Netherlands and is suggested to have fewer side effects than the predominantly used opioids so far. Paracetamol is metabolized in the liver mostly into APAP-sulphate, to a lesser extent into APAP-glucuronide due to an immature glucuronide conjugation system. Only a small portion is oxidized into the toxic metabolites (chapter 5).
- Intravenously administered paracetamol is not effective in relieving acute pain, for example, pain associated with placement of a central venous catheter (chapter 6).
- The use of paracetamol to achieve PDA closure is debatable; administration of paracetamol after incomplete or complete ibuprofen treatment is useless (chapter 7).

Future perspectives

In terms of expanding our knowledge on neonatal pain management, assessment and treatment we have come a long way. Still numerous questions remain unanswered. Findings from research settings combined with clinical experience should be incorporated into new pain management guidelines used in the daily care of critically ill neonates. Repeated monitoring of compliance with the guidelines is potentially more important. Effectiveness of these new guidelines should be evaluated and adjustments should be made, if necessary, to prevent that nurses develop pain assessment 'fatigue'.

Instead of developing more and newer pain assessment instruments it is worthwhile to further evaluate the existing instruments that have been validated for only a specific painful procedure or patient group. For example, specificity and sensitivity of the COMFORTneo scale in the extreme preterm infants should also be tested during sleep and skin-to-skin care. Exploring the applicability of novel methods, such as NIRS and biomarkers like hair cortisol, should also be included in future research on stress in neonates of all gestational ages. Note, however, that only studies in which relevant differences in stress levels are expected will be of any help.

In this thesis we found that paracetamol is not suitable to relieve acute pain. The role of fentanyl or remifentanil herein could be a field for future research. Paracetamol as an adjuvant to other analgesics is also a field to explore. A possible reduction of opioid needs post- operatively by adding paracetamol or replacing morphine with paracetamol

is certainly worth investigating. For example, in preterm neonates after surgical ligation or surgery for NEC or focal intestinal perforation. Future research should also take safety measures into account: data on the effects of high doses or prolonged use of paracetamol treatment on the short term (considering liver and kidney function) and on the long term (neurodevelopment) are still scarce.
We should not ignore the lack of PK/PD data as well as safety aspects and blindly copy the dose regimen of a newer drug established in other studies that only focused on positive effects. The use of paracetamol in PDA treatment is a good example of this. Future research should, at least include a randomized controlled trial in a population of patients with different gestational ages comparing paracetamol (15 mg/kg/6 hour) with placebo to rule out cases of spontaneous PDA closure.
The next step logical step would be a PK/PD study investigating the relationship between the dose-concentration-effect of paracetamol on PDA closure. Large samples are needed to investigate the safety of the used doses of paracetamol, but it is worth attempting so as to deal with this issue for once and for all.
PK modeling of available data on PDA treatment with paracetamol in different gestational age groups can lead to different dosing recommendations per age group. A proof of principle study with dose recommendations of paracetamol for PDA is another option for future research.

Our search for effective and safe analgesics suitable for even the extreme preterm infants will continue as the use of paracetamol did not answer all our research questions. Both the role of paracetamol in multimodal analgesic treatment and the indications for maintenance paracetamol treatment need to be elucidated. Meanwhile we should also focus on improving pain assessment. Instead of developing new instruments, the area of technology-based pain assessment instruments probably in combination with a validated pain assessment instrument is a promising field to explore. These new instruments should be ideally non-invasive, easy to use and applicable for infants of all gestational ages. Although critically ill infants need to undergo painful procedures in the context of intensive care, and pain prevention obviously is preferable, with proper pain assessment - and consequently adequate pain treatment - we can contribute to their survival with bright prospects for their future.

References

1. Anand KJ, Hickey PR. Pain and its effects in the human neonate and fetus. *N Engl J Med.* Nov 19 1987;317(21):1321-1329.
2. Doyle LW, Victorian Infant Collaborative Study G. Neonatal intensive care at borderline viability--is it worth it? *Early Hum Dev.* Nov 2004;80(2):103-113.
3. Batton DG, DeWitte DB, Pryce CJ. One hundred consecutive infants born at 23 weeks and resuscitated. *Am J Perinatol.* Apr 2011;28(4):299-304.
4. Anand KJ, Hall RW, Desai N, et al. Effects of morphine analgesia in ventilated preterm neonates: primary outcomes from the NEOPAIN randomised trial. *Lancet.* May 22 2004;363(9422):1673-1682.
5. Simons SH, van Dijk M, van Lingen RA, et al. Routine morphine infusion in preterm newborns who received ventilatory support: a randomized controlled trial. *JAMA.* 2003;290(18):2419-2427.
6. Bellu R, de Waal KA, Zanini R. Opioids for neonates receiving mechanical ventilation. *Cochrane Database Syst Rev.* 2005(1):CD004212.
7. Ng E, Taddio A, Ohlsson A. Intravenous midazolam infusion for sedation of infants in the neonatal intensive care unit. *Cochrane database of systematic reviews (Online).* 2012;6:CD002052.
8. Anand KJ, Barton BA, McIntosh N, et al. Analgesia and sedation in preterm neonates who require ventilatory support: results from the NOPAIN trial. Neonatal Outcome and Prolonged Analgesia in Neonates. *Arch Pediatr Adolesc Med.* Apr 1999;153(4):331-338.
9. Gascon E, Klauser P, Kiss JZ, Vutskits L. Potentially toxic effects of anaesthetics on the developing central nervous system. *Eur J Anaesthesiol.* Mar 2007;24(3):213-224.
10. Roofthooft DW, Simons SH, Anand KJ, Tibboel D, van Dijk M. Eight years later, are we still hurting newborn infants? *Neonatology.* 2014;105(3):218-226.
11. Carbajal R, Eriksson M, Courtois E, et al. O-103 Sedation And Analgesia For Neonates In Nicus Across Europe: The Europain Survey. *Archives of Disease in Childhood.* October 1, 2014 2014;99(Suppl 2):A64.
12. Lee SJ, Ralston HJ, Drey EA, Partridge JC, Rosen MA. Fetal pain: a systematic multidisciplinary review of the evidence. *JAMA.* Aug 24 2005;294(8):947-954.
13. Humphrey T. The development of human fetal activity and its relation to postnatal behavior. *Adv Child Dev Behav.* 1970;5:1-57.
14. Kostovic I, Judas M. Transient patterns of cortical lamination during prenatal life: do they have implications for treatment? *Neurosci Biobehav Rev.* 2007;31(8):1157-1168.
15. Dubin AE, Patapoutian A. Nociceptors: the sensors of the pain pathway. *J Clin Invest.* Nov 2010;120(11):3760-3772.
16. Fitzgerald M, Beggs S. The neurobiology of pain: developmental aspects. *Neuroscientist.* Jun 2001;7(3):246-257.
17. Slater R, Worley A, Fabrizi L, et al. Evoked potentials generated by noxious stimulation in the human infant brain. *Eur J Pain.* Mar 2010;14(3):321-326.
18. Gibbins S, Stevens B, McGrath PJ, et al. Comparison of pain responses in infants of different gestational ages. *Neonatology.* 2008;93(1):10-18.
19. Johnston CC, Stevens BJ. Experience in a neonatal intensive care unit affects pain response. *Pediatrics.* Nov 1996;98(5):925-930.
20. Carbajal R, Rousset A, Danan C, et al. Epidemiology and treatment of painful procedures in neonates in intensive care units. *JAMA.* Jul 2 2008;300(1):60-70.

21. Larsson BA, Tannfeldt G, Lagercrantz H, Olsson GL. Venipuncture is more effective and less painful than heel lancing for blood tests in neonates. *Pediatrics.* May 1998;101(5):882-886.

22. van Lingen RA, Brand PL. [Assessment and treatment of pain in children. Advice for the clinic] Meting en behandeling van pijn bij kinderen. Advies voor de praktijk. *Ned Tijdschr Geneeskd.* Mar 21 2009;153(12):532-534.

23. Weissman A, Aranovitch M, Blazer S, Zimmer EZ. Heel-lancing in newborns: behavioral and spectral analysis assessment of pain control methods. *Pediatrics.* Nov 2009;124(5):e921-926.

24. Carbajal R, Eriksson M, Courtois E, et al. O-113 Pain Assessment In Ventilated And Non-ventilated Neonates In Nicus Across Europe: European Pain Audit In Neonates (europain Survey). *Archives of Disease in Childhood.* October 1, 2014 2014;99(Suppl 2):A68.

25. Lagercrantz H. The emergence of consciousness: Science and ethics. *Semin Fetal Neonatal Med.* Oct 2014;19(5):300-305.

26. Johnston CC, Stevens BJ, Franck LS, Jack A, Stremler R, Platt R. Factors explaining lack of response to heel stick in preterm newborns. *J Obstet Gynecol Neonatal Nurs.* 1999;28(6):587-594.

27. Hummel P, Lawlor-Klean P, Weiss MG. Validity and reliability of the N-PASS assessment tool with acute pain. *J Perinatol.* Jul 2010;30(7):474-478.

28. Hummel P, Puchalski M, Creech SD, Weiss MG. Clinical reliability and validity of the N-PASS: neonatal pain, agitation and sedation scale with prolonged pain. *J Perinatol.* Jan 2008;28(1):55-60.

29. Stevens BJ, Gibbins S, Yamada J, et al. The premature infant pain profile-revised (PIPP-R): initial validation and feasibility. *Clin J Pain.* Mar 2014;30(3):238-243.

30. Ambuel B, Hamlett KW, Marx CM, Blumer JL. Assessing distress in pediatric intensive care environments: the COMFORT scale. *J Pediatr Psychol.* Feb 1992;17(1):95-109.

31. van Dijk M, Roofthooft DW, Anand KJ, et al. Taking up the challenge of measuring prolonged pain in (premature) neonates: the COMFORTneo scale seems promising. *Clin J Pain.* Sep 2009;25(7):607-616.

32. Lindh V, Wiklund U, Hakansson S. Heel lancing in term new-born infants: an evaluation of pain by frequency domain analysis of heart rate variability. *Pain.* Mar 1999;80(1-2):143-148.

33. Lindh V, Wiklund U, Sandman PO, Hakansson S. Assessment of acute pain in preterm infants by evaluation of facial expression and frequency domain analysis of heart rate variability. *Early Hum Dev.* Apr 25 1997;48(1-2):131-142.

34. Oberlander T, Saul JP. Methodological considerations for the use of heart rate variability as a measure of pain reactivity in vulnerable infants. *Clin Perinatol.* Sep 2002;29(3):427-443.

35. Storm H. "Pain monitoring in anesthetized children: first assessment of skin conductance and analgesia-nociception index at different infusion rates of remifentanil", recommended preset values for the skin conductance equipment was not used. *Paediatr Anaesth.* Aug 2013;23(8):761-763.

36. Gunther AC, Bottai M, Schandl AR, Storm H, Rossi P, Sackey PV. Palmar skin conductance variability and the relation to stimulation, pain and the motor activity assessment scale in intensive care unit patients. *Crit Care.* 2013;17(2):R51.

37. de Jesus JA, Tristao RM, Storm H, da Rocha AF, Campos D, Jr. Heart rate, oxygen saturation, and skin conductance: a comparison study of acute pain in Brazilian newborns. *Conf Proc IEEE Eng Med Biol Soc.* 2011;2011:1875-1879.

38. Bartocci M, Bergqvist LL, Lagercrantz H, Anand KJ. Pain activates cortical areas in the preterm newborn brain. *Pain.* May 2006;122(1-2):109-117.

39. Maxwell LG, Malavolta CP, Fraga MV. Assessment of pain in the neonate. *Clin Perinatol.* Sep 2013;40(3):457-469.

40. Slater R, Cornelissen L, Fabrizi L, et al. Oral sucrose as an analgesic drug for procedural pain in newborn infants: a randomised controlled trial. *Lancet.* Oct 9 2010;376(9748):1225-1232.

41. Storm H. Skin conductance and the stress response from heel stick in preterm infants. *Arch Dis Child Fetal Neonatal Ed.* Sep 2000;83(2):F143-147.

42. Valkenburg AJ, Niehof SP, van Dijk M, Verhaar EJ, Tibboel D. Skin conductance peaks could result from changes in vital parameters unrelated to pain. *Pediatr Res.* Apr 2012;71(4 Pt 1):375-379.

43. Munsters J, Wallstrom L, Agren J, Norsted T, Sindelar R. Skin conductance measurements as pain assessment in newborn infants born at 22-27 weeks gestational age at different postnatal age. *Early Hum Dev.* Jan 2012;88(1):21-26.

44. Fabrizi L, Slater R, Worley A, et al. A shift in sensory processing that enables the developing human brain to discriminate touch from pain. *Curr Biol.* Sep 27 2011;21(18):1552-1558.

45. Slater R, Cantarella A, Franck L, Meek J, Fitzgerald M. How well do clinical pain assessment tools reflect pain in infants? *PLoS Med.* Jun 24 2008;5(6):e129.

46. Worley A, Fabrizi L, Boyd S, Slater R. Multi-modal pain measurements in infants. *J Neurosci Methods.* Apr 15 2012;205(2):252-257.

47. Bakke M, Tuxen A, Thomsen CE, Bardow A, Alkjaer T, Jensen BR. Salivary cortisol level, salivary flow rate, and masticatory muscle activity in response to acute mental stress: a comparison between aged and young women. *Gerontology.* Nov-Dec 2004;50(6):383-392.

48. Vining RF, McGinley RA, Maksvytis JJ, Ho KY. Salivary cortisol: a better measure of adrenal cortical function than serum cortisol. *Ann Clin Biochem.* Nov 1983;20 (Pt 6):329-335.

49. Vanaelst B, Huybrechts I, Bammann K, et al. Intercorrelations between serum, salivary, and hair cortisol and child-reported estimates of stress in elementary school girls. *Psychophysiology.* Aug 2012;49(8):1072-1081.

50. Michels N, Sioen I, De Vriendt T, Huybrechts I, Vanaelst B, De Henauw S. Children's morning and evening salivary cortisol: pattern, instruction compliance and sampling confounders. *Horm Res Paediatr.* 2012;77(1):27-35.

51. Shibata M, Kawai M, Matsukura T, Heike T, Okanoya K, Myowa-Yamakoshi M. Salivary biomarkers are not suitable for pain assessment in newborns. *Early Hum Dev.* Jul 2013;89(7):503-506.

52. Yamada J, Stevens B, de Silva N, et al. Hair cortisol as a potential biologic marker of chronic stress in hospitalized neonates. *Neonatology.* 2007;92(1):42-49.

53. Purcell-Jones G, Dormon F, Sumner E. Paediatric anaesthetists' perceptions of neonatal and infant pain. *Pain.* May 1988;33(2):181-187.

54. Franck LS, Bruce E. Putting pain assessment into practice: why is it so painful? *Pain Res Manag.* Jan-Feb 2009;14(1):13-20.

55. Deindl P, Unterasinger L, Kappler G, et al. Successful implementation of a neonatal pain and sedation protocol at 2 NICUs. *Pediatrics.* Jul 2013;132(1):e211-218.

56. Aukes DI, Roofthooft DW, Simons SH, Tibboe D, van Dijk M. Pain Management in Neonatal Intensive Care: Evaluation of the Compliance with Guidelines. *Clin J Pain.* Nov 3 2014.

57. Beggs S, Salter MW. Stereological and somatotopic analysis of the spinal microglial response to peripheral nerve injury. *Brain Behav Immun.* Jul 2007;21(5):624-633.

58. Parry G, Tucker J, Tarnow-Mordi W, Group UKNSSC. CRIB II: an update of the clinical risk index for babies score. *Lancet.* May 24 2003;361(9371):1789-1791.

59. Weisman SJ, Bernstein B, Schechter NL. Consequences of inadequate analgesia during painful procedures in children. *Arch Pediatr Adolesc Med.* Feb 1998;152(2):147-149.

60. Taddio A, Katz J, Ilersich AL, Koren G. Effect of neonatal circumcision on pain response during subsequent routine vaccination. *Lancet.* Mar 1 1997;349(9052):599-603.

61. Brummelte S, Grunau RE, Chau V, et al. Procedural pain and brain development in premature newborns. *Ann Neurol.* Mar 2012;71(3):385-396.

62. Smith GC, Gutovich J, Smyser C, et al. Neonatal intensive care unit stress is associated with brain development in preterm infants. *Ann Neurol.* Oct 2011;70(4):541-549.

63. Jevtovic-Todorovic V, Hartman RE, Izumi Y, et al. Early exposure to common anesthetic agents causes widespread neurodegeneration in the developing rat brain and persistent learning deficits. *J Neurosci.* Feb 1 2003;23(3):876-882.

64. Fredriksson A, Ponten E, Gordh T, Eriksson P. Neonatal exposure to a combination of N-methyl-D-aspartate and gamma-aminobutyric acid type A receptor anesthetic agents potentiates apoptotic neurodegeneration and persistent behavioral deficits. *Anesthesiology.* Sep 2007;107(3):427-436.

65. Duhrsen L, Simons SH, Dzietko M, et al. Effects of repetitive exposure to pain and morphine treatment on the neonatal rat brain. *Neonatology.* 2013;103(1):35-43.

66. Zhang GH, Sweitzer SM. Neonatal morphine enhances nociception and decreases analgesia in young rats. *Brain Res.* Mar 14 2008;1199:82-90.

67. McPherson RJ, Gleason C, Mascher-Denen M, Chan M, Kellert B, Juul SE. A new model of neonatal stress which produces lasting neurobehavioral effects in adult rats. *Neonatology.* 2007;92(1):33-41.

68. Traudt CM, Tkac I, Ennis KM, Sutton LM, Mammel DM, Rao R. Postnatal morphine administration alters hippocampal development in rats. *J Neurosci Res.* Jan 2012;90(1):307-314.

69. Bryant RA, Creamer M, O'Donnell M, Silove D, McFarlane AC. A study of the protective function of acute morphine administration on subsequent posttraumatic stress disorder. *Biol Psychiatry.* Mar 1 2009;65(5):438-440.

70. Hsiao PN, Chang MC, Cheng WF, et al. Morphine induces apoptosis of human endothelial cells through nitric oxide and reactive oxygen species pathways. *Toxicology.* Feb 4 2009;256(1-2):83-91.

71. Kim MS, Cheong YP, So HS, et al. Protective effects of morphine in peroxynitrite-induced apoptosis of primary rat neonatal astrocytes: potential involvement of G protein and phosphatidylinositol 3-kinase (PI3 kinase). *Biochem Pharmacol.* Apr 1 2001;61(7):779-786.

72. Shu L, Li T, Han S, et al. Inhibition of neuron-specific CREB dephosphorylation is involved in propofol and ketamine-induced neuroprotection against cerebral ischemic injuries of mice. *Neurochem Res.* Jan 2012;37(1):49-58.

73. Hu S, Sheng WS, Lokensgard JR, Peterson PK. Morphine induces apoptosis of human microglia and neurons. *Neuropharmacology.* May 2002;42(6):829-836.

74. Ohlsson A, Jacobs SE. NIDCAP: a systematic review and meta-analyses of randomized controlled trials. *Pediatrics.* Mar 2013;131(3):e881-893.

75. Johnston C, Campbell-Yeo M, Fernandes A, Inglis D, Streiner D, Zee R. Skin-to-skin care for procedural pain in neonates. *Cochrane Database Syst Rev.* 2014;1:CD008435.

76. Wilkinson DJ, Savulescu J, Slater R. Sugaring the pill: ethics and uncertainties in the use of sucrose for newborn infants. *Arch Pediatr Adolesc Med.* Jul 1 2012;166(7):629-633.

77. Lasky RE, van Drongelen W. Is sucrose an effective analgesic for newborn babies? *Lancet.* Oct 9 2010;376(9748):1201-1203.

78. Heaton PA, Fernando AM, Herd D. Oral sucrose for procedural pain in infants. *Lancet.* Jan 1 2011;377(9759):25; author reply 27-28.

79. Chen W, Bambang Oetomo S, Tetteroo D, et al. Mimo Pillow - an Intelligent Cushion Designed with Maternal Heart Beat Vibrations for Comforting Newborn Infants. *IEEE J Biomed Health Inform.* Aug 18 2014.

80. de Graaf J, van Lingen RA, Simons SH, et al. Long-term effects of routine morphine infusion in mechanically ventilated neonates on children's functioning: five-year follow-up of a randomized controlled trial. *Pain.* Jun 2011;152(6):1391-1397.

81. de Graaf J, van Lingen RA, Valkenburg AJ, et al. Does neonatal morphine use affect neuropsychological outcomes at 8 to 9 years of age? *Pain.* Mar 2013;154(3):449-458.

82. Ferguson SA, Ward WL, Paule MG, Hall RW, Anand KJ. A pilot study of preemptive morphine analgesia in preterm neonates: effects on head circumference, social behavior, and response latencies in early childhood. *Neurotoxicol Teratol.* Jan-Feb 2012;34(1):47-55.

83. Ceelie I, James LP, Gijsen V, et al. Acute liver failure after recommended doses of acetaminophen in patients with myopathies. *Crit Care Med.* Apr 2011;39(4):678-682.

84. Shaheen SO, Newson RB, Sherriff A, et al. Paracetamol use in pregnancy and wheezing in early childhood. *Thorax.* Nov 2002;57(11):958-963.

85. Snijder CA, Kortenkamp A, Steegers EA, et al. Intrauterine exposure to mild analgesics during pregnancy and the occurrence of cryptorchidism and hypospadia in the offspring: the Generation R Study. *Hum Reprod.* Apr 2012;27(4):1191-1201.

86. Viberg H, Eriksson P, Gordh T, Fredriksson A. Paracetamol (acetaminophen) administration during neonatal brain development affects cognitive function and alters its analgesic and anxiolytic response in adult male mice. *Toxicol Sci.* Mar 2014;138(1):139-147.

87. Naranjo CA, Busto U, Sellers EM, et al. A method for estimating the probability of adverse drug reactions. *Clin Pharmacol Ther.* Aug 1981;30(2):239-245.

88. Flint R, Simons S, Burger D, de Groot R, Reiss I, Tibboel D. O-102 Analyses Of Current Unlicensed And Off-label For Age Drug Prescriptions At A Neonatal Intensive Care Unit. *Archives of Disease in Childhood.* October 1, 2014 2014;99(Suppl 2):A63.

89. Wang C, Allegaert K, Tibboel D, et al. Population pharmacokinetics of paracetamol across the human age-range from (pre)term neonates, infants, children to adults. *J Clin Pharmacol.* Dec 30 2013.

90. Bonnefont J, Courade JP, Alloui A, Eschalier A. [Antinociceptive mechanism of action of paracetamol] Mecanisme de l'action antinociceptive du paracetamol. *Drugs.* 2003;63 Spec No 2:1-4.

91. Ceelie I, de Wildt SN, van Dijk M, et al. Effect of intravenous paracetamol on postoperative morphine requirements in neonates and infants undergoing major noncardiac surgery: a randomized controlled trial. *JAMA.* Jan 9 2013;309(2):149-154.

92. Hammerman C, Bin-Nun A, Markovitch E, Schimmel MS, Kaplan M, Fink D. Ductal closure with paracetamol: a surprising new approach to patent ductus arteriosus treatment. *Pediatrics.* Dec 2011;128(6):e1618-1621.

93. Hammerman C, Bin-Nun A, Kaplan M. Managing the patent ductus arteriosus in the premature neonate: a new look at what we thought we knew. *Semin Perinatol.* Apr 2012;36(2):130-138.

94. Oncel MY, Yurttutan S, Degirmencioglu H, et al. Intravenous paracetamol treatment in the management of patent ductus arteriosus in extremely low birth weight infants. *Neonatology.* 2013;103(3):166-169.

95. Oncel MY, Yurttutan S, Uras N, et al. An alternative drug (paracetamol) in the management of patent ductus arteriosus in ibuprofen-resistant or contraindicated preterm infants. *Arch Dis Child Fetal Neonatal Ed.* Jan 2013;98(1):F94.

96. Sinha R, Negi V, Dalal SS. An Interesting Observation of PDA Closure with Oral Paracetamol in Preterm Neonates. *J Clin Neonatol.* Jan 2013;2(1):30-32.

97. Yurttutan S, Oncel MY, Arayici S, et al. A different first-choice drug in the medical management of patent ductus arteriosus: oral paracetamol. *J Matern Fetal Neonatal Med.* May 2013;26(8):825-827.

98. Alan S, Karadeniz C, Okulu E, et al. Management of patent ductus arteriosus in preterm infants: clinical judgment might be a fair option. *J Matern Fetal Neonatal Med.* Dec 2013;26(18):1850-1854.

99. El-Khuffash A, Jain A, Corcoran D, et al. Efficacy of paracetamol on patent ductus arteriosus closure may be dose dependent: evidence from human and murine studies. *Pediatr Res.* Sep 2014;76(3):238-244.

100. El-Khuffash A, James AT, Cleary A, Semberova J, Franklin O, Miletin J. Late medical therapy of patent ductus arteriosus using intravenous paracetamol. *Arch Dis Child Fetal Neonatal Ed.* Feb 4 2015.

101. Dang D, Wang D, Zhang C, Zhou W, Zhou Q, Wu H. Comparison of Oral Paracetamol versus Ibuprofen in Premature Infants with Patent Ductus Arteriosus: A Randomized Controlled Trial. *PLoS One.* 2013;8(11):e77888.

102. Oncel MY, Yurttutan S, Erdeve O, et al. Oral paracetamol versus oral ibuprofen in the management of patent ductus arteriosus in preterm infants: a randomized controlled trial. *J Pediatr.* Mar 2014;164(3):510-514 e511.

103. Shetty N, Patil AK, Ganeshkar SV, Hegde S. Comparison of the effects of ibuprofen and acetaminophen on PGE2 levels in the GCF during orthodontic tooth movement: a human study. *Prog Orthod.* 2013;14:6.

104. Hirt D, Van Overmeire B, Treluyer JM, et al. An optimized ibuprofen dosing scheme for preterm neonates with patent ductus arteriosus, based on a population pharmacokinetic and pharmacodynamic study. *Br J Clin Pharmacol.* May 2008;65(5):629-636.

105. Stevens B, McGrath P, Gibbins S, et al. Determining behavioural and physiological responses to pain in infants at risk for neurological impairment. *Pain.* Jan 2007;127(1-2):94-102.

106. Sinner B, Becke K, Engelhard K. General anaesthetics and the developing brain: an overview. *Anaesthesia.* Sep 2014;69(9):1009-1022.

Chapter 9

Summary

"Pain is inevitable; suffering is optional". This phrase by Haruki Murakami summarizes what we have learned in the past three decades where far-reaching changes took place in pain perception and pain management of newborns. For example, the misconception that preterm infants cannot feel pain due to an immature central nervous system was corrected. Furthermore, it was demonstrated that untreated pain can have acute, short term but also long-lasting physiologic and neurodevelopmental negative outcomes. Advances in technology made it possible to treat and keep the very ill and extremely preterm infants alive. They have to endure numerous painful procedures on their way to survival, however, and adequate and effective pain management is therefore an indispensable part of their treatment.

Research in the past years led to profound interest in improving and optimizing pain assessment, prevention and treatment. This thesis presents various facets of pain assessment and management in newborn infants and of our search for effective and safe analgesics, suitable even for extreme preterm infants. This search focused on paracetamol, which worldwide has various applications including the treatment of fever and control of mild-to moderate pain as a single agent or as part of multimodal analgesia.

Pain should be considered the fifth vital sign according to the American Pain Society and proper pain assessment tools are indispensable for adequate dosing of analgesics in neonates. A wide selection of multidimensional pain measurement instruments is available, many of which combine behavioral items (facial expression, movements) with physiological parameters like change in heart rate and oxygen saturation. The majority of these instruments target acute procedural pain whereas prolonged pain, such as that induced by mechanical ventilation, is much more difficult to assess. In a clinical observational study described in **Chapter 2**, we tested a modified version of the COMFORT-behavioral scale, called the COMFORTneo scale, for its psychometric qualities in the NICU setting. Nurses assessed patients with the COMFORTneo scale and Numeric Rating Scales (NRS) for pain and distress. The COMFORTneo scale showed good interrater reliability (median weighted Cohen κ 0.79). COMFORTneo cut off scores of 14 or higher had good sensitivity (r=0.81) and specificity (r=0.90) using NRS-pain and NRS-distress scores of 4 or higher as criterion. No major differences were found in cut-off values for low birth weight, small for gestational age, neurological impairment risk levels or gender.

In 2005 a pain management protocol was implemented in our NICU including individual pain assessments and pain treatment guidelines with an accompanying decision tree for treatment. The pain management protocol demanded that nurses assess pain with the COMFORTneo scale in all patients three times every 24 hour (once every 8-hour shift). Extra COMFORTneo assessments were required in case of suspected pain or distress, after

an acute painful procedure, in case of suspected oversedation and in case of administration of analgesics and/or sedatives for five days or longer. **Chapter 3** describes to what extent the medical and nursing staff complied with this pain protocol in calendar year 2011. The majority of pain scores (86%) suggested that patients were comfortable, with scores between 9 and 14. The requirement of pain assessments three times daily was not always complied with. Compliance with pain assessment and treatment was far from perfect generally. In cases of high pain scores (>=14), the required reassessment within 60 minutes took place in 31% of cases and in 40% treatment was started or adjusted. In cases of low pain scores (<=8) during treatment, 13% of the 457 assessments were reassessed within 120 minutes and in 17% a dose reduction was performed. There is room for improvement with respect to reassessments after adjustment of analgesic/sedative treatment. Lastly we recommended justified and individualized adaptation of pain protocols to increase compliance and get rid of useless and time-consuming procedures not resulting in adjustment of treatment. Tailoring the number of pain assessments to the individual patient could also contribute to better compliance.

In a prospective observational study reported in **Chapter 4**, we counted the total number of notably painful procedures 175 neonates admitted to our NICU were subjected to in the first two weeks of life. We also registered the number of attempts needed for certain painful procedures and quantified analgesic therapy used in these patients. From a study performed in 2001 it appeared that in our NICU neonates underwent a mean of 14 painful procedures per day and that pharmacological analgesic therapy was given to no more than 60.3% of neonates per study day. After this study we introduced sucrose and NIDCAP on our ward, placing a focus on non-pharmacological pain management. New pain guidelines and a decision tree for pharmacological treatment were implemented at the same time. This new study brought to light that neonates still had to endure a mean number of 11 painful procedures per day in the first two weeks of life and that no more than 36.6% received analgesics, most likely because more non-pharmacological pain- or stress reducing strategies like NIDCAP and sucrose were applied. As further reduction of the number of painful procedures is unlikely we should apply more non-pharmacological interventions and explore newer pharmacological agents.

We therefore focused on the metabolism of intravenous paracetamol in **Chapter 5**. The use of paracetamol in very preterm infants is limited due to the lack of data on dosing, efficacy and safety. We conducted a multicenter, blinded and randomized trial in 60 very preterm infants (n= 60) with a gestational age between 24 and 32 weeks. They were randomly allocated to one of three different single doses of intravenous paracetamol (10, 15, 20 mg/kg) before central venous catheter placement in the first week of life. Plasma concentrations of paracetamol and its non-toxic and toxic metabolites were determined.

In neonates paracetamol is primarily metabolized into APAP-Sulphate (APAP-Sulph) and to a lesser extent into APAP-Glucuronide (APAP-Gluc) due to an immature glucuronide conjugation system. An even smaller part of APAP is metabolized via oxidation with iso-enzymes CYP2E1, 1A2 and 3A4 of Cytochrome P450 into the toxic metabolite NAPQI. The enzyme gluthatione S transferase converts NAPQIs into APAP-Gluthatione (APAP-Glut), which is metabolized into APAP-Cysteine (APAP-Cys) and APAP-N-acyl-Cysteine (APAP-NAC). All AUCs of APAP and its metabolites increased statistically significantly with increasing paracetamol doses, with p values ranged from $p<0.001$ for AUCs of APAP and APAP-Sulph to $p=0.04$ for AUCs of APAP-NAC.
Gestational age determines the glucuronidation pathway of paracetamol as AUC of APAP-Gluc for the older gestational age group was statistically significantly larger than that for the younger age group

Whether polymorphisms of different CYP-isoenzymes play a role in APAP-induced hepatotoxicity is unknown. Still, paracetamol-induced hepatotoxicity caused by N-acetyl-p-benzoquinone imine (NAPQI) formation produced by the oxidative enzyme CYP2E1 has been described. We therefore determined the relation between CYP2E1 polymorphism and the formation of oxidized metabolites of APAP as an indirect measurement for NAPQI formation. In 58 patients we performed DNA sampling and CYP2E1-wild type was found in 45 patients (77.6%) and in 13 patients (22.4%) a polymorphism for CYP2E1: 2 homozygous and 11 heterozygous, respectively. In a multiple regression analysis with CYP2E1 polymorphism as a predictor and controlling for gestational age and paracetamol dose, there was a trend towards lower AUCs of APAP-Cys for patients with the CYP2E1 polymorphisms ($p=0.07$). This trend was not found for APAP-NAC ($p=0.15$). These "premature" data might suggest a protective role of the CYP2E1 polymorphism in the formation of reactive metabolites. This finding is in line with limited literature available in adults, where a similar decrease of the proteins as well as the catalytic activity of this CYP2E1 variants was found suggesting that polymorphism of CYP2E1 iso-enzymes leads to less protein formation or less stable proteins.

Besides the pharmacokinetic properties of paracetamol we also studied the pharmacodynamics of paracetamol in the same study population in **Chapter 6**. The objective of the study was to determine the analgesic effect of different doses of paracetamol, and a group of age-matched neonates who underwent the same procedure after administration of sucrose only, served as control group. Pain during central venous catheter placement was assessed with two pain assessment tools: the Premature Infant Pain Profile (PIPP), a multidimensional tool that has been well validated to assess procedural pain in preterm and term neonates. As second assessment tool we used the COMFORTneo scale, which had good psychometric qualities in our NICU (chapter 2). No

statistically significant differences in pain scores were found between the paracetamol treatment groups and the sucrose group. The median PIPP score was 7 for the 15 mg/kg paracetamol group and 8 for all other treatment groups, reflecting mild to moderate pain. COMFORTneo scores of 14 or higher, suggesting pain or distress, were assigned to 25% of infants who received sucrose and to up to 50% of infants in the 15 mg/kg and 20 mg/kg paracetamol groups. None of the infants showed abnormal kidney or liver function after treatment with a single dose of paracetamol, but the number of patients in this study is too limited to draw conclusions about safety of paracetamol in general.

The phrase "Old medication with a new indication" applies to **Chapter 7**. The ductus arteriosus fails to close after birth in 30 to 60% of prematurely born infants. This condition – patent ductus arteriosus (PDA) – is associated with a prolonged ventilation need and carries an increased risk of morbidity (i.e., necrotizing enterocolitis, chronic lung disease) and even mortality. Pharmacological closure with non-steroidal anti-inflammatory drugs (NSAIDs), mainly ibuprofen and indomethacin, is currently the standard of care and was found effective in around 70% of patients. Moreover, NSAIDs are contra-indicated in a considerable proportion of newborns, notably those with renal failure, intracerebral hemorrhage, gastro-intestinal problems and thrombocytopenia. If NSAIDs fail or are contra-indicated, the only currently available solution is surgical ligation, which is associated with the risks of cardiothoracic surgery and impaired neurological outcome. Paracetamol has been suggested as an alternative drug for PDA closure. More than 10 observational and retrospective studies have described oral or intravenous high-dose paracetamol treatment with varying effectiveness. Two recent prospective randomized controlled trials comparing oral paracetamol with ibuprofen both showed a slightly favorable effect of paracetamol. We conducted a prospective observational cohort study in which we evaluated the effectiveness of intravenous paracetamol in closing a PDA in very low birth weight infants with a hemodynamically significant PDA who either did not respond to ibuprofen or had a contra-indication for ibuprofen. They received high dose paracetamol therapy (15mg/kg/6h intravenous) for 3-7 days. Cardiac ultrasounds were performed before and 3 and 7 days after treatment. We included 33 patients with a median gestational age of $25^{1/7}$ weeks (IQR 1.66), a median birth weight of 750 grams (IQR 327) and a median post-natal age of 14 days (IQR 12). Paracetamol was ineffective in 27/33 patients (82%). This percentage even rose to 100% after previous exposure to ibuprofen. Patients with a primary contra-indication for ibuprofen (n=13; 39.4%) received the first dose of paracetamol after a median of 12 (IQR 11.5) postnatal days and the median duration of the course was 6 (IQR 3) days. In six patients (46%) the ductus arteriosus was closed or not hemodynamically significant after paracetamol and further treatment was not indicated.

In view of the findings from this study, we do not recommend the use of intravenous paracetamol for hemodynamic significantly PDA closure in VLBW infants after ibuprofen failure. Still, as we did not rule out effectiveness of paracetamol as early PDA treatment, it might be recommended when started in the first week of life. On the other hand, as long as data on long term safety are lacking, high dosages of paracetamol should be used with caution. Better designed PK/PD studies are needed to shed a light on safety aspects and the optimal dose-concentration-effect relationship of paracetamol and PDA closure.

In **Chapter 8** the results of our studies are discussed and recommendations for future perspectives and research are given.

Samenvatting

"Pijn is onvermijdelijk, lijden is een keuze". Deze uitspraak van de schrijver Haruki Murakami verwoordt goed wat we hebben geleerd in de afgelopen drie decennia, waarin grote veranderingen hebben plaatsgevonden in onze inzichten over pijn en het pijnbeleid bij pasgeborenen. De eerdere opvatting dat te vroeg geboren kinderen geen pijn kunnen voelen omdat hun centraal zenuwstelsel zich nog moet ontwikkelen is inmiddels weerlegd. Verder is aangetoond dat onbehandelde pijn negatieve effecten kan hebben op de ontwikkeling van het kind, zowel op de korte als de lange termijn. Tegenwoordig is het technisch beter mogelijk om ernstig zieke en extreem prematuur (=veel te vroeg) geboren kinderen te behandelen en in leven te houden. Hun medische behandeling in de eerste weken na de geboorte brengt echter talrijke pijnlijke handelingen met zich mee, en het is uiteraard belangrijk om de pijn goed te bestrijden.

Er is in de afgelopen jaren al veel onderzoek gedaan naar betere pijnmeting, -preventie en –behandeling in het algemeen. Dit proefschrift gaat in op deze facetten met betrekking tot pasgeboren kinderen, en in het bijzonder op onze zoektocht naar effectieve pijnstillers die zelfs geschikt zijn voor veel te vroeg geboren kinderen. We hebben ons voornamelijk gericht op paracetamol, een geneesmiddel dat wereldwijd wordt gebruikt voor de behandeling van koorts en lichte tot matige pijn.

Volgens de American Pain Society zou pijn beschouwd moeten worden als de vijfde vitale functie en zijn goede pijnmeetinstrumenten onmisbaar om pijnmedicatie bij pasgeborenen goed te kunnen doseren. Veel van de geschikte en gebruikte pijnmeetinstrumenten voor pasgeborenen combineren zogenaamde gedragsindicatoren (bijvoorbeeld gelaatsexpressie en bewegingen) met lichamelijke kenmerken zoals veranderingen in de hartslag en zuurstofverzadiging. De meeste pijnmeetinstrumenten zijn bedoeld om acute pijn te meten, zoals bijvoorbeeld van een hielprik. Langdurige pijn, bijvoorbeeld door mechanische ventilatie, is veel moeilijker te beoordelen.

Hoofdstuk 2 beschrijft een onderzoek in onze NICU naar de sensitiviteit en specificiteit van een aangepaste versie van de COMFORT-gedragsschaal, genaamd de COMFORTneo schaal. Verpleegkundigen beoordeelden pijn en onrust van de pasgeborenen met zowel de COMFORTneo schaal als de Numerieke Rating Schaal (NRS). Voor toepassing van de COMFORTneo schaal werd goede overeenkomst gevonden tussen de verschillende beoordelaars (mediaan gewogen Cohen κ 0.79). Bij afkapwaarden van de COMFORTneo schaal van 14 of hoger hebben een goede sensitiviteit (r=0.81) en specificiteit (r=0.90) met als criterium een NRS-pijn en NRS-onrust score van 4 of hoger. Geboortegewicht, dysmaturiteit (=te laag geboortegewicht voor de zwangerschapsduur), neurologische ontwikkeling of geslacht speelden hierbij geen rol.

In 2005 werd op onze NICU een protocol voor pijnbeleid ingevoerd dat voorziet in verplichte pijnmetingen en richtlijnen geeft voor de behandeling van pijn aan de hand van een beslisboom. De verpleegkundigen worden geacht bij alle patiënten minstens drie keer per 24 uur (1 maal per 8-uurs dienst) pijn te beoordelen met de COMFORTneo schaal. Extra metingen moeten worden uitgevoerd bijvoorbeeld bij verdenking op pijn of onrust, na een pijnlijke handeling, of bij verdenking op te veel sedatie of in geval van langdurige toediening (5 dagen of meer) van pijnstillers al dan niet in combinatie met sedatie. **Hoofdstuk 3** beschrijft de mate waarin de medische en verpleegkundige staf dit pijnprotocol opvolgden in het kalenderjaar 2011. De meerderheid van de pijn scores (86%) waren tussen de 9 en 14 wat suggereert dat patiënten comfortabel waren. De pijnmeting met de COMFORTneo schaal drie keer per dag werd niet altijd nageleefd. Ook werd in geval van hoge pijnscores (>=14) maar in 31% van de gevallen de verplichte herbeoordeling binnen 60 minuten gedaan en werd maar in 40% van de gevallen waarin dit op grond van de beoordeling nodig was, behandeling van pijn gestart of aangepast. In geval van lage pijn scores (<=8) gedurende de behandeling werd in 13% een herbeoordeling gedaan binnen 120 minuten en in 17% werd de dosering verlaagd. De ruimte voor verbetering ligt in het uitvoeren van herbeoordelingen na aanpassingen van de medicatie. Ook kan het afstemmen van het aantal pijnmetingen op de individuele patiënt bijdragen tot een betere naleving van het protocol.

Hoofdstuk 4 beschrijft een prospectieve observationele studie naar het totaal aantal pijnlijke procedures die 175 pasgeborenen op onze NICU moesten ondergaan in de eerste twee levensweken. Verder hebben we ook het aantal pogingen genoteerd die nodig waren voor een bepaalde pijnlijke procedures zoals intubatie, inbrengen van navellijnen en centrale lijnen en het prikken van een perifere arterielijn. Verder is genoteerd welke en hoeveel pijnstillende medicatie is gegeven aan deze patiënten in die eerste twee levensweken. Uit een soortgelijke eerdere studie in 2001 op onze NICU, was een gemiddeld aantal van 14 pijnlijke procedures per kind per dag naar voren gekomen; ook bleek dat niet meer dan 60,3% van de pasgeborenen pijnmedicatie kreeg. Naar aanleiding van die studie hebben we de toediening van sucrose voor pijnlijke procedures en NIDCAP- als niet-farmacologische pijnbestrijding ingevoerd op onze NICU; een nieuw pijnprotocol met een beslisboom voor farmacologische pijnbehandeling werd tegelijkertijd geïntroduceerd. Onze nieuwe studie, uitgevoerd in 2009, liet zien dat pasgeborenen gemiddeld nog steeds 11 pijnlijke procedures per dag ondergaan in de eerste twee levensweken en dat maar 36.6% van hen pijnstillers heeft gekregen, waarschijnlijk omdat niet-farmacologische pijn- en stress reducerende strategieën zoals NIDCAP en sucrose werden toegepast. Aangezien een verdere vermindering van het aantal pijnlijke procedures per patiënt per dag onwaarschijnlijk is, zullen we meer niet-

farmacologische interventies moeten gaan toepassen en op zoek moeten gaan naar nieuwere farmacologische middelen.

Het gebruik van paracetamol bij veel te vroeg geboren kinderen is beperkt ten gevolge van het gebrek aan data over dosering, effectiviteit en veiligheid. In **Hoofdstuk 5** hebben we ons gefocust op het metabolisme van intraveneus toegediende paracetamol en beschrijft een gerandomiseerde, geblindeerde studie bij 60 pasgeborenen met een zwangerschapsduur tussen de 24 en 32 weken. Ze werden willekeurig toegewezen aan een van de drie verschillende doseringen van intraveneus toegediende paracetamol (10, 15 of 10 mg/kg) voorafgaande aan het plaatsen van een centraal veneuze katheter, dat een pijnlijke handeling is. Plasma concentraties van paracetamol en de metabolieten werden bepaald in het bloed. Bij pasgeborenen wordt paracetamol primair gemetaboliseerd in APAP-Sulfaat (APAP-Sulph) en in mindere mate in APAP-Glucuronide (APAP-Gluc) ten gevolge van een onrijp glucuronide conjugatie systeem. Een nog kleiner deel van de paracetamol wordt via oxidatie met iso-enzymen CYP2E1, 1A2 en 3A4 van Cytochroom P450 omgezet in de toxische metaboliet N-acetyl-p-benzoquinone imine (NAPQI). Het enzym glutathion S transferase zet NAPQIs direct om in APAP-Glutathion (APAP-Glut), dat vervolgens wordt gemetaboliseerd in APAP-Cysteine (APAP-Cys) en APAP-N-acetyl-Cysteine (APAP-NAC). Alle 'area's under the curve' van paracetamol en de metabolieten namen statistisch significant toe met een hogere dosis paracetamol dosis, met p-waardes variërend van $p<0.001$ voor APAP en APAP-Sulph tot $p=0.04$ voor APAP-NAC.

Of polymorfisme van de verschillende CYP-iso-enzymen een rol spelen in de door paracetamol veroorzaakte hepatotoxiciteit (leverschade) is onbekend. Hoewel hepatoxiciteit veroorzaakt door de vorming van NAPQIs uit paracetamol door het oxidatieve enzym CYP2E1 wel beschreven is. We hebben onderzoek gedaan naar polymorfismen van CYP2E1en de vorming van toxische metabolieten van paracetamol. DNA sampling bij 58 van de 60 patiënten gaf als resultaat dat 45 patiënten (77.6%) het CYP2E1 wild type hadden en 13 patiënten (22.4%) een polymorfisme voor CYP2E1: 2 homozygoot en 11 heterozygoot. In een multipele regressieanalyse met CYP2E1 polymorfisme als predictor, en gecontroleerd voor zwangerschapsduur en paracetamol dosis, was er een trend naar lagere 'area's under the curve' voor APAP-Cys voor patiënten met het CYP2E1 polymorfisme ($p=0.07$). Deze trend werd niet gevonden voor APAP-NAC ($p=0.15$). Deze 'premature' data zouden kunnen wijzen op een beschermende rol van CYP2E1 polymorfismen bij de vorming van reactieve metabolieten. Deze bevinding komt overeen met de beperkte beschikbare literatuur bij volwassenen, waar zowel een vergelijkbare daling van de gevormde metabolieten als een verlaagde de katalyserende activiteit van deze CYP2E1 varianten was gevonden; Dit wijst er op dat polymorfismen van CYP2E1

iso-enzymen kunnen leiden tot minder metaboliet formatie of tot minder stabiele metabolieten.

Naast de farmacokinetische eigenschappen van paracetamol hebben we ook de farmaco-dynamiek bestudeerd van paracetamol bij de kinderen beschreven in **Hoofdstuk 6.** Het doel van de studie was het bepalen van het pijnstillende effect van verschillende doseringen paracetamol en een groep leeftijd-gematchte pasgeborenen die dezelfde procedure moesten ondergaan maar met sucrose als pijnstilling fungeerde als controle groep. Pijn tijdens het plaatsen van een centraal veneuze katheter werd gemeten met twee pijnmeetinstrumenten: de eerder genoemde COMFORTneo schaal (hoofdstuk 2) en de Premature Infant Pain Profile (PIPP), een multidimensioneel instrument dat gevalideerd is voor de beoordeling van acute procedurele pijn bij prematuren en terme pasgeborenen. Er werden geen statistisch significante verschillen in pijnscores gevonden tussen de verschillende paracetamol doseringen en de sucrose groep. De mediane PIPP score was 7 voor de groep kinderen die 15 mg/kg paracetamol hadden gekregen en 8 voor de overige groepen, duidend op mild tot matige pijn. COMFORTneo scores van 14 of hoger, wijzend op pijn of onrust, werden gevonden bij 25% van de kinderen die sucrose hadden gekregen en bij maximaal 50% van de kinderen die of 15mg/kg of 20mg/kg paracetamol hadden gekregen. Geen van de kinderen vertoonde nier- of leverfunctiestoornissen als gevolg van de behandeling met een enkele dosis paracetamol, maar het aantal patiënten in dit onderzoek is te klein om conclusies te mogen trekken over de veiligheid van paracetamol in het algemeen.

De uitdrukking "Oude medicatie met een nieuwe indicatie' zou goed kunnen passen bij **Hoofdstuk 7**. Bij 30 tot 60% van de te vroeg geboren kinderen sluit de ductus arteriosus niet spontaan na de geboorte, wat wel het geval zou moeten. De ductus arteriosus is een bloedvat dat de longslagader verbindt met de lichaamsslagader. Dit bloedvat is vooral belangrijk tijdens het intra-uteriene leven van een foetus. Als dit bloedvat open blijft na de geboorte, is langdurige mechanische ventilatie nodig en is er grote kans op ernstige aandoeningen, zoals necrotiserende enterocolitis en chronische longziekte, en zelfs op overlijden. De huidige standaardtherapie is toediening met zogenaamde NSAIDs, vooral ibuprofen en indomethacine, maar dit werkt slechts bij ongeveer 70% van de patiënten. Bovendien kunnen deze medicijnen niet worden gegeven aan kinderen met aandoeningen zoals nierfalen, hersenbloedingen, maagdarmklachten en trombocytopenie. Dan blijft alleen chirurgische sluiting als behandelmogelijkheid voor een open ductus over, maar deze operatie draagt ook risico's met zich mee met een verhoogde kans op ook een slechtere neurologische uitkomst.

Paracetamol wordt genoemd als alternatief medicijn voor het sluiten van een open ductus. Meer dan 10 observationele en retrospectieve studies hebben de orale of intraveneuze hoog gedoseerde paracetamol behandeling voor een open ductus beschreven met wisselende effectiviteit. In twee recente prospectieve, gerandomiseerde, gecontroleerde trials waarin paracetamol werd vergeleken met ibuprofen bleek paracetamol een iets gunstiger effect te hebben op het sluiten van de ductus. Wij hebben een prospectieve, observationele cohortstudie verricht waarin we gekeken hebben in hoeverre intraveneuze toediening van paracetamol effectief was om een open ductus arteriosus te sluiten. Het ging hierbij om 33 kinderen met een geboortegewicht < 1500 gram met een open ductus die aanleiding gaf tot klinische symptomen (= hemodynamisch significante ductus) en waarvoor ibuprofen behandeling faalde of gecontra-indiceerd was. Ze kregen 3 tot maximaal 7 dagen een hoge dosis paracetamol (15 mg/kg/6 uur intraveneus) toegediend, en echo's van het hart werden gemaakt voorafgaande aan de behandeling met paracetamol en 3 en 7 dagen daarna ter controle van de sluiting.

De paracetamol was ineffectief bij 27 van de 33 kinderen (82%). Dit percentage was zelfs 100% bij de kinderen die voorafgaande aan de paracetamol een behandeling met ibuprofen hadden gekregen. Van de 33 kinderen hadden er 13 een contra-indicatie voor een behandeling met ibuprofen en zij kregen primair paracetamol. In 6 van deze 13patiënten (46%) was na behandeling met paracetamol de ductus arteriosus gesloten of niet meer hemodynamisch significant dat verdere behandeling nodig was.

Gezien het hoge percentage kinderen bij wie een behandeling met intraveneuze paracetamol niet leidde tot sluiting van een hemodynamisch significante ductus zien wij geen reden om dit toe te passen bij kinderen met een geboortegewicht < 1500 gram als ibuprofen eerder al niet effectief was gebleken. We sluiten de effectiviteit van paracetamol voor de vroege open ductus behandeling in de eerste levensweek niet uit. Niettemin is voorzichtigheid geboden in het gebruik van hoge doseringen paracetamol zolang er weinig bekend is over de veiligheid op de lange termijn. Weldoordachte PK/PD studies kunnen inzicht geven in de veiligheidsaspecten en de optimale dosis- concentratie- effect relatie van paracetamol om sluiting van de ductus arteriosus te bewerkstelligen.

In **Hoofdstuk 8** zullen de resultaten van onze studies bediscussieerd worden en aanbevelingen gedaan worden voor toekomstig onderzoek.

Chapter 10

Appendices

List of abbreviations

ALAT	Aspartaat-aminotransferase
APAP	Acetyl-para-aminophenol
APAP-Cys	APAP-Cysteine
APAP-Gluc	APAP-Glucuronide
APAP-Glut	APAP-Glutathione
APAP-NAC	APAP-N-acetyl-cysteine
APAP-Sulph	APAP-sulphate
ASAT	Alanine-aminotransferase
AUC	Area Under the Curve
BUN:	Blood Urea Nitrogen
CI	Confidence Interval
CPAP	Continuous Positive Pressure Ventilation
CRIB	Critical Risk Index for Babies
CVC:	Central venous catheter
EDIN	Echelle Douleur Inconfort Nouveau-Né
EEG	Electroencephalogram
ELBW	Extreme Low Birth Weight
GA	Gestational Age
hsPDA	hemodynamically significant Patent Ductus Arteriosus
IQR	Interquartile range
IRDS	Infant Respiratory Distress Syndrome
i.v.	Intravenous
IVH	Intraventriculair hemorrhage
LA/Ao ratio	Left Atrial to Aortic Root ratio
LLOQ	lower limit of quantification
LPA	Left Pulmonary Artery
mg	Milligram
NAPQI	N-acetyl-p-benzoquinone imine
NEC	Necrotizing Enterocilitis
NI	Neurologic Impairment
NICU	Neonatal Intensive Care Unit
NIDCAP	Newborn Individualized Developmental Care and Assessment Program
NIRS	Near-infrared Spectroscopy
N-PASS	Neonatal Pain, Agitation and Sedation Scale
NPT	Nasopharyngeal Tube

NRS	Numeric rating Scale
NSAID	Non-Steroidal Anti-Inflammatory Drug
PD	Pharmacodynamic
PDA	Patent Ductus Arteriosus
PDMS	Patient Data Management System
PICU	Pediatric Intensive Care Unit
PIPP	Premature Infant Pain Profile
PK	Pharmacokinetic
ROC	Receiver Operator Characteristic
SD	Standard Deviation
SDM	Standardized Mean Difference
SGA	Small for Gestational Age
SPSS	Statistical Package for the Social Sciences
VLBW	Very Low Birth Weight

PhD Portfolio

Name PhD student	Daniëlla W.E. Roofthooft
Erasmus MC Department	Neonatology
PhD period	2009-2015
Promotors	Prof. dr. D. Tibboel
	Prof. dr. J.N. van den Anker
Copromotor	Dr. M. van Dijk

1. PhD training

	Year	Workload ECTS
General academic skills and Research skills		
- LACDR course on Pharmacokinetics	2009	1.0
Center for Bio-Pharmaceutical Science of the University of Leiden		
- Systematic Literature Search and Endnote	2010	0.4
Erasmus University		
- BROK ('Basiscursus Regelgeving Klinisch Onderzoek")	2012	1.0
Erasmus University		
- CPO (Centrum voor patiënt gebonden onderzoek) minicursus	2013	0.6
In-depth courses (e.g. Research school, Medical Training)		
- NLS cursus	2010	0.4
Presentations and National/ International conferences		
- Symposium Extreme prematuren, Rotterdam	2010	0.3
- EAPS Kopenhagen, Denemarken	2010	1.0
- Grand Round: 10 jaar verpleegkundig specialist op de Neonatologie (oral presentation)	2010	0.3
- Pass meeting, Denver, USA	2011	1.0
- 3rd International Conference on Clinical neonatology, Turino, Italy	2012	1.0
- Presentation Annual Pharmacology meeting, Erasmus MC	2012, 2013	0.4
- Zomercongres Sectie Kinderanesthesiologie, Bergen	2012	0.6
- EASP Istanbul, Turkey (invited speaker)	2012	1.0
- New Insights into Neonatal Resuscitation, Boerhaave nascholing, Leiden	2012	0.3
- Masterclass Pijn bij pasgeborenen, Erasmus MC (invited speaker)	2013	0.3
- Neonatologie aan de Maas, Tegelen, Nederland	2009	1.0
	2011	1.0
	2013	1.0
- Lof der Geneeskunst	2013	0.1
- KNMG, Taakherschikking in de zorg met verpleegkundig specialisten (invited speaker)	2014	0.3

-	2e Erasmus MC Critical Care Day	2014	0.3
-	6e nationale Pijncongres, Ede, Nederland (invited speaker)	2014	0.5
-	EAPS Barcelona, Spain	2014	1.0
-	Interklinische avond Kindergeneeskunde (3x/jaar)	2009-2015	0.4
-	Refereeravond neonatologie (regio 4x/jaar)	2009-2015	0.4
Seminars and workshops			
-	Neonatale reanimatie workshops –Neonatologie aan de Maas, Tegelen, Nederland	2011	0.5
-	28th International Workshop on Surfactant Replacement, Helsinki. Finland	2013	1.0
-	Themaweek Neonatal Resuscitation	2012, 2014	2.0
-	Research meeting neonatology/pharmacology (weekly)	2009-2015	1.0
Didactic skills			
-	Teach the teacher course	2011	0.3
2. Teaching activities			
-	Teaching medical students (internship Pediatrics)	2009-2015	3.0
-	Teaching paediatric residents	2009-2015	6.0
-	Teaching nurses (Neonatology/Obstetrics and Gynaecology)	2009-2015	6.0
-	Teaching Neonatal Nurse Practitioners	2009-2015	3.0
-	Neonatal Life support (department neonatology)	2009-2015	3.0

ECTS = European Credit Transfer and Accumulation System
1 ECTS represents 28 hours

List of Publications

Daniëlla W.E. Roofthooft, Ingrid M. van Beynum, Johan C.A. de Klerk, Monique van Dijk, John N. van den Anker, Irwin K.M. Reiss, Dick Tibboel, Sinno H.P. Simons.
Limited effects of intravenous paracetamol on patent ductus arteriosus in very low birth weight infants with contra-indications for ibuprofen or after ibuprofen failure.
Eur J Pediatr. 2015 april 30. (epub ahead of print)

D.W.E. Roofthooft, S.H.P. Simons, R.A. van Lingen, D. Tibboel, J.N. van den Anker, I.K.H. Reiss, M. van Dijk. (submitted Pediatrics 2015).
Intravenous acetaminophen is not effective for pain management during central venous catheter placement in very preterm infants.
(submitted Pediatrics 2015)

Bijvank SW, Visser W, Duvekot JJ, Steegers EA, Edens MA, **Roofthooft DW**, Vulto AG, Hanff LM. Ketanserin versus dihydralazine for the treatment of severe hypertension in early-onset preeclampsia: a double blind randomized controlled trial.
Eur J Obstet Gynecol Reprod Biol. 2015 Mar 10. pii: S0301-2115(15)00037-8. doi: 10.1016/j.ejogrb.2015.02.002.
[Epub ahead of print]

Aukes DI, **Roofthooft DW**, Simons SH, Tibboel D, van Dijk M.
Pain Management in Neonatal Intensive Care: Evaluation of the Compliance with Guidelines.
Clin J Pain. 2014 Nov 3.

Roofthooft DW, Simons SH, Anand KJ, Tibboel D, van Dijk M.
Eight years later, are we still hurting newborn infants? Neonatology. 2014;105(3):218-26. doi: 10.1159/000357207.
Epub 2014 Feb 4.

Durrmeyer X, Hummler H, Sanchez-Luna M, Carnielli VP, Field D, Greenough A, Van Overmeire B, Jonsson B, Hallman M, Mercier JC, Marlow N, Johnson S, Baldassarre J; European Union Nitric Oxide Study Group.
Two-year outcomes of a randomized controlled trial of inhaled nitric oxide in premature infants.
Pediatrics. 2013 Sep;132(3):e695-703.

Roofthooft DW, van Beynum IM, Helbing WA, Reiss IK, Simons SH. Paracetamol for ductus arteriosus closure: not always a success story. Concerning the article by M.Y. Oncel et al: intravenous paracetamol treatment in the management of patent ductus arteriosus in extremely low birth weight infants [Neonatology 2013;103:166-169].
Neonatology. 2013;104(3):170

de Graaf J, van Lingen RA, Simons SH, Anand KJ, Duivenvoorden HJ, Weisglas-Kuperus N, **Roofthooft DW**, Groot Jebbink LJ, Veenstra RR, Tibboel D, van Dijk M.
Long-term effects of routine morphine infusion in mechanically ventilated neonates on children's functioning: five-year follow-up of a randomized controlled trial.
Pain. 2011 Jun;152(6):1391-7.

Mercier JC, Hummler H, Durrmeyer X, Sanchez-Luna M, Carnielli V, Field D, Greenough A, Van Overmeire B, Jonsson B, Hallman M, Baldassarre J; EUNO Study Group.
Inhaled nitric oxide for prevention of bronchopulmonary dysplasia in premature babies (EUNO): a randomised controlled trial.
Lancet. 2010 Jul 31;376(9738):346-54.

van Dijk M, **Roofthooft DW**, Anand KJ, Guldemond F, de Graaf J, Simons S, de Jager Y, van Goudoever JB, Tibboel D.
Taking up the challenge of measuring prolonged pain in (premature) neonates: the COMFORTneo scale seems promising.
Clin J Pain. 2009 Sep;25(7):607-16.

Maingay-de Groof F, Lequin MH, **Roofthooft DW**, Oranje AP, de Coo IF, Bok LA, van der Spek PJ, Mancini GM, Govaert PP. Extensive cerebral infarction in the newborn due to incontinentia pigmenti.
Eur J Paediatr Neurol. 2008 Jul;12(4):284-9.

Smit LS, **Roofthooft D**, van Ruissen F, Baas F, van Doorn PA.
Congenital hypomyelinating neuropathy, a long term follow-up study in an affected family.
Neuromuscul Disord. 2008 Jan;18(1):59-62.

Hanff LM, Visser W, **Roofthooft DW**, Vermes A, Hop WC, Steegers EA, Vulto AG.
Insufficient efficacy of intravenous ketanserin in severe early-onset pre-eclampsia.
Eur J Obstet Gynecol Reprod Biol. 2006 Sep-Oct;128(1-2):199-203.

Hanff LM, Vulto AG, Bartels PA, **Roofthooft DW**, Bijvank BN, Steegers EA, Visser W. Intravenous use of the calcium-channel blocker nicardipine as second-line treatment in severe, early-onset pre-eclamptic patients.
J Hypertens. 2005 Dec;23(12):2319-26.

Simons SH, **Roofthooft DW**, van Dijk M, van Lingen RA, Duivenvoorden HJ, van den Anker JN, Tibboel D. Morphine in ventilated neonates: its effects on arterial blood pressure.
Arch Dis Child Fetal Neonatal Ed. 2006 Jan;91(1):F46-51..

Simons SH, van Dijk M, van Lingen RA, **Roofthooft DW**, Boomsma F, van den Anker JN, Tibboel D. Randomised controlled trial evaluating effects of morphine on plasma adrenaline/noradrenaline concentrations in newborns.
Arch Dis Child Fetal Neonatal Ed. 2005 Jan;90(1):F36-40.

Hanff LM, Visser W, **Roofthooft DW**, Bulsink MJ, Vermes A, Steegers EA, Vulto AG.
Ketanserin in pre-eclamptic patients: transplacental transmission and disposition in neonates.
BJOG. 2004 Aug;111(8):863-6.

Simons SH, van Dijk M, van Lingen RA, **Roofthooft DW**, Duivenvoorden HJ, Jongeneel N, Bunkers C, Smink E, Anand KJ, van den Anker JN, Tibboel D.
Routine morphine infusion in preterm newborns who received ventilatory support: a randomized controlled trial.
JAMA. 2003 Nov 12;290(18):2419-27.

Simons SH, van Dijk M, Anand KS, **Roofthooft DW**, 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 Nov;157(11):1058-64.

Bloem BR, **Roofthooft DW**, Lammers GJ, de Beaufort AJ, Brouwer OF.
Withdrawal symptoms in a neonate following exposure to venlafaxine during pregnancy.
Ned Tijdschr Geneeskd. 2003 Sep 20;147(38):1885-6; author reply 1886. Dutch. No abstract available.

Bloem BR, **Roofthooft DW**, Lammers GJ, de Beaufort AJ, Brouwer OF.
Clomipramine withdrawal in newborns.
Arch Dis Child Fetal Neonatal Ed. 1999 Jul;81(1):F77.

Curriculum vitae

Daniëlla Roofthooft was born on the 25th of March 1967 in Leiden, the Netherlands. She went to secondary school at the Bonaventura College in Leiden and graduated from Gymnasium B in 1985. The same year she started her medical training at the Erasmus University in Rotterdam. Before achieving her medical degree in January 1992, she worked for 6 months at the Children's department of the Rumah Sakit Dr. Soetomo in Surabaya, Indonesia. She did residencies in Pediatrics at the Slotervaart Hospital in Amsterdam (1992) and at St Franciscus Gasthuis in Rotterdam (1993) and started her official pediatric training in 1994 at the Leiden University Medical Center (Head Prof. dr. J.M. Wit). The non-academic part of the training was done at the Juliana Children's Hospital in The Hague (Head Prof. dr. A. van der Heijden) where she finished in 1999. Directly afterwards she started her fellowship Neonatology at the Erasmus MC-Sophia under the supervision of Prof. dr. J.N. van den Anker and finished in 2001. Since then she works as a staff member and medical coordinator at the department of Neonatology, Erasmus MC-Sophia. In 2001 she played a leading role in the introduction of Nurse Practitioners on the NICU and hitherto this profession has become indispensable in the care for critically ill and preterm infants. Her special interest has always been in pain and in 2009 Prof. dr. J.B. van Goudoever (Head of the department of Neonatology at that time) encouraged her and made it possible to start this PhD thesis. Under the supervision of Prof. dr.Tibboel, Prof. dr. J.N. van den Anker and Dr. M. van Dijk she wrote the PhD thesis and today this task is accomplished!

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Alles zelf doen is optellen, samenwerken is vermenigvuldigen.

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Daarnaast wil ik de overige leden van de promotiecommissie, Dr. van Lingen, Dr. Simons en Prof. dr. Carbajal danken voor de bereidheid om plaats te nemen in de grote commissie.

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Prof. dr. Carbajal, dear Ricardo, I am truly honored that you would like to participate in my PhD committee.

Prof. dr. van Goudoever, beste Hans. Jij was het die tegen mij zei dat ik iedere vijf jaar een nieuwe uitdaging in mijn vak moest zoeken en dat iets doen dat je niet makkelijk afgaat maar veel moeite en energie kost, je uiteindelijk meer voldoening zal geven. Je had helemaal gelijk! Dank voor het scheppen van de mogelijkheden om dit promotietraject te starten in 2009. Op de goede afloop zullen we zeker samen een glaasje drinken.

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Som-hi tots-♥ Dave