MORPHINE MORE FINE?
ITS EFFECTS IN CRITICALLY ILL NEWBORNS

Maakt morfine beter?
Onderzoek naar de werking bij ernstig zieke pasgeborenen

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Proefschrift

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Chapter 1

INTRODUCTION

Introduction

The pharmacist Sertürner first isolated morphine from opium in 1803 and named it after Morpheus, the god of dreams in Greco-Roman mythology. Ever since, it has been one of the most frequently used drugs to relieve pain, for a variety of age groups. In our days, however, there is still debate whether morphine and analgesic therapy should serve as standard of care for hospitalized newborns.

Until the last decade of the 20th century, premature neonates were generally believed to have little pain sensation[1] and thus not in need of analgesic therapy. The studies of Anand et al., which showed decreased morbidity and mortality in neonatal patients receiving adequate analgesic therapy after surgery,[2,3] were instrumental in altering this notion. At present it is widely recognized that even the most premature neonates can feel pain. While neonatal pain experiences have been suggested to bring about short and long-term negative consequences,[4] analgesic therapy in the vulnerable newborns can also carry risks, such as increased incidence of seizures.[5] This leaves us with the question whether the benefits of treatment will outweigh the side effects and potential hazards of analgesic treatment. Or, in other words, is morphine more fine?

Scope of this thesis
The studies described in this thesis generally aim to improve neonatal pain treatment, by investigating the beneficial and adverse effects of neonatal morphine use. They also aim at improving our knowledge of how newborns respond to pain and how to measure pain objectively: pain assessment.

To investigate the current management of pain in our Neonatal Intensive Care Unit (NICU), we prospectively studied the frequency and painfulness of daily-performed procedures in 151 neonates during their first fourteen days in the NICU (**Chapter 2**). We simultaneously analyzed the use of analgesic therapy in these neonates. **Chapter 3.1** reviews the current knowledge about analgesics in neonates and infants, and **Chapter 3.2** focuses on the available neonatal pain assessment instruments and their shortcomings. To gain more insight into the effects and safety of the routine administration of continuous morphine in ventilated neonates, we conducted a blind randomized placebo controlled trial among 150 neonates admitted to either our NICU or a different one in the Netherlands. They were allocated to receive either placebo or a 100 μg/kg morphine loading-dose followed by a continuous morphine dosage of 10 μg/kg/h. We describe the effects of routine continuous morphine infusion in these ventilated neonates on the level of pain experience and clinical outcome (**Chapter 4**), on stress

responses, measured by epinephrine and norepinephrine plasma concentrations (**Chapter 5**), and on blood pressure and blood pressure variability (**Chapter 6**), .

In neonates and infants, morphine is mainly metabolized by glucuronidation into morphine-3-glucuronide (M3G) and the analgesically active metabolite morphine-6-glucuronide (M6G).[6] This pathway is catalyzed by the enzyme UDP-glucuronosyl-transferase 2B7 (UGT2B7).[7] Small parts of morphine are sulphated[8] or eliminated as free unbound morphine. In **Chapter 7** the pharmacokinetics of morphine were studied in critically ill neonates receiving extra-corporeal membrane oxygenation (ECMO) and compared with morphine and metabolite plasma-levels obtained in neonates and infants after major surgery.

In **Chapter 8** we evaluated three pain assessment tools, the Premature Infants Pain Profile (PIPP),[9] the Neonatal Infant Pain Scale (NIPS),[10] validated for acute neonatal pain, and the COMFORT scale, validated for postoperative pain in newborns and widely implemented in our hospital,[11] in order to determine which is the most appropriate to assess neonatal pain. Furthermore, we analyzed if physiological indicators such as heart rate, blood pressure and oxygen saturation, are specific enough as indicators of pain. For these aims we performed an in-depth analysis of the data generated from our randomized controlled trial in ventilated preterm neonates, comparing morphine with placebo.

Evaluation of the randomized controlled trial revealed large inter-individual differences in morphine requirements of neonates. In **Chapter 9**, we therefore investigated whether an infant's DNA can predict the morphine requirements for adequate analgesia. To this end we studied two polymorphisms in relationship with morphine requirements of neonates during intensive care and postoperative treatment. DNA was used from patients participating in three different randomized controlled trials evaluating the effects of morphine in neonates and infants. The one polymorphism was $asn^{40}asp$, in the gene encoding the human μ-receptor (OPRM)[12], the other polymorphism was COMT $val^{158}met$,[13] in a gene encoding the catechol-O-methyltransferase enzyme. Both have been suggested to influence experience of pain and need for analgesia in humans.

In **Chapter 10** the results of our studies are incorporated into the discussion of the main remaining questions about neonatal pain and analgesia. Furthermore, suggestions are given for future research.

References

1. Swafford LI, Allan DA. Pain relief in the pediatric patient. Med Clin North Am. 1968;52(1):131-136.

2. Anand KJS, Hickey PR. Pain and its effects in the human neonate and fetus. N Engl J Med. 1987;317(21):1321-1329.

3. Anand KJS, Sippell WG, Aynsley-Green A. Randomised trial of fentanyl anaesthesia in preterm babies undergoing surgery: effects on the stress response. Lancet. 1987;1(8524):62-66.

4. Goldschneider KR, Anand KJS. Long-term consequences of pain in neonates, Chapter 4. In: Schechter NL, Berde C, Yaster M, eds. Pain in Infants, Children, and Adolescents. 2nd ed. Philadelphia: Lippencott Williams & Wilkens; 2003:58-70.

5. Koren G, Butt W, Pape K, Chinyanga H. Morphine-induced seizures in newborn infants. Vet Hum Toxicol. 1985;27(6):519-520.

6. Kart T, Christrup LL, Rasmussen M. Recommended use of morphine in neonates, infants and children based on a literature review: Part 1--Pharmacokinetics. Paediatr Anaesth. 1997;7(1):5-11.

7. Coffman BL, Rios GR, King CD, Tephly TR. Human UGT2B7 catalyzes morphine glucuronidation. Drug Metab Dispos. 1997;25(1):1-4.

8. Choonara I, Ekbom Y, Lindstrom B, Rane A. Morphine sulphation in children. Br J Clin Pharmacol. 1990;30(6):897-900.

9. Stevens B, Johnston C, Petryshen P, Taddio A. Premature Infant Pain Profile: development and initial validation. Clin J Pain. 1996;12(1):13-22.

10. Lawrence J, Alcock D, McGrath P, Kay J, MacMurray SB, Dulberg C. The development of a tool to assess neonatal pain. Neonatal Netw. 1993;12(6):59-66.

11. van Dijk M, de Boer JB, Koot HM, Tibboel D, Passchier J, Duivenvoorden HJ. The reliability and validity of the COMFORT scale as a postoperative pain instrument in 0 to 3-year-old infants. Pain. 2000;84(2-3):367-377.

12. Lotsch J, Zimmermann M, Darimont J, et al. Does the A118G polymorphism at the mu-opioid receptor gene protect against morphine-6-glucuronide toxicity? Anesthesiology. 2002;97(4):814-819.

13. Zubieta J, Heitzeg MM, Smith YR, et al. COMT val153met genotype affects μ-opioid neurotransmitter responses to a pain stressor. Science. 2003;299(5610):1240-1243.

Chapter **2**

DO WE STILL HURT NEWBORN BABIES?
A PROSPECTIVE STUDY OF
PROCEDURAL PAIN AND ANALGESIA IN NEONATES

Abstract

Background

Despite an increasing awareness regarding pain management in neonates and the availability of published guidelines for the treatment of procedural pain, preterm neonates experience pain leading to short- and long-term detrimental effects.

Objective

To assess the frequency of use of analgesics and of invasive procedures in neonates and the associated pain burden in this population.

Methods

For 151 neonates, we prospectively recorded all painful procedures, including the number of attempts required, and analgesic therapy used during the first 14 days of Neonatal Intensive Care Unit admission. These data were linked to estimates of the pain of each procedure, obtained from the opinions of experienced clinicians.

Results

On average, each neonate was subjected to a mean ± SD of 14 ± 4 procedures per day. The highest exposure to painful procedures occurred during the first day of admission and most (63.6%) procedures consisted of suctioning. Many procedures were estimated to be painful (painfulness scores above 4.0 on a 10-point scale for 26 out of 31 procedures on a questionnaire). Pre-emptive analgesic therapy was provided to fewer than 35% of neonates studied each day, while 39.7% of the neonates did not receive any analgesic therapy in the neonatal intensive care unit.

Conclusions

Clinicians estimated that most Neonatal Intensive Care Unit procedures are painful, but only a third of the neonates received appropriate analgesic therapy. Despite the accumulating evidence that neonatal procedural pain is harmful, analgesic treatment for painful procedures is limited. Systematic approaches are required to reduce the occurrence of pain and to improve the analgesic treatment of repetitive pain in neonates.

Introduction

Advances in perinatal care have increased the survival of very preterm neonates in the Neonatal Intensive Care Units (NICUs) worldwide. The physiological instability and underlying diseases of these infants necessitate various invasive procedures, such as endotracheal intubation, heelsticks, insertion of indwelling venous and arterial catheters, as well as oral, nasal, tracheal, and gastric suctioning. Although previous studies[1-4] have reported the frequency of daily procedures in the NICU (table 1), many procedures such as nasopharyngeal tube insertions and nasal suctioning were not included, and the number of failed procedures have never been evaluated, to our knowledge.

Despite the current knowledge that low birth weight infants are able to experience pain, many daily procedures are still performed without pharmacological or non-pharmacological analgesic therapy.[2,5-9]
Indeed, preterm neonates are highly sensitive to pain[10-12] and amass acute responses to painful procedures.[13,14] Short- and long-term effects of painful procedures in neonates[15,16] occur as a consequence of their immature and vulnerable nervous systems, reflected in an altered pain response,[17] possibly leading to changes in neural development. Studies in animals indicate developmental changes in the brain[18,19] and in the spinal dorsal horn that are associated with neonatal pain.[20] A higher frequency of painful invasive procedures in low birth weight infants has been associated with a greater pain response at 32 weeks compared with controls.[21] Although it is unknown whether these changes in pain response persist until older age,[22,23] more cognitive[24] and psychopathological problems[25-32] have been reported in children born preterm.[33,34]

Table 1 Overview of studies investigating the numbers of procedures in the Neonatal Intensive Care Unit (NICU)

Source	Duration of study	No. Of infants	Total procedures	No. of Procedures	Most frequently performed
Barker & Rutter,[1] 1995	Total NICU stay	54	3283	60.8 per patient	Heelstick (56%)
Johnston et al,[2] 1997	First 7 days	23	2134	2-10 per day	Heelstick (61%)
Porter & Anand,[4] 1998	Total NICU stay	144	7672	53.3 per patient	Heelstick (87%)
Benis & Suresh,[5] 2001	Total NICU stay	15	5663	6 per day	Suctioning (51%)
Present study	First 14 days	151	19674	14 per day	Suctioning (63.6%)

A growing awareness in the past several years of the importance of adequate neonatal pain management led to the discussion whether analgesia should be given to all ventilated newborns.[35,36] Although there is still controversy about the risks and benefits of continuous opioid administration (and as a consequence neonatologists are reluctant to prescribe them) recently developed international consensus statements have provided neonatal pain management guidelines, including those for procedural interventions.[37,38] Therefore, we hypothesized that analgesic therapy is frequently used and that procedural pain is minimal in neonates admitted to the NICU. Prospectively, we collected bedside data on the number and type of all daily painful procedures performed in 151 neonates during their stay in a tertiary-care NICU, including failed procedures (e.g. multiple attempts to insert peripheral venous catheters). These data were integrated with the results of a questionnaire evaluating the opinions of experienced clinicians about the pain of different procedures. Furthermore the analgesic therapy used during the studied days was evaluated and compared to current guidelines.

Methods

A pain research team (including a neonatologist [D.R.], pediatric intensivist [D.T.], research-nurse and a psychologist[M. van D.]) with extensive research[39-50] and clinical experience, compiled a checklist containing all daily painful procedures, invasive and non-invasive, including procedures that require multiple attempts. The checklist designed for this study was tested and refined during a pilot study in December 2000 and January 2001, and 34 procedures were included in the final version.

From February 1, 2001 to July 31, 2001, we documented all procedures performed in all neonates during the first fourteen days of admission in a tertiary-care NICU. The unit is part of the biggest children's hospital in the Netherlands, which comprises a perinatal center and pediatric surgery setting. Patients older than 3 days at admission and infants discharged or transferred within 24 hours after admission, were excluded from the study. Nurses and physicians noted all procedures performed in real time each day, including the number of attempts for each procedure. The recorded procedures were cross-checked by the researchers every day to ensure accuracy.

Background variables and mode of respiratory support were noted daily and the Clinical Risk Index for Babies (CRIB)[51] was scored as a measure of severity of illness.

In addition, all administered analgesics were noted during the studied days. Standardized pharmacological analgesic therapy for procedural pain in our NICU is limited to a morphine loading dose (100 μg/kg of body weight) before tracheal intubation (unless intubation occurs in the delivery room) and before the insertion of thoracostomy tubes. Infants with indwelling thoracostomy tubes receive a continuous morphine infusion (10 μg/kg per hour) until the tube is removed.

To estimate the pain of NICU procedures we developed a questionnaire listing all invasive procedures from our checklist, with two non-invasive procedures (diaper change and cranial ultrasound) included as control variables. The questionnaire was distributed among the nurses and physicians of two NICUs and one Pediatric Surgical Intensive Care Unit, also present in this children's hospital, where newborns with major congenital anomalies are admitted. Participants were asked to estimate a rating from 0 (not painful) to 10 (most painful) for each procedure, without taking specific circumstances into account.

Statistics

Procedures were counted per calendar day. Because first and last study day are usually shorter than 24 hours, the numbers of procedures were corrected for the actual length of stay on these days.

Random regression modeling (PROC MIXED; SAS Institute, Cary, NC) was used to simultaneously estimate the effect of the time-varying covariates respiratory support (no support, nasal oxygen, continuous positive airway pressure, and mechanical ventilation), postnatal age, gestational age and length of study, on the number of procedures performed. Because a procedural volume difference on the first day compared to the other days was expected, the study days were dichotomized into 2 variables: 0 (1 day) and 1(2-14 days). The outcome variable, ie, frequency of procedures, was log-transformed (base 10) to achieve a normal distribution. The model incorporated random intercepts and random slopes.

Multiple regression analysis was performed to estimate the effect of background variables (profession, sex, age, unit, hospital [Erasmus MC-Sophia Children's Hospital or Isala Clinics], parent [yes or no], and years of ICU experience) on the painfulness scoring of the participants. Data are presented as mean ± SD.

Results

Patients

One hundred fifty-one neonates were included in the study; 89 other newborns who were discharged within 24 hours were excluded. Table 2 lists the study subjects' background characteristics and primary diagnoses. Most neonates (n = 129) were admitted and enrolled on the first postnatal day. Gestational ages ranged from 25.3 to 42.0 weeks, with a mean of 32.4 ± 4.5 weeks. The CRIB[51] scores, ranged from 0 to 16 with a mean of 3.9 ± 3.3. Study subjects required respiratory support consisting of mechanical ventilation on 49.6% (one third high frequency oscillation and two thirds conventional ventilation), continuous positive airway pressure on 22.5%, nasal oxygen on 15.6%, and subjects required no respiratory support on 12.2% of study days. During 55.2% of the study days, patients had an arterial line (42.8% peripheral arterial lines, 12.3% umbilical arterial lines).

Frequency

During 1375 patient-days, 19674 procedures were performed, with a mean NICU stay of 9.1 ± 4.4 days per patient. Table 3 gives the procedures rank-ordered by their frequency. Suctioning of nasal, endotracheal, and nasopharyngeal tubes constituted almost 63.6% of the performed procedures. The mean number of procedures per neonate per day was 14.3 ± 4, with a range of 0 to 53 procedures per day. Almost one third (30.9%) of the 1076 insertions of intravenous cannulae were not successful. Procedures for placement of central venous catheters, peripheral arterial catheters and umbilical catheters were not successful in 45.6%, 37.5% and 34.6% of attempts, respectively. Failure rates for venepunctures and lumbar punctures were 21.0% and 17.5% respectively (Figure 1). Random regression modelling (Table 4) showed significantly higher frequencies of procedures during the first study day compared with day 2 to 14 ($P < 0.001$). The frequency of procedures was not predicted by gestational age ($P = 0.51$), study day ($P = 0.50$), or postnatal age ($P = 0.72$).

Procedures were performed with significantly higher frequencies in patients receiving nasal oxygen ($P < 0.0001$), continuous positive airway pressure, and ventilation ($P < 0.001$ for all) compared to those without respiratory support.

Table 2 Background Characteristics and Primary Diagnoses in 151 infants

| Background characteristics	Value
male/female	82 / 69
Gestational age, mean ± SD (range), wk	32.4 ± 4.5 (25.3 to 42.0)
Birth weight, mean ± SD, g	1734 ± 979
Clinical Risk Index for Babies score, mean ± SD (range)	3.9 ± 3.3 (0 to 16)
Duration of admission, mean ± SD, d	9.2 ± 4.4
Respiratory support, % of total days	
No support	12.2
Nasal oxygen	15.6
Continuous positive airway pressure	22.5
Conventional ventilation	32.9
High-frequency oscillation ventilation	16.7
Primary diagnoses, No	
Prematurity	104
Small for gestational age (> 2 SDs under mean birth weight)	23
Asphyxia	15
Respiratory insufficiency	93
Respiratory distress syndrome	58
Wet Lung	17
Meconium aspiration syndrome	5
Pneumothorax	9
Persistent pulmonary hypertension of the newborn	7
Infections	20
Sepsis	42
Meningitis	2
Necrotizing Enterocolitis	5
Hyperbilirubinaemia	47
Interventricular hemorrhage	12
Other cerebral abnormalities	19
Patent Ductus Arteriosus	28
Indomethacin therapy	25
Surgical closure	1
No therapy	2
Congenital cardiac defects	8
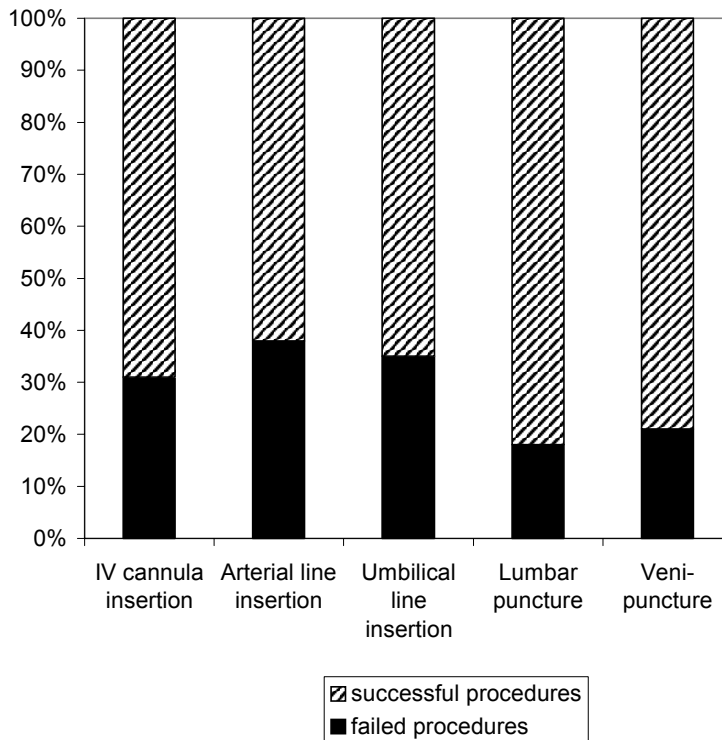
Figure 1   Percentages of failed and successful procedures

Painfulness of procedures

Two hundred forty-seven questionnaires were distributed, with a response rate of 59.9% (n = 148), which was similar for nurses and physicians (Table 5 gives their background characteristics). The mean pain score, across all respondents and all procedures, on the 10-point scale was 5.2 ± 1.3. The mean pain score per procedure varied from 1.7 ± 1.6 for diaper change to 8.9 ± 1.4 for intubation. Pain ratings given by the nurses and physicians are shown in figure 2.

Because procedures were scored on a 10-point scale, results of this questionnaire can be compared with a frequently used pain score, the Visual Analog Scale (VAS). VAS scores above 4 are generally used as a criterion for extra analgesic therapy.[45] Therefore, procedures with pain scores above 4 can be considered as moderately painful. Procedures that received lower scores included the control variables, diaper change and cranial ultrasound, as well as insertion of nasal cannulae, X-rays and the removal of nasogastric tubes. All other procedures were considered moderately to severely painful.
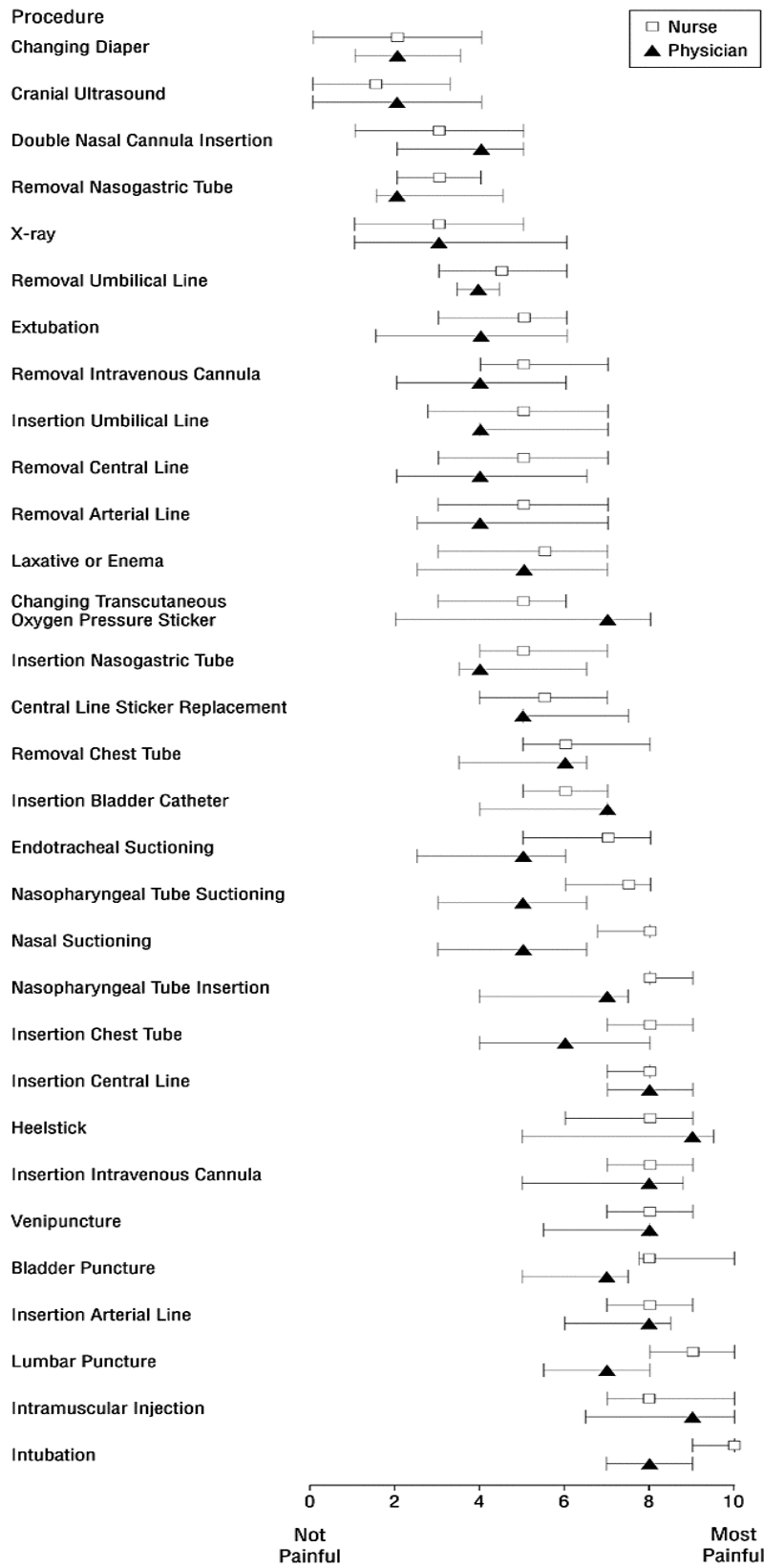
Figure 2 Questionnaire results per procedure are shown by median scores of physicians (triangles) and nurses (squares) and their interquartile ranges.

Table 3   Incidence of procedures with frequencies per infant per day

Procedure	% of total procedures	Frequency per infant per day, mean ± SD
Nasal suctioning	31.2	4.5 ± 2.3
Endotracheal suctioning	23.0	3.3 ± 4.0
NPT suctioning	9.4	1.3 ± 2.4
Heelstick	7.1	1.0 ± 1.6
IV cannula insertion	3.8	0.5 ± 0.6
Nasogastric tube insertion	3.8	0.5 ± 0.6
IV cannula removal	3.2	0.5 ± 0.7
Nasogastric tube removal	3.1	0.4 ± 0.5
X-ray	2.9	0.4 ± 0.9
NPT insertion	2.4	0.3 ± 0.6
Attempt IV cannula insertion	1.7	0.2 ± 0.9
Laxative or enema	1.2	0.2 ± 0.5
Nasal oxygen cannula insertion	1.0	0.2 ± 0.4
Intubation	0.9	0.1 ± 0.4
Peripheral arterial line insertion	0.8	0.1 ± 0.3
$TCPO_2$ sticker removal	0.8	0.1 ± 0.6
Extubation	0.7	0.1 .± 0.3
Peripheral arterial line removal	0.6	< 0.1 ± 0.3
Attempt arterial line insertion	0.5	< 0.1 ± 0.5
Venipuncture	0.4	< 0.1 ± 0.3
Insertion umbilical. Line	0.4	< 0.1 ± 0.2
Lumbar puncture	0.3	< 0.1 ± 0.2
Changing central line sticker	0.3	< 0.1 ± 0.2
Removal umbilical.line	0.3	< 0.1 ± 0.2
Bladder puncture	0.2	< 0.1 ± 0.2
Attempt umbilical line insertion	0.2	< 0.1 ± 0.2
Insertion central line	0.2	< 0.1 ± 0.2
Insertion chest tube	0.1	< 0.1 ± 0.2
Attempt to insert central line	0.1	< 0.1 ± 0.2
Venipuncture attempt	0.1	< 0.1 ± 0.2
Removal central line	0.1	< 0.1 ± 0.1
Removal chest tube	0.1	< 0.1 ± 0.1
Lumbar puncture attempt	0.1	< 0.1 ± 0.1
Intramuscular injection	< 0.1	< 0.1 ± 0.1

Abbreviations: IV, intravenous; NPT, nasopharyngeal tube; $TCPO_2$-sticker, transcutaneous oxygen-pressure sticker.

Table 4   Random regression model for predicting the frequency of procedures

variable*	ß	SE of ß	t	P Value
Nasal oxygen†	**0.18**	**0.02**	**8.57**	**< 0.001**
Continuous positive airway pressure	**0.40**	**0.02**	**19.40**	**< 0.001**
Ventilation†	**0.42**	**0.02**	**22.38**	**< 0.001**
Gestational age	-0.001	0.002	-0.65	**0.51**
Postnatal age	-0.00003	0.00008	-0.35	**0.72**
Day of admission	0.001	0.002	0.67	**0.50**
Day 1 vs day 2-14‡	**-0.21**	**0.03**	**-8.18**	**0.0001**
Length of stay	**0.02**	**0.001**	**13.81**	**0.0001**

The outcome variable was the frequency of procedures. Boldface indicates significant predictor variables (P < 0.05)
† Dummy coding was used, with no respiratory support as reference group.
‡ Day of admission was dichotomized as 0 (day 1) vs 1 (day 2-14).

Table 5   Background characteristics of 148 questionnaire respondents

Characteristic	Nurses (n = 119)	Physicians (n = 29)
Participants		
NICU Rotterdam, the Netherlands	49	9
PSICU Rotterdam	31	6
NICU Zwolle, the Netherlands	39	14
Age, mean ± SD, y	37 ± 7	34 ± 7
Male / female	7 / 112	12 / 17
Parent / not parent	64 / 55	12 / 17
NICU experience, mean ± SD, y	7.9 ± 6.2	4.2 ± 6.6

Abbreviations: NICU, neonatal intensive care unit; PSICU, pediatric surgical intensive care unit.

Multiple regression analyses (Table 6) showed that nurses scored various procedures as more painful than physicians ($\beta = 0.30$, $P = 0.001$), caregivers who were parents themselves scored procedures lower than persons without children ($\beta = 0.27$, $P = 0.002$), and ratings between the 2 hospitals were somewhat different ($\beta = 0.20$, $P = 0.03$). The multiple regression coefficient (R = 0.43) explained about 15% of the variance in the model.

Table 6   Multiple regression model for pain scores of 148 respondents

Variable*	ß (95% confidence interval)	t	*P* value
Nurse vs physician	**-0.30 (-0.91 to 0.31)**	**-3.30**	**0.001**
Sex	0.02 (-0.67 to 0.71)	0.17	0.87
Age	-0.01 (-0.05 to 0.03)	-0.12	0.91
Parent, no vs yes	**-0.27 (-0.70 to 0.16)**	**-3.12**	**0.002**
NICU experience, y	0.10 (0.06 to 0.14)	0.91	0.36
Hospital	**0.20 (-0.26 to 0.66)**	**2.2**	**0.03**
Unit, NICU vs PSICU	0.17 (-0.33 to 0.67)	1.9	0.06

Abbreviations: NICU, neonatal intensive care unit; PSICU, pediatric surgical intensive care unit
* The outcome variable was the mean pain score.
Boldface indicates significant predictor variables (P < 0.05)

Analgesic treatment
Pharmacological analgesic treatment was studied in 126 patients (Figure 3): 25 neonates were excluded because they were enrolled in a double-blind randomized controlled trial comparing morphine with placebo. Fifty patients (39.7%) did not receive any analgesic therapy during the study. Analgesic treatment regimens consisted of a morphine loading-dose (given before intubation in 67.8% of administrations), continuous morphine infusion, or morphine in combination with vecuronium bromide, midazolam hydrochlorate, or acetaminophen supplements. Fewer than 35% of newborns received analgesic therapy each day. Most therapy was used during the first day, when 41 infants (32.5%) received analgesics, and decreased within two weeks to 12.2 and 14.6% of infants receiving analgesics on days 13 and 14, respectively.

Analgesic therapy, pharmacological and nonpharmacological, was rarely applied before invasive procedures. Analgesics were generally used only before tracheal intubation or insertion of a chest tube and usually were not given in association with other procedures with high pain scores. Although some nurses used pacifiers and tried to comfort infants during and after procedures by handling, nonpharmacological analgesic treatment was not given routinely for any of the procedures.
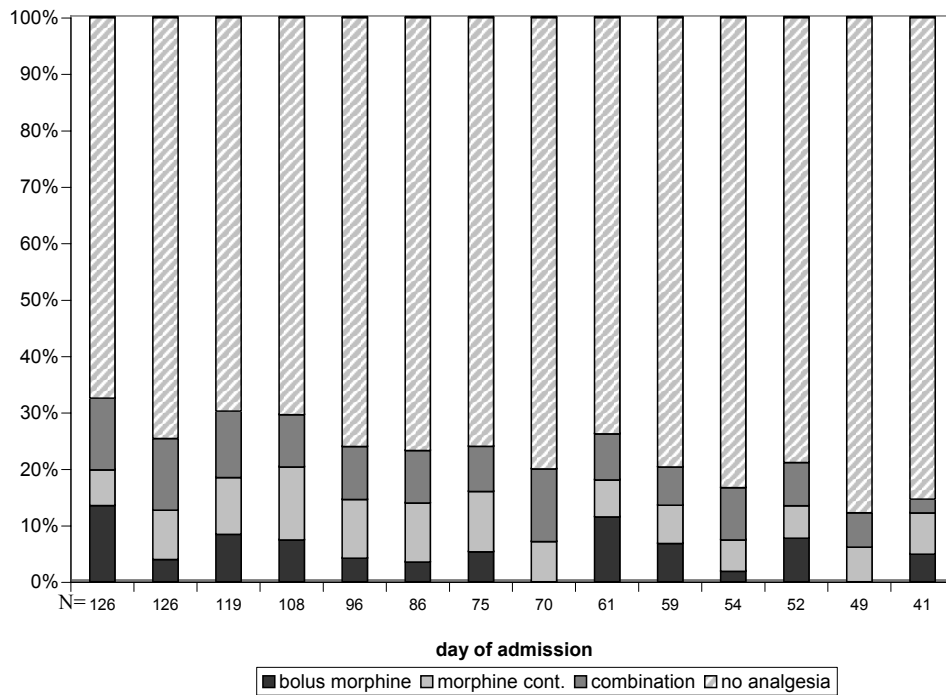
Figure 3   Pharmacological analgesic treatment per day of admission in percentages

Comment

In sharp contrast to the accumulating evidence that repetitive pain is harmful in newborns and despite major clinical advances over the past 10 years, neonates experience up to 14 painful procedures per day, and, remarkably, more than 65% of the patients in this study did not receive appropriate analgesic therapy.

We report a higher number of painful procedures in this study compared with previous studies, perhaps because we used a more extensive list of procedures and accounted for failed procedures (Table 1). For instance, Benis and Suresh[3] reported a mean of 6 procedures per day during the entire NICU stay of 15 neonates. Barker and Rutterl[1] reported an increased number of procedures in infants younger than 31 weeks' gestation compared with older infants, but did not mention length of NICU stay, which may explain the exposure to a greater number of invasive procedures. In the present study, however, the frequency of procedures per study day was not related to gestational age, perhaps because term neonates admitted to the NICU may have a severity of illness comparable to that of preterm neonates.

To our knowledge, we are the first to report prospective data on a substantial number of failed procedures in the NICU. Although attempts by even the most experienced

clinicians may be unsuccessful, the relative inexperience of trainees may partly explain the high proportion of failed procedures in this study. These findings may, therefore, be applicable to NICUs located in other academic centers.

Efforts should be aimed at minimizing the number of invasive procedures as stated in recent consensus guidelines.[37,38] Our study showed that the number of procedures is significantly higher in neonates requiring nasal oxygen, continuous positive airway pressure, or ventilation and that procedures occur mostly on the first day of admission, because of initial stabilization, monitoring, and diagnostic evaluation. Furthermore the number of heelsticks and venipunctures is subsequently decreased in patients having arterial lines. Our nursing protocols require tracheal suctioning every 4 hours and as needed for ventilated neonates, whereas a recent study showed comparable ventilatory outcomes when the frequency of routine suctioning was decreased from 4 to every 8 hours.[52]

Nurses and physicians agreed that most neonatal procedures cause moderate or severe pain,[45] with pain scores above 4 estimated for 26 of the 31 procedures. Physicians ascribed lower pain scores; because they are mostly responsible for prescribing analgesics, this may contribute to the limited use of analgesic therapy in neonates.[2] Others have reported comparable significant differences in pain scores of invasive procedures between nurses and physicians.[53,54] The clinicians not returning the questionnaire might be unmotivated and not interested in pain management in the NICU. However, a formal analysis of their motives was not undertaken. This lack of interest is probably related to their belief that these daily procedures are not that painful. As the response rate was not very high, the results of our questionnaire might show some overestimation of clinicians' overall opinion about the pain level of these procedures.

In our NICU, nurses tried to cluster procedures during routine nursing care, after which they comforted patients by touch, pacifiers or positioning (e.g. swaddling), whereas other behavioral and environmental approaches were used irregularly. Similar to our results, a multicenter study in France also showed minimal use of analgesics and a lack of standardization in the pharmacological regimens used in the NICU.[55] Kahn et al[56] reported a 28.6-fold variation in the use of opioids among 6 NICUs. Variations in attitude toward pain may limit the generalizability of our study findings to other centers around the world.

Restrained use of opioids by neonatologists can be explained by the fact that that there is wide disagreement as to whether the evidence base is sufficient to justify prolonged exposure to opioids in this vulnerable population. There is some evidence, from studies in rats, that neonatal morphine exposure causes specific long-term behavioral effects[57] and might cause retarded growth and motor development.[58] Underlying pathological mechanisms have been demonstrated by morphine-induced apoptosis in human fetal cell cultures,[59] and μ-opiate receptor down-regulation following morphine treatment in neonatal rat brain.[60] Prolonged use of high doses of opioids in animal and in-vitro models complicates extrapolation of these findings to daily NICU practice. The only study investigating long-term effects of human neonatal morphine treatment showed no effects in 5- to 6-year-olds.[61] Anand and colleagues[62,63] reported decreased mortality with the use of postoperative analgesia in premature infants. However, a similar benefit of routine use of morphine have not been reported in this population without a surgical operation. Grunau et al found that altered pain responses in preterm neonates were predicted by the number of previous painful procedures and were normalized by the early use of morphine analgesia.[21] Although accumulating data suggests that analgesic therapy with morphine might be useful to prevent some of the long-term effects of repetitive neonatal pain, further evidence about the safety of prolonged use of opioids is needed.

Conclusion

We recommend that a continuous intravenous infusion of opioids should be considered for infants requiring respiratory support during the first 24 hours of admission in combination with well validated pain scores as part of routine nursing care. Use of an algorithm would enable caregivers to respond immediately and in a structured way when they observe pain in these infants. Although this recommendation may be supported by preliminary studies,[39,64-67] results from larger randomized controlled trials are needed to decide if ventilated neonates should be routinely treated with continuous opioids.[68] As treatment regimens evolve, consistent practices in the NICU should be developed to minimize invasive procedures that continue to hurt our newborns.

Acknowledgements

What this study adds

As evidence about the short- and long-term harmful effects of pain in neonates accumulates, effort should be made to decrease the number of pain experiences and to improve analgesic therapy. This study reliably reports the number of painful procedures that neonates experience during an NICU stay. In contrast to the high incidence of painful procedures, we show the limited use of analgesic treatment, despite the awareness of nurses and physicians that most procedures are indeed painful.

References

1. Barker DP, Rutter N. Exposure to invasive procedures in neonatal intensive care unit admissions. Arch Dis Child Fetal Neonatal Ed. 1995;72(1):F47-48.

2. Johnston CC, Collinge JM, Henderson SJ, Anand KJS. A cross-sectional survey of pain and pharmacological analgesia in Canadian neonatal intensive care units. Clin J Pain. 1997;13(4):308-312.

3. Benis MM, Suresh GK. Frequency of invasive procedures in very low birth weight (VLBW) infants in the neonatal intensive care unit (NICU), abstract. Pediatric Research. 2001;49(2):392A, 2253.

4. Porter FL, Anand KJS. Epidemiology of pain in neonates. Res Clin Forums. 1998;20:9-18.

5. Franck LS. A national survey of the assessment and treatment of pain and agitation in the neonatal intensive care unit.PG - 387-93. J Obstet Gynecol Neonatal Nurs. 1987;16(6):387-393.

6. Tohill J, McMorrow O. Pain relief in neonatal intensive care. Lancet. 1990;336(8714):569.

7. Bauchner H, May A, Coates E. Use of analgesic agents for invasive medical procedures in pediatric and neonatal intensive care units. J Pediatr. 1992;121(4):647-649.

8. McLaughlin CR, Hull JG, Edwards WH, Cramer CP, Dewey WL. Neonatal pain: a comprehensive survey of attitudes and practices. J Pain Symptom Manage. 1993;8(1):7-16.

9. Fernandez CV, Rees EP. Pain management in Canadian level 3 neonatal intensive care units. Cmaj. 1994;150(4):499-504.

10. Giannakoulopoulos X, Teixeira J, Fisk N, Glover V. Human fetal and maternal noradrenaline responses to invasive procedures. Pediatr Res. 1999;45(4 Pt 1):494-499.

11. Smith RP, Gitau R, Glover V, Fisk NM. Pain and stress in the human fetus. Eur J Obstet Gynecol Reprod Biol. 2000;92(1):161-165.

12. Vanhatalo S, van Nieuwenhuizen O. Fetal pain? Brain Dev. 2000;22(3):145-150.

13. Craig KD, Whitfield MF, Grunau RV, Linton J, Hadjistavropoulos HD. Pain in the preterm neonate: behavioural and physiological indices. Pain. 1993;52(3):287-299.

14. Johnston CC, Stevens B, Yang F, Horton L. Developmental changes in response to heelstick in preterm infants: a prospective cohort study. Dev Med Child Neurol. 1996;38(5):438-445.

15. Anand KJS. Pain, plasticity, and premature birth: a prescription for permanent suffering? Nat Med. 2000;6(9):971-973.

16. Porter FL, Grunau RE, Anand KJS. Long-term effects of pain in infants. J Dev Behav Pediatr. 1999;20(4):253-261.

17. Fitzgerald M, Andrwes K. Neurophysiological and neurobiological correlates of supraspinal pain processing measurement techniques. In: McGrath PJ, ed. Progress in pain research and management. Seattle: IASP Press; 1998:21-46.

18. Anand KJS, Scalzo FM. Can adverse neonatal experiences alter brain development and subsequent behavior? Biol Neonate. 2000;77(2):69-82.

19. Anand KJS, Coskun V, Thrivikraman KV, Nemeroff CB, Plotsky PM. Long-term behavioral effects of repetitive pain in neonatal rat pups. Physiol Behav. 1999;66(4):627-637.

20. Ruda MA, Ling QD, Hohmann AG, Peng YB, Tachibana T. Altered nociceptive neuronal circuits after neonatal peripheral inflammation. Science. 2000;289(5479):628-631.

21. Grunau RE, Oberlander TF, Whitfield MF, Fitzgerald C, Lee SK. Demographic and therapeutic determinants of pain reactivity in very low birth weight neonates at 32 Weeks' postconceptional Age. Pediatrics. 2001;107(1):105-112.

22. Oberlander TF, Grunau RE, Whitfield MF, Fitzgerald C, Pitfield S, Saul JP. Biobehavioral pain responses in former extremely low birth weight infants at four months' corrected age. Pediatrics. 2000;105(1):e6.

23. Whitfield MF, Grunau RE. Behavior, pain perception, and the extremely low-birth weight survivor. Clin Perinatol. 2000;27(2):363-379.

24. Bhutta AT, Cleves MA, Casey PH, Cradock MM, Anand KJ. Cognitive and behavioral outcomes of school-aged children who were born preterm: a meta-analysis. Journal of the American Mediacal Association. 2002;288(6):728-737.

25. Grunau RV, Whitfield MF, Petrie JH, Fryer EL. Early pain experience, child and family factors, as precursors of somatization: a prospective study of extremely premature and fullterm children. Pain. 1994;56(3):353-359.

26. Peterson BS, Vohr B, Staib LH, et al. Regional brain volume abnormalities and long-term cognitive outcome in preterm infants. Jama. 2000;284(15):1939-1947.

27. Miceli PJ, Goeke-Morey MC, Whitman TL, Kolberg KS, Miller-Loncar C, White RD. Brief report: birth status, medical complications, and social environment: individual differences in development of preterm, very low birth weight infants. J Pediatr Psychol. 2000;25(5):353-358.

28. Cherkes-Julkowski M. Learning disability, attention-deficit disorder, and language impairment as outcomes of prematurity: a longitudinal descriptive study. Journal of Learning Disabilities. 1998;31(3):294-306.

29. Nadeau L, Boivin M, Tessier R, Lefebvre F, Robaey P. Mediators of behavioral problems in 7-year-old children born after 24 to 28 weeks of gestation. J Dev Behav Pediatr. 2001;22(1):1-10.

30. Rushe TM, Rifkin L, Stewart AL, et al. Neuropsychological outcome at adolescence of very preterm birth and its relation to brain structure. Dev Med Child Neurol. 2001;43(4):226-233.

31. Tideman E, Ley D, Bjerre I, Forslund M. Longitudinal follow-up of children born preterm: somatic and mental health, self-esteem and quality of life at age 19. Early Hum Dev. 2001;61(2):97-110.

32. Botting N, Powls A, Cooke RW, Marlow N. Attention deficit hyperactivity disorders and other psychiatric outcomes in very low birthweight children at 12 years. J Child Psychol Psychiatry. 1997;38(8):931-941.

33. Taylor HG, Klein N, Hack M. School-age consequences of birth weight less than 750 g: a review and update. Dev Neuropsychol. 2000;17(3):289-321.

34. Taylor HG, Klein N, Minich NM, Hack M. Middle-school-age outcomes in children with very low birthweight. Child Dev. 2000;71(6):1495-1511.

35. Ambalavanan N, Carlo WA. Analgesia for ventilated neonates: where do we stand? J Pediatr. 1999;135(4):403-405.

36. Kennedy KA, Tyson JE. Narcotic analgesia for ventilated newborns: are placebo-controlled trials ethical and necessary? J Pediatr. 1999;134(2):127-129.

37. Anand KJS. Consensus statement for the prevention and management of pain in the newborn. Arch Pediatr Adolesc Med. 2001;155(2):173-180.

38. Prevention and management of pain and stress in the neonate. American Academy of Pediatrics. Committee on Fetus and Newborn. Committee on Drugs. Section on Anesthesiology. Section on Surgery. Canadian Paediatric Society. Fetus and Newborn Committee. Pediatrics. 2000;105(2):454-461.

39. Bouwmeester NJ, Anand KJS, van Dijk M, Hop WC, Boomsma F, Tibboel D. Hormonal and metabolic stress responses after major surgery in children aged 0-3 years: a double-blind, randomized trial comparing the effects of continuous versus intermittent morphine. Br J Anaesth. 2001;87(3):390-399.

40. Lingen van RA, Deinum HT, Quak CM, Okken A, Tibboel D. Multiple-dose pharmacokinetics of rectally administered acetaminophen in term infants. Clin Pharmacol Ther. 1999;66(5):509-515.

41. Lingen van RA, Deinum JT, Quak JM, et al. Pharmacokinetics and metabolism of rectally administered paracetamol in preterm neonates. Arch Dis Child Fetal Neonatal Ed. 1999;80(1):F59-63.

42. Lingen van RA, Quak CM, Deinum HT, et al. Effects of rectally administered paracetamol on infants delivered by vacuum extraction. Eur J Obstet Gynecol Reprod Biol. 2001;94(1):73-78.

43. van Dijk M, Bouwmeester NJ, Duivenvoorden HJ, et al. Efficacy of continuous versus intermittent morphine administration after major surgery in 0-3-year-old infants; a double-blind randomized controlled trial. Pain. 2002;98(3):305-313.

44. van Dijk M, de Boer JB, Koot HM, Tibboel D, Passchier J, Duivenvoorden HJ. The reliability and validity of the COMFORT scale as a postoperative pain instrument in 0 to 3-year-old infants. Pain. 2000;84(2-3):367-377.

45. Van Dijk M, Koot HM, Saad HH, Tibboel D, Passchier J. Observational visual analog scale in pediatric pain assessment: useful tool or good riddance? Clin J Pain. 2002;18(5):310-316.

46. van Dijk M, Tibboel D. Review of Pain Assessment Measures for Postoperative Pain in Vulnerable Infants. Clin Perinatol. September 2002;29:469-491.

47. van Lingen R, Simons S, Anderson B, Tibboel D. The Effects of Analgesia in the Vulnerable Infant During Transition from the Intrauterine to the Extrauterine Environment. Clin Perinatol. September 2002;29.

48. van der Marel CD, van Lingen RA, Pluim MA, et al. Analgesic efficacy of rectal versus oral acetaminophen in children after major craniofacial surgery. Clin Pharmacol Ther. 2001;70(1):82-90.

49. van Lingen RA, Simons SH, Anderson BJ, Tibboel D. The effects of analgesia in the vulnerable infant during the perinatal period. Clin Perinatol. 2002;29(3):511-534.

50. Peters JW, Koot HM, de Boer JB, et al. Major surgery within the first 3 months of life and subsequent biobehavioral pain responses to immunization at later age: a case comparison study. Pediatrics. 2003;111(1):129-135.

51. The CRIB (clinical risk index for babies) score: a tool for assessing initial neonatal risk and comparing performance of neonatal intensive care units. The International Neonatal Network. Lancet. 1993;342(8865):193-198.

52. Cordero L, Sananes M, Ayers LW. A comparison of two airway suctioning frequencies in mechanically ventilated, very-low-birthweight infants. Respir Care. 2001;46(8):783-788.

53. Porter FL, Wolf CM, Gold J, Lotsoff D, Miller JP. Pain and pain management in newborn infants: a survey of physicians and nurses. Pediatrics. 1997;100(4):626-632.

54. Quinn MW, Baker J. Staff perception of pain on a neonatal intensive care unit. Arch Dis Child Fetal Neonatal Ed. 2001;85(1):F77.

55. Debillon T, Bureau V, Savagner C, Zupan-Simunek V, Carbajal R. Pain management in French neonatal intensive care units. Acta Paediatr. 2002;91(7):822-826.

56. Kahn DJ, Richardson DK, Gray JE, et al. Variation among neonatal intensive care units in narcotic administration. Arch Pediatr Adolesc Med. 1998;152(9):844-851.

57. Bhutta AT, Rovnaghi C, Simpson PM, Gossett JM, Scalzo FM, Anand KJ. Interactions of inflammatory pain and morphine in infant rats: long-term behavioral effects. Physiol Behav. 2001;73(1-2):51-58.

58. Handelmann GE, Dow-Edwards D. Modulation of brain development by morphine: effects on central motor systems and behavior. Peptides. 1985;6(Suppl 2):29-34.

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

60. Tempel A. Visualization of mu opiate receptor downregulation following morphine treatment in neonatal rat brain. Brain Res Dev Brain Res. 1991;64(1-2):19-26.

61. MacGregor R, Evans D, Sugden D, Gaussen T, Levene M. Outcome at 5-6 years of prematurely born children who received morphine as neonates. Arch Dis Child Fetal Neonatal Ed. 1998;79(1):F40-43.

62. Anand KJS, Sippell WG, Aynsley-Green A. Randomised trial of fentanyl anaesthesia in preterm babies undergoing surgery: effects on the stress response. Lancet. 1987;1(8524):62-66.

63. Anand KJS, Hickey PR. Halothane-morphine compared with high-dose sufentanil for anesthesia and postoperative analgesia in neonatal cardiac surgery. N Engl J Med. 1992;326(1):1-9.

64. Anand KJS, 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. 1999;153(4):331-338.

65. Dyke MP, Kohan R, Evans S. Morphine increases synchronous ventilation in preterm infants. J Paediatr Child Health. 1995;31(3):176-179.

66. Goldstein RF, Brazy JE. Narcotic sedation stabilizes arterial blood pressure fluctuations in sick premature infants. J Perinatol. 1991;11(4):365-371.

67. Saarenmaa E, Huttunen P, Leppaluoto J, Meretoja O, Fellman V. Advantages of fentanyl over morphine in analgesia for ventilated newborn infants after birth: A randomized trial. J Pediatr. 1999;134(2):144-150.

68. NEOPAIN Multicenter Group. Effects of Morphine Therapy on Neurologic Outcomes in Ventilated Preterm Neonates: Primary Outcomes from the NEOPAIN (Neurologic Outcomes & Pre-emptive Analgesia In Neonates) Multicenter Trial. Pediatric Research. 2002:abstract 2101.

Chapter 3

CURRENT STATUS OF PAIN MANAGEMENT IN NEONATES

Chapter **3.1**

ANALGESICS IN NEWBORNS AND INFANTS

Partly based on:

Richard A. van Lingen, Sinno H.P. Simons, Brian J. Anderson, Dick Tibboel.

The effects of analgesia in the vulnerable infant during the perinatal period

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&

Sinno H.P. Simons, Brian J. Anderson, Dick Tibboel.

Analgesic Agents

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Introduction

Neonates were believed to be unable to experience pain until the late 1980s. Several studies have changed our understanding of pain,[1] pain assessment[2] and analgesia in newborn neonates and infants.[3-6] As a consequence the importance of anti-nociceptive therapy in newborns has been increasingly acknowledged, leading to a burst of pain research in newborns. There is still limited knowledge about the metabolism for 80% of commonly used drugs,[7] such as analgesics. Moreover, rational drug therapy in newborns is often confounded by a combination of unpredictable and often poorly investigated pharmacokinetic and pharmacodynamic interactions.[8] The studies focussing on analgesic drug use during the newborn period are often limited to one dose, or to the first 24 or 48 hours of life.[3,4] Clinicians, however, seek information about effects during the neonatal intensive care stay, a period that extends from 1 to 2 weeks in selected babies. Therefore analgesics, such as morphine, fentanyl or acetaminophen, are still restrainedly used in the newborn infant.

Research has concentrated on the development of pain assessment instruments and clinical trials investigating the effectiveness and safety of a variety of analgesics in infants.[9,10] As neonates cannot verbalize pain, many observational instruments, containing behavioral and physiologic items have been developed and proved to be useful and reliable measures of postoperative and procedural pain in different age groups and to a lesser extent for those mentally handicapped. Knowledge about analgesic effects has been enlarged using pain assessment tools in randomized trials comparing different dose regimen and different agents.[11,12] These trials have given more insight into the specific pharmacokinetics (PK) and pharmacodynamics (PD) of analgesics in infants.[9]

Pediatric pharmacokinetics are dependent of the maturation of enzyme systems and physiological processes responsible for absorption and elimination. Changing body composition alters disposition.[13-15] Pharmacodynamic changes with age are poorly documented. Proported pharmacodynamic differences between children and adults for drugs such as morphine, for example, disappear once PK differences are accounted for.

This chapter concentrates on what is known from literature about pharmacokinetics and dynamics of opioids in neonates and infants. Special attention is given to the transition from fetal analgesia towards postnatal neonatal analgesia. Frequently used opioids in neonates are discussed.

Analgesia in utero

Adverse drug reactions during pregnancy, eventually contributing to the development of congenital anomalies, and uncertainty about short and long term adverse effects of drug administration in the neonate have made clinicians careful in their use of drugs that have not been attentively studied in neonates. Intrauterine surgery and prenatal procedures[16-18] have raised the controversies regarding the use of analgesia in utero. Giving analgesia to either the pregnant woman or directly to the fetus has few consequences with regard to fetal pharmacokinetics, as most of this is taken care of by the maternoplacental unit. The fetus is capable of metabolizing drugs. Wang et al,[19] for example, reported the presence of acetaminophen glucuronide and acetaminophen sulphate in fetal plasma; neither metabolite can cross the placenta and are thus produced by the fetus. There are, however, indications that giving analgesics does have pharmacodynamic effects.[16] The necessity of fetal analgesia is dictated by the ability of the fetus to feel pain and by the adverse effects of noxious stimuli on future sensory development.

Whether the fetus feels pain and from what gestational age has been widely discussed recently.[20,21] In the neuro-anatomical development of the pain-pathways the thalamo-cortical fibers, which are considered to be crucial for nociception, are present somewhere between the 20th and 34th week of gestation. The afferent fibers grow into the cortical plate at a gestational age of at least 26 weeks,[22] but between 20 and 26 weeks there are synaptic circuits between the subplate and the cortical plate possibly enabling the fetus to feel pain.[23] Evoked potential studies show that the distinct component signalling the arrival of sensory impulses at the cortex cannot be detected before 29 weeks of gestation.[24] So although the exact gestational age of nociceptive capability to feel pain has not readily been determined, the fetus is likely to have the nociceptive capability to feel pain from around 20-24 weeks of gestation.
The fetus can mount a stress response as shown by increases in noradrenaline from 18 weeks of gestation onwards,[25] ß-endorphin from 18 weeks of gestation,[16,17] and cortisol from 20 weeks of gestation.[16,26] Another indicator for the fetal stress response has been found in the redistribution of blood flow in the human fetus in response to stimuli.[16] The consciousness to experience pain might be associated with cerebral cortex activity. The suggested minimum gestation for this consciousness to be present is still under debate, and varies from six[27] to 26 weeks of gestation,[23] although it is also believed that consciousness develops at the moment of birth.[28]

The Royal College of Obstetricians and Gynaecologists recommends that practitioners who undertake diagnostic or therapeutic surgical procedures upon the fetus at or after 24 weeks should consider the requirements of fetal analgesia and sedation, either by agents given to the mother or directly.[23] Fisk et al.,[26] however, show that fetuses can react to intrauterine needling as early as 20 weeks, as fentanyl attenuates the fetal stress response, as shown by a decrease of the ß-endorphin response, but not the cortisol response.

The study of reactions in utero of single fetuses towards touch would lead to ethical and practical constraints. However twins are exposed to cutaneous stimulation from the co-twin, as can be seen with real-time ultrasound. Monoamniotic multiplets responded to tactile stimulation as early as 9 weeks.[29] Contact patterns vary according to the speed of initiatives and reactions of distinct parts of the body. Monoamniotic and monochoriotic twin pregnancies show more numerous contacts compared to dichorionic pregnancies because the membranes may prevent early reaching and touch in utero.[30] From the study of early fetal heart rate (FHR)/fetal movement analysis of singletons compared to twins it was concluded that inter-twin reactions contribute to an increased number of simultaneous FHR accelerations.[30] As such, both direct view by ultrasound and FHR can be used as indicators of pain. Next to the relief of possible fetal pain, analgesics reduce postoperative myometrial contraction of pregnant women after intrauterine fetal surgery and as a consequence may be of importance in preventing premature labor.[31]

Transition in drug metabolism

Although our knowledge of pain and its management in the perinatal period has increased,[32] still little is known about the first hours and days of life, when major physiological transition events occur. Transition from the intra-uterine to the extra-uterine environment in both preterm and term born neonates is a complicated process. Adaptation to extra-uterine life takes place in various organs during the first 24 to 48 hours. This process is already complicated in a term baby born after an uneventful pregnancy and delivery, but is especially troublesome in critically ill newborns with disturbed liver and kidney function and resulting aberrations in drug metabolism. In critically ill term born neonates and in preterm newborn neonates circulatory and pulmonary problems of prematurity and underlying pathology add to this. For example, prenatal and postnatal events that promote inflammation and infection may blunt the effects of resuscitation efforts, as increasing levels of circulating cytokines can result in persistent respiratory problems, hypovolemia and hypotension.[33]

Pharmacokinetics and drug metabolism change in the last trimester[34,35] and pain sensitivity may be altered after 32 weeks of gestation.[36] Consequently, dose and dose interval may vary considerably between neonates and within an individual during the first days of life. First of all, neonates might be in an unstable condition after birth with hypovolemia and hypotension. Analgesics and other drugs with α or β-mimetic effects given to the mother during labor might exacerbate adverse effects (e.g. hypotension) from fentanyl or morphine given to the neonate, hampering the doctor's efforts to stabilize the neonate. Whether analgesics given to the neonate increase stability is still unclear. Increased morbidity and mortality have been shown in infants receiving placebo infusions after surgery compared to infants receiving analgesics[37] highlighting the negative consequences of pain in infants. On the other hand adverse effects of analgesics, e.g. hypotension, may be harmful in critically ill infants.

Drug dose is determined by PK and PD considerations. Both the PK and PD in neonates are quite different from those reported in older children, as illustrated in figures 1 and 2. Dramatic changes in PK parameter estimates occur in the first weeks of life in term neonates because of enzyme system maturation, body composition changes and the withdrawal of maternal hormones, e.g gastrin/glucagon affecting stomach emptying.[46] These changes may be even more dramatic in the premature neonate. This sub-population is not homogenous and drug doses in a term neonate with a post-natal age of 2 weeks may be quite different from those at birth and are certainly different from those in a premature neonate. There is also large inter-individual parameter variability, making prediction in any one individual imprecise.

In view of different condition and body composition of preterm infants compared to older infants and adults there are both arguments for better analgesia and arguments for the opposite.[36] There are, however, so many changes going on in the neonate with regard to neural tissue, fat stores and muscle stores, that it is difficult to make predictions about any one drug. Reduced plasma albumin results in more unbound circulating drug but is of importance only with drugs that have a low V_D and high protein binding. Greater loss in the tissue with edema due to capillary leak will again only add significantly if the drug distributes to the extra-cellular fluid volume. Shifts in intracellular and extra-cellular water might also be of importance.
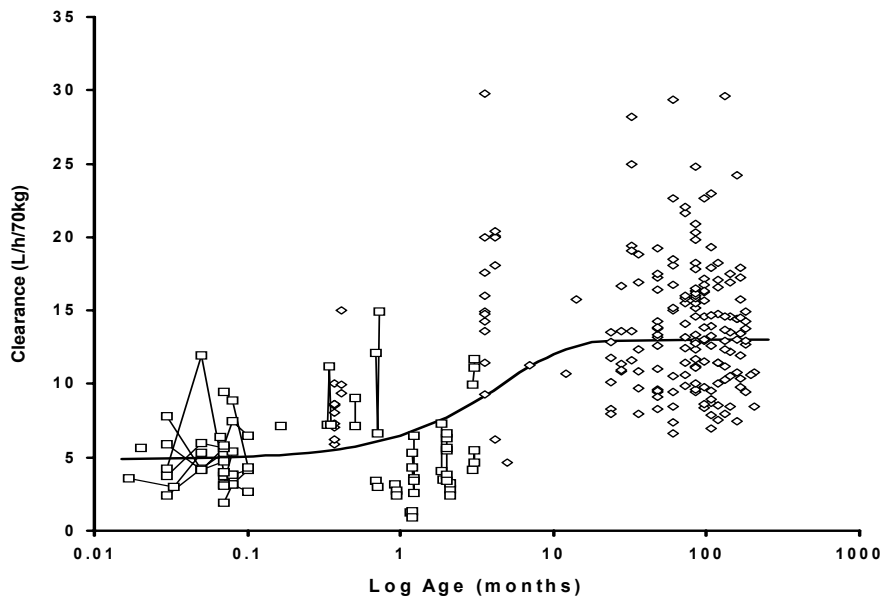
Figure 1 Clearance changes with age. The between occasion variability for each individual is demonstrated by linking estimates with a fine line. Individual predicted clearances, standardized to a 70 kg person, from NONMEM's post hoc step are plotted against age. The solid line demonstrates the predicted non-linear relationship between clearance and age (from Anderson et al. Br J Clin Pharmacol 2000;50:125-34).
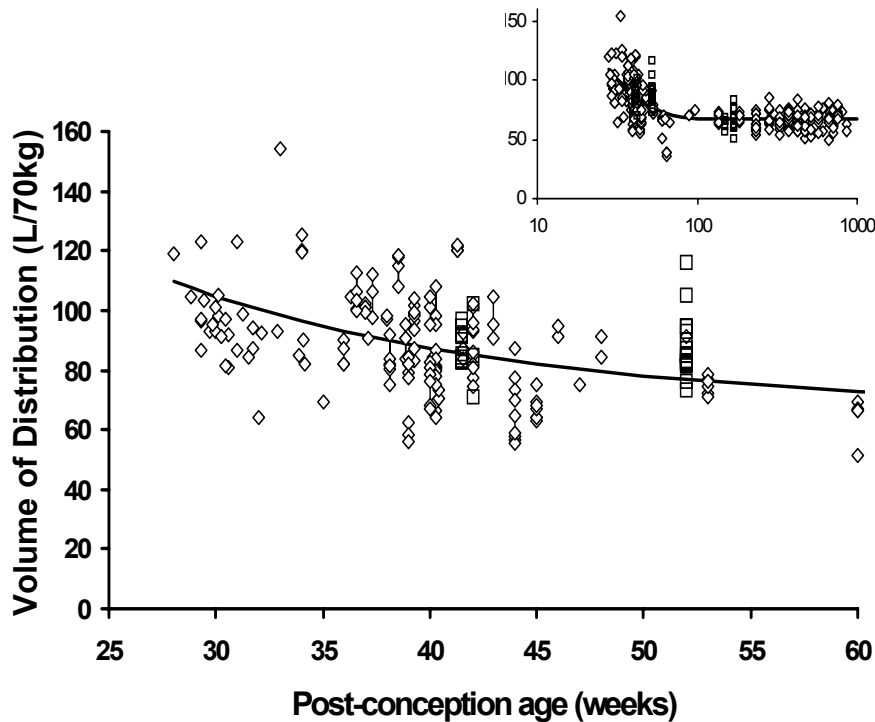
Figure 2 Volume of distribution changes with age up to 60 weeks post-conception. Individual predicted volumes, standardised to a 70 kg person, against age. The solid line demonstrates the non-linear relationship between volume of distribution and age. The figure inset includes older children and shows the Vstd of 66.6 l/70kg reached out of infancy. The x-axis (post-conception age in weeks) of the inset figure uses a log scale (from Anderson et al. Anaesthesiology 2002;96:1336-45).

Opioids in neonates

Opioid analgesics include naturally occurring agents (opium-alkaloids) and synthetic opioid-agonists that elicit morphine-like activity. The analgesic effects of opioids occur by activation of μ (mu), κ (kappa), and/or δ (delta) receptors in the CNS.[47] Each class of receptors is divided into subtypes that have different clinical effects. Analgesia is obtained by spinal or supraspinal activation of opioid-receptors, leading to decreased neurotransmitter release from nociceptive neurons inhibiting the ascending neuronal pain pathways and altering the perception and response to pain.[48] Opioid receptors also exist outside the central nervous system in the dorsal root ganglia and on peripheral terminals of primary afferent neurons.[49]

The World Health Organization (WHO) Analgesic Ladder is a generally accepted guideline for the supply of analgesics, originally developed for the treatment of cancer pain. Mild pain should be treated with non-opioid analgesics, acetaminophen or NSAIDs, moderate pain with 'weaker' opioids or combination products, and severe pain with stronger opioids (WHO, 1986). Opioids in children, infants and newborns are therefore reserved for moderate to severe types of pain, such as postoperative pain, sickle cell disease, and pain due to cancer, or as an additive to acetaminophen or NSAIDs if pain is moderate. Furthermore opioids may be used in the ICU for pain or stress related to artificial ventilation, surgical procedures (chest tube placement, vessel canulation for extracorporeal membrane oxygenation (ECMO)) or painful conditions like necrotizing enterocolitis. The most frequently used opioids are fentanyl and morphine, but codeine, oxycodone, methadone, hydromorphone and meperidine are used in children, as well as fentanyl derivatives such as alfentanil and sufentanil. Recommended starting doses are recently reviewed.[9] All doses should, however, be adjusted to clinical circumstances and titrated to the individual patient's needs. Doses aim to achieve a target concentration but the correlations between the analgesic plasma concentrations and validated pain scores are weak.[50,51]

Opioids produce adverse effects that may be minimized by appropriate drug selection and dosing. Respiratory depression, hypotension, glottic and chest wall rigidity, constipation, urinary retention, seizures, sedation, bradycardia are well described. Continuous monitoring and frequent assessment of vital signs should be performed during opioid administration. Naloxone is a competitive opioid receptor agonist that reverses many of these side-effects in appropriate dosage. Naloxone also antagonizes endorphin effects and

some morphine side-effects can be managed alternatively (e.g. neuromuscular blocking drugs for fentanyl muscle rigidity).

Although opiates are frequently used in newborn infants, individual differences in effectiveness of these drugs and their side-effects still seem to limit effective pain treatment. It has now been indicated that the variable effect of opiates results from genetic differences in receptor expression rates.[52,53]

The opioid system contains three major classes of opioid receptors: μ, κ, and δ, which have been further subclassified (μ1, μ 2, κ1, κ2, κ3, δ1, δ2) all modulating pain perception, with the exception of the κ2 receptor, which has not been adequately examined. Supraspinal systems have been described for μ1, κ3, and δ 2 receptors, while μ2, κ1, and δ1 receptors modulate pain at the spinal level. In addition to their ability to act independently, the various systems also interact synergistically with each other making the relief of pain a complex interaction of at least six receptor systems.[54] The opioid receptors mediate the potent analgesic actions of drugs and, when activated by endorphins regulate responses to pain, stress and emotions. The μ-opiate receptor (MOR) has been indicated to be the major site for the analgesic action of most clinically important opiate drugs. Transgenic knockout mice have shown that morphine is not analgesic in mice without MORs.[55-57] Furthermore, murine studies have shown that changes in MOR densities of 50%, or even less, can produce differences in nociceptive responses, as well as differences in modulation of opiates.[52,53] Polymorphisms of the human MOR gene, predicting the level of μ-receptor expression, would contribute to the individualisation of drug treatment (e.g. morphine, fentanyl). However, until now extensive research of the human MOR protein coding sequence failed in identifying variants that dramatically change the receptor function.

Morphine

Mechanism of action / metabolism

The pharmacist Sertürner first isolated morphine from opium in 1803 and named it after Morpheus, the god of dreams in Greco-Roman mythology. Ever since, it has been one of the most frequently used drugs to relieve pain in a variety of age groups. It also is the commonest used opioid for pediatric pain. Morphine is a member of the morphinan-framed alkaloids. The drug is soluble in water, but its solubility in lipids is poor compared with other opioids (table 1). Although morphine may also act on κ-opioid receptor subtypes,[58] the analgesic effect of morphine is mainly caused by an activation of μ-

receptors, as confirmed by a lack of analgesic effect of morphine in murine studies using μ-receptor knockout mice.[55-57] Alterations of the morphine molecular structure change the pharmacological activity and may have important clinical consequences (Figure 3). The most important positions on the morphine molecule, next to the nitrogen atom (probably responsible for the analgesic activity, as modifications reduce penetration into the central nervous system) are the phenolic hydroxyl at position 3 and the alcoholic hydroxyl at position 6. Morphine is mainly metabolized by the enzyme UDP-glucuronosyl transferase 2B7 (UGT2B7) into morphine-3-glucuronide (M3G) and morphine-6-glucuronide (M6G).[59] The latter has been shown to have higher analgesic potency than morphine[60,61] and also has respiratory depressive effects.[62,63] M3G has been suggested to antagonize the anti-nociceptive and respiratory depressive effects of morphine and M6G,[64,65] and contributes to the development of tolerance. The enzyme responsible for morphine glucuronidation, UGT2B7, is mainly found in the liver, but also exists in the intestines and kidneys.[66] Sulphation is a minor pathway.[67,68] The metabolites,[68] are cleared by the kidneys and partly by biliary excretion. Some recirculation of morphine occurs due to β-glucuronidase activity in the gut.[69] Impaired renal function leads to accumulation of M3G and M6G.[70]

Morphine may be administered by different routes. Administration in premature newborns is limited to the intravenous route. The painful administration of intra-muscular morphine injections is frowned upon. Subcutaneous intermittent boluses through an indwelling catheter offer an alternative route.[71] The large variability in plasma-levels observed after rectal administration is a major disadvantage of this route.[72] Oral morphine, either as elixir or slow release formulation offers a good alternative despite a high first pass effect. Epidural or intrathecal administration may cause delayed respiratory depression.[73] Patient controlled analgesia (PCA) is possible in some children aged as young as six years, nurse controlled analgesia (NCA) can be used effectively in younger children.[74]

Table 1   Onset, peak and duration of effects as well as lipid solubility of opioids

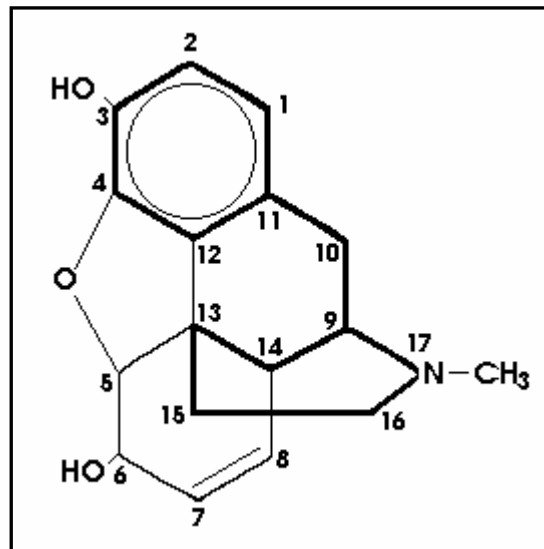|	**Morphine**	**Meperidine**	**Fentanyl**	**Sufentanil**	**Alfentanil**	**Remifentanil**
Peak effect (min)	45 – 90	20	3 - 4	5 – 6	1 - 2	1
Duration	4-5 hrs	2-4 hrs	30 min	30 min	15 min	5-10 min
Oil / H_2O	1.4	39	860	1.8	13.4	17.9

Adapted from: www.anaesthesist.com

Figure 3   The morphine molecule. The structure of morphine and all opium derivatives is characterized by the piperidine ring, which is indicated with bold lines. (From Goodman and Gilman's The Pharmacological Basis of Therapeutics, 1990).

Pharmacodynamics

A concentration-response relationship for morphine in children has not been described. The effectiveness of intravenous morphine in infants has been studied using validated pain assessment tools in different age groups. After major surgery continuous morphine doses of 10 to 40 $\mu g.kg^{-1}$ have been shown to be effective in alleviating pain in infants aged 0 to 14 years.[12,75-78] No difference in analgesic effect was found between continuous and intermittent dosing.[12] Intravenous morphine was shown to decrease pain in premature neonates requiring artificial ventilation with continuous doses of 10 to 30 $\mu g.kg^{-1}$.[4,11,79] Chay et al. have reported mean morphine concentrations required to produce adequate sedation in 50% of neonates to be 125 ng/ml,[80] but analgesic target plasma concentrations are generally believed to be around 15 to 20 ng/ml.[81,82] The large PK and PD variability means that morphine is often titrated to effect using small incremental doses (0.02 mg/kg) in children suffering postoperative pain.[83]

Pharmacokinetics

The effect compartment equilibration half-time (Teq) for morphine is approximately 17 minutes[84]. Morphine clearance matures with postconceptual age[13,85] and reaches adult rates at 6-12 months (table 2).[86-88] Fetuses have been shown to be capable of metabolizing morphine from 15 weeks gestation.[89,90] Morphine pharmacokinetic parameters show large inter-individual variability (CV 50% for clearance) that contributes to the range of

morphine serum concentrations observed during constant infusion.[88] Clinical circumstances, such as type of surgery, concurrent illness[68,82,91] and ECMO,[92,93] also influence morphine pharmacokinetics. Protein binding of morphine is low (from 20% in premature neonates[68,94] to 35% in adults[95]) and has no impact on disposition changes with age.[96]

Respiratory depression may occur at concentrations of 20 ng/ml.[82] Respiratory depression, as measured by carbon dioxide response curves or by arterial oxygen tension, is similar in children from 2 to 570 days of age at the same morphine concentration.[44] Further, intrathecal dosing causes similar respiratory depression at similar CSF concentrations in children aged 4 months to 15 years.[97]

Although morphine is considered in many NICU's as a factor contributing to hypotension, different settings and morphine dosage regimens used in studies make comparison of the available data difficult. Morphine has been shown to cause significant[38] and non-significant[39,40] decreases in blood pressure, as well as no effect at blood pressure at all.[41,42] Goldstein et al.[43] showed that morphine stabilized arterial blood pressure in sick premature infants. The hypotensive effect of morphine seems to be apparent when high dosages are used (e.g. 200 µg/kg/2h followed by 25 µg/kg/h or more).[38] Higher morphine plasma concentrations are accompanied by more, or more severe, side-effects of morphine.[40,44] Therefore the hypotensive effect of morphine will probably be minimal with the currently recommended analgesic dosages.[45] On the other hand the hypotensive effect may be an indirect effect of analgesia established by morphine, since pain may be accompanied by heightened cardiac sympathetic activity.

Table 2   Age related clearances of morphine and fentanyl standardized to a 70 kg person.

	Morphine		**Fentanyl**
	CL allometric ¾ (l.h^{-1}.70kg^{-1})		CL allometric ¾ (l.h^{-1}.70kg^{-1})
Neonate (<1 week)	13.1 (8.7)	Neonate (< 1 month)	31.5 (5.2)
Neonate (1 wk-2 months)	18.4 (7.9)		
Infant (2-6 months)	48.8 (15.6)	Infant (1-6 months)	41.1 (3.2)
Infant (6 months-2.5 yrs)	51 (13-68)	Child (1-5 yrs)	34.1 (12.3)
Adult	63 (4)	Adult	43.0 (7.3)

Adapted from B.J. Anderson and G.H. Meakin, Scaling for size: some implications for paediatric anaesthesia dosing. Paediatric Anaesthesia 2002;12:205-219.

Hypotension, bradycardia and flushing are part of the histamine response to morphine and are associated with a rapid intravenous bolus administration.[98] The incidence of vomiting in children after tonsillectomy is related to morphine dose. Doses above 0.1 mg/kg were associated with a greater than 50% incidence of vomiting.[99]

Codeine

Codeine, or methylmorphine, is a morphine-like opioid, with one tenth the potency of morphine. It is mainly metabolized by glucuronidation, but minor pathways are N-demethylation to norcodeine and O-demethylation to morphine. Around 10% of codeine is metabolized to morphine. As codeine's affinity for opioid receptors is very low, the analgesic effect of codeine is mainly due to its metabolite morphine.[100] The p450 enzyme CYP2D6 catalyzes the metabolism of codeine to morphine. A genetic polymorphism of this enzyme causes distinct phenotypes, responsible for the presence of slow, extensive, and ultra-rapid extensive codeine metabolizers in the population.[101,102] A proportion of the population between 7 and 10 % is believed to be slow metabolizer of codeine,[102-104] but, this proportion has also been reported to be much higher.[102,103] Although codeine has been shown to cause no analgesic effect in the poor metabolizers, side-effects persist.[104] High incidences of adverse effects might be expected in patients who have an ultra-rapid extensive metabolism. These patients achieve higher morphine concentrations. Codeine metabolites and 10% of unmetabolized codeine are excreted in the urine. The plasma half-life of codeine is 3-4 hours.

Codeine can be given by intra-muscular, oral and rectal routes. Intravenous codeine is not recommended because of hypotensive effects.[105] Rectal codeine achieves lower concentrations than intra-muscular because of incomplete, slower, and more variable absorption.[106] In children, it is generally given in doses of 1 to 3 $mg.kg^{-1}.day^{-1}$. Codeine is often used in combination with acetaminophen or NSAIDs. The addition of codeine to acetaminophen has been shown to improve post-operative pain relief in infants.[107] The analgesic effect of acetaminophen (10-15 mg/kg) and codeine (1-1.5mg/kg) was comparable with that of ibuprofen (5-10 mg/kg) in children after tonsillectomy.[108]

Peak plasma concentration (Cmax) occurs 1 hour (Tmax) after oral administration. The plasma half-life is 3-3.5 hours. Following intra-muscular injection Cmax is reached after 30 minutes.[100] The half-life of codeine has been shown to be increased in infants with low

body weight[109] and administration (especially of codeine preparations with an antihistamine and decongestant) in the neonate may cause intoxication.[110] Codeine has been used in infants and neonates after major surgery as an adjunct to acetaminophen or NSAIDs (optimal doses oral codeine dose 1-1.5 mg/kg every 4-6 h, oral acetaminophen 20 mg/kg every 6 h in infants > 3months).[111]

The side-effects of codeine are broadly similar to those of other opioids. As codeine is metabolized into morphine, side-effects appear to be directly related to, and caused by, morphine plasma concentrations.[112] It is widely believed that codeine causes less side-effects, such as sedation and respiratory depression, compared with other opioids,[100] but there is little evidence for this.

The analgesic effect of codeine is dependent on its conversion to morphine. Consequently other medications competing for the CYP2D6 enzyme (e.g. quinidine) may decrease the analgesic effect of codeine.

Oxycodone

Oxycodone is a semi-synthetic analgesic that is available as an immediate release product (oral solution and capsule), as well as a controlled release tablet for 12-hourly administration.

Immediate-release and controlled-release preparations of oxycodone have similar efficacy and comparable side-effect profiles in adults.[113] Oxycodone is very expensive and drugs such as controlled-release morphine and methadone offer cheaper alternatives.[113] Controlled-release oxycodone may be appropriate if the patient cannot tolerate other controlled-release or long-acting opioid analgesics. Olkkola et al showed that oxycodone (0.1 mg.kg^{-1}) in children after ophthalmic surgery caused greater ventilatory depression compared to other opioids. A large multi-center trial is currently investigating safety and efficacy of immediate-release, controlled-release, as well as liquid oxycodone in the pediatric population.

Methadone

Methadone is a synthetic opioid with an analgesic potency similar to that of morphine but with a more rapid distribution and a slower elimination. Methadone is used as a maintenance drug in opioid addicted adults to prevent withdrawal. Methadone might have

beneficial effects because it is a long acting synthetic opioid, with a very high bioavailability by the enteral route. Although only few data on the efficacy and safety of methadone are available, methadone is widely used for the treatment of opioid withdrawal in neonates and children.[114,115] Intravenous methadone has been shown to be an effective analgesic for postoperative pain relief[116] and oral administration has been recommended as the first line opioid for severe and persistent pain in children.[117] Although a predominant role for methadone in the management of prolonged pain in neonates has been suggested, its use first needs to be further evaluated in a clinical research setting.[118] The few data on methadone pharmacokinetics show a slow elimination half-life with large interindividual variability (3.8-62 hours).[118] Methadone's lipid solubility is greater than that of morphine.[119] The increased lipid solubility and longer duration of effect give this drug potential for single shot epidural use.

Hydromorphone

Hydromorphone is a semi-synthetic congener of morphine with a potency of around 5-7.5 times that of morphine.[120] Hydromorphone is mainly metabolized into hydromorphone-3-glucuronide.[123]
Hydromorphone is used for chronic cancer pain and for post-operative analgesia. Its side-effect profile is comparable to that of other strong opioids and it does not convincingly demonstrate clinical superiority in adults over other strong opioid analgesics.[124] Only few data in children are available. Goodarzi showed that epidural hydromorphone caused less side-effects compared to morphine and fentanyl in children undergoing orthopedic procedures.[125] Patient-controlled analgesia with hydromorphone seems to result in similar analgesia and side-effects compared to morphine in children for the management of mucositis pain after bone marrow transplantation.[120] Plasma concentrations of around 4.7 ng/ml (range 1.9 to 8.9 ng/ml) relieve mucositis in children given PCA devices. In children time to peak concentration is 4 - 6 hours and clearance 51.7 ml/min/kg.[120,126]

Meperidine (pethidine)

Meperidine is a weak opioid, primarily μ-receptor, agonist that has a potency of 1/10th that of morphine. The analgesic effects are detectable within 5 minutes of intravenous administration and peak effect is reached within 10 minutes.[69,127] In adults meperidine is

metabolized to meperidinic acid and normeperidine. Meperidine was initially synthesized as an anti-cholinergic agent but was soon discovered to have analgesic properties. Although meperidine's anti-cholinergic effects were demonstrated in vivo, the anti-cholinergic effects on the biliary and renal tracts have not been demonstrated in vivo. Studies have clearly demonstrated that meperidine is no more efficacious in treating biliary or renal tract spasm than comparative μ-opioids. Meperidine was portrayed in practice and teaching as having unique clinical advantages.[128] As morphine results in better analgesia with less side-effects, there are no particular advantages of meperidine as an analgesic.[129] Accumulation of the metabolite normeperidine result in seizures and dysphoria.[130] Intramuscular administration of meperidine was frequently used in pediatric patients but this route of administration is used uncommonly now because it is painful. Meperidine's local anesthetic properties have been found useful for epidural techniques.[131]

Fentanyl

Fentanyl is a synthetic opioid acting as a "morphine-like agonist". Its potency is about 50 to 100 fold that of morphine, with a large postulated effect on the μ-receptor. Fentanyl has a wide margin of safety and beneficial effects on hemodynamic stability.[132,133] Furthermore, it has a rapid onset (Teq = 6.6 min) and short duration of action. This is probably due to the relative increased lipid solubility and molecular conformation, enabling efficient penetration of the blood-brain barrier. Fentanyl may be the preferred analgesic agent for critically ill patients with hemodynamic instability, patients with symptoms related to histamine release during morphine infusion or those with morphine tolerance. Because of its rapid onset of action and short duration of effect, fentanyl efficiently alleviates procedural pain.[134] It has been used in neonates on artificial ventilation[135] with broncho-pulmonary dysplasia, pulmonary hypertension and/or diaphragmatic hernia. One study showed a need to escalate dose during ECMO, indicating rapid development of tolerance.[136] Overall the use of synthetic opioids shows a more rapid tolerance (3-5 days) compared to morphine (2 weeks) and heroin (weeks).[118,137]

Fentanyl metabolism is related to the activity of the hepatic cytochrome p450 system (CYP3A4) and fentanyl is metabolized by oxidative N-dealkylation into nor-fentanyl and

hydroxylized.[138,139] All metabolites are inactive and a small amount of fentanyl is renally eliminated.[140]

Fentanyl has been shown to effectively prevent preterm neonates from surgical stress responses and to improve postoperative outcome.[37] Single fentanyl doses (3 μg.kg^{-1}) and infusion (1.1 μg.kg^{-1}.hr^{-1}) reduced physiologic and behavioral measures of pain and stress during mechanical ventilation in preterm neonates[3,141] as effectively as morphine.[135] Internationally recommended starting doses are, however, smaller. In older infants and children, fentanyl has been shown to be effective for the management of peri- and postoperative pain[142] and for the management of procedural pain. Fentanyl clearance may be impaired due to decreased hepatic blood flow (e.g. from increased intra-abdominal pressure)[143] in neonates after major abdominal surgery (e.g. omphalocele). Fentanyl also has a propensity for muscular rigidity.[144] Transdermal fentanyl can be used for severe cancer-related pain[145] or in palliative pediatric care.[146] Fentanyl plasma concentrations are not measurable until 2 hr after application, and there is a 8-16 hr latency until full clinical fentanyl effects are observed. Following removal, serum fentanyl concentrations decline gradually and fall to 50% in approximately 16 hours. This prolonged elimination half-life occurs because fentanyl continues to be absorbed from the skin where a fentanyl depot concentrates.[147] The systemic availability of fentanyl by this route is approximately 30% of that found using the intravenous route.[148]

Oral transmucosal fentanyl provides consistent analgesia for brief painful procedures.[149] Transdermal and transmucosal fentanyl use have not been studied in newborns.

Because fentanyl has very high lipid solubility, it is widely distributed in tissues. Its short duration of effect is due to redistribution to deep, lipid-rich compartments. Accumulation of fentanyl in lipid-rich tissues may redistribute slowly after discontinuation of therapy, resulting in prolonged periods of sedation and respiratory depression after an extended period of use.[150] The context-sensitive half-life after an infusion of 1 h is approximately 20 min, but it is 270 min after an 8 h infusion.[151] The clearance of fentanyl appears to be somewhat immature at birth but increases dramatically after birth. Fentanyl clearance is 70-80% of adult values in term neonates (table 2) and, standardized to a 70 kg person, already appears to reach adult levels within the first 2 weeks of life.[88,150] The volume of distribution of fentanyl at steady state is around 5.9 l/kg in term born neonates and decreases with age to 4.5 l/kg during infancy, 3.1 l/kg during childhood, and 1.6 l/kg in adults.[152] Initial plasma concentrations in pediatric patients are lower compared with adults due to larger distribution volumes

The intra-operative use of 3 µg.kg^{-1} fentanyl in infants did not result in respiratory depression or hypoxemia in a placebo controlled trial.[142] Only 3 out of 2000 non-intubated infants and children experienced short apneic episodes after a low dose of fentanyl for the repair of facial lacerations.[153] Fentanyl has similar respiratory depression in infants and adults when plasma concentrations are similar.[154]

Fentanyl, alfentanil and sufentanil are metabolized by CYP3A4, and other drugs that also use this enzyme (e.g. cyclosporine, erythromycin) may decrease clearance leading to an increased fentanyl plasma concentrations.[155,156] Acetaminophen has been shown to interact with fentanyl metabolism in in vitro,[157] although the clinical importance of this interaction is probably negligible.

Research investigating DNA polymorphisms has shown genetic variability of CYP3A4 with slow and rapid metabolizers. One of the main explanations for inter-individual variability in pharmacokinetics across a patient population appears to be the efficiency of drug metabolism arising from differences in enzyme expression levels and/or from the presence of allelic variants of the enzyme with compromised catalytic ability. In the near future individual tapering of drugs will be possible due to the increasing knowledge of this genetic make-up, so called pharmacogenetics.[158,159]

Alfentanil

Alfentanil is a synthetic opioid that is chemically a derivate of fentanyl. It has a rapid onset (Teq = 0.9 min), a brief duration of action, and 1/4 the potency of fentanyl. Alfentanyl has lower lipid solubility and causes less histamine release[51] than fentanyl. It is used as a procedural analgesic for pediatric patients because the onset of analgesia is rapid.[160] Sufficient analgesia during endotracheal intubation and suctioning has been found using 10-20 µg.kg^{-1} alfentanil in preterm neonates.[160-162] A target plasma concentration of 400 ng/ml is used in anesthesia. Metabolism is comparable to that of adults i.e. phase 1 via oxidative N-dealkalatiom, by CYP3A4,[138] and O-dealkalation, and then phase 2 conjugation to renally excreted end-products.[163] Alfentanil plasma protein binding increases from 65% in preterm neonates and 79% in term infants to around 90% in adults.[164,165] The volume of distribution is smaller in infants compared to adults.[166] Clearances of alfentanil, standardized to a 70 kg person, are similar at different ages (± 250-500 ml.min^{-1}.70kg^{-1}) except for the neonatal age group, in which clearances are decreased (± 20-60 ml.min^{-1}.70kg^{-1}). Consequently elimination half-life in children (40 to 68 minutes) is higher in the neonatal period. In premature neonates the half-life is as long as 6-9 hours.[167,168] Children with chronic renal failure or chronic hepatic disease do not

show impaired clearance of alfentanil.[169] Alfentanil must be used with neuromuscular blocking drugs in newborns, because of a very high incidence of rigidity.[160,170]

Sufentanil

Sufentanil is a most potent opioid analgesic that is 5-10 times more potent than fentanyl with a Teq of 6.2 min. Elimination of sufentanil has been suggested by *O*-demethylation and *N*-dealkylation in animal studies. Like fentanyl and alfentanil the P450 CYP3A4 enzyme is responsible for the *N*-dealkylation.[138] The amount of free sufentanil decreases with age (neonates: 19%, infants: 11%, children/adults: 8%) and is strongly correlated with the alpha 1-acid glycoprotein plasma concentration.[164] The lower concentration of alpha 1-acid glycoprotein in newborns and infants contributes to the increased free fraction of sufentanil in these age groups. Although sufentanil, fentanyl, alfentanil and remifentanil have high protein binding (> 70%) and have high hepatic (or non-hepatic for remifentanil) extraction ratios, protein binding changes are probably clinically unimportant[96] because dose is titrated to effect and clearance variability has greater impact.

Remifentanil

Remifentanil resembles fentanyl, sufentanil and alfentanil in chemical structure. It is a selective μ-receptor agonist with a higher potency compared with alfentanil. As the inhibitory neurotransmitter glycine is used as a carrier for remifentanil, it should not be used for spinal or epidural applications[171] and because of its short duration of action it is usually given as an infusion (Teq = 1.16 min).[172,173] Remifentanil is metabolized to carbonic acid. The metabolism is independent from liver and renal function. Remifentanil reacts with nonspecific esterase in tissue and erythrocytes.[174,175] Carbonic acid is excreted through the kidneys. Little pharmacokinetic data about remifentanil in children are available. Per kilogram models show decreasing clearances with age, with rates of 90 ml.kg^{-1}.min^{-1} in infants less than 2 years old, 60 mL.kg^{-1}.min^{-1} between 2 and 12 years of age and 40 ml.kg^{-1}.min^{-1} in adults.[73,176] Volume of distribution in children is smaller (± 200-300 ml.kg^{-1}) compared with adults (± 400 ml.kg^{-1}), and might be increased in young infants (± 450 ml.kg^{-1}).[73,176] Elimination half-life seems to be constant around 3 to 6 minutes.[176]

Administration of 1 µg.kg^{-1} intravenously followed by 0.1 to 1.0 µg.kg^{-1}.min^{-1} results in sufficient analgesia during surgery in children.[177-179] Because of its short duration, remifentanil seems to be ideally suited for pediatric neurosurgical patients who may require neurological assessment at completion of surgery.[180] Its use is accompanied by a high incidence of life-threatening respiratory depression already at sub-therapeutic concentrations.[181] As a result of a rapid development of µ-receptor tolerance with remifentanil use, higher subsequent opioid doses are required.

Intravenous remifentanil doses of 0.25 µg.kg^{-1}.min^{-1} appear to be safe and effective in neonates[182,183] but data concerning the use of remifentanil in this group are few.

Summary/conclusion

As is shown in this chapter, analgesia in newborns differs in many ways from that in older infants and adults. Both pharmacokinetics and pharmacodynamics need to mature during the first years of life, making pediatric analgesia even more complicated than in adults. Although much about analgesics in infants is already known, many gaps in current knowledge have been shown in this chapter, requiring more research in this area.

References

1. Anand KJS, Hickey PR. Pain and its effects in the human neonate and fetus. N Engl J Med. 1987;317(21):1321-1329.

2. Craig KD, Whitfield MF, Grunau RV, Linton J, Hadjistavropoulos HD. Pain in the preterm neonate: behavioural and physiological indices. Pain. 1993;52(3):287-299.

3. Lago P, Benini F, Agosto C, Zacchello F. Randomised controlled trial of low dose fentanyl infusion in preterm infants with hyaline membrane disease. Arch Dis Child Fetal Neonatal Ed. 1998;79(3):F194-197.

4. Scott CS, Riggs KW, Ling EW, et al. Morphine pharmacokinetics and pain assessment in premature newborns. J Pediatr. 1999;135(4):423-429.

5. van Lingen RA, Deinum HT, Quak CME, Okken A, Tibboel D. Multiple-dose pharmacokinetics of rectally administered acetaminophen in term infants. Clin Pharmacol Ther. 1999;66:509-515.

6. van Lingen RA, Deinum JT, Quak JM, et al. Pharmacokinetics and metabolism of rectally administered paracetamol in preterm neonates. Arch Dis Child Fetal Neonatal Ed. 1999;80(1):F59-63.

7. Kauffman RE. Status of drug approval processes and regulation of medications for children. Curr Opin Pediatr. 1995;7(2):195-198.

8. Blumer JL RM. Principles of neonatal pharmacology. In: Pediatric Pharmacology. Therapeutic principles in practice. Yaffe SJ, Arande JV (eds) WB Saunders Co Philadelphia. 1992:164-177.

9. Berde CB, Sethna N. Analgesics for the treatment of pain in children. N Engl J Med. 2002;347(14):1094-1103.

10. van Dijk M, Peters JWB, Bouwmeester J, Tibboel D. Are postoperative pain instruments useful for specific groups of vulnerable infants. Clin Perinatol. September 2002;29:469-491.

11. Anand KJS, 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. 1999;153(4):331-338.

12. van Dijk M, Bouwmeester NJ, Duivenvoorden HJ, et al. Efficacy of continuous versus intermittent morphine administration after major surgery in 0-3-year-old infants; a double-blind randomized controlled trial. Pain. 2002;98(3):305-313.

13. Faura CC, Collins SL, Moore RA, McQuay HJ. Systematic review of factors affecting the ratios of morphine and its major metabolites. Pain. 1998;74(1):43-53.

14. de Wildt SN, Kearns GL, Leeder JS, van den Anker JN. Glucuronidation in humans. Pharmacogenetic and developmental aspects. Clin Pharmacokinet. 1999;36(6):439-452.

15. de Wildt SN, Kearns GL, Leeder JS, van den Anker JN. Cytochrome P450 3A: ontogeny and drug disposition. Clin Pharmacokinet. 1999;37(6):485-505.

16. Teixeira J, Fogliani R, Giannakoulopoulos X, Glover V, Fisk NM. Fetal haemodynamic stress response to invasive procedures. Lancet. 1996;347(9001):624.

17. Giannakoulopoulos X, Sepulveda W, Kourtis P, Glover V, Fisk NM. Fetal plasma cortisol and beta-endorphin response to intrauterine needling. Lancet. 1994;344(8915):77-81.

18. Adzick NS, Harrison MR. Fetal surgical therapy. Lancet. 1994;343(8902):897-902.

19. Wang LH, Rudolph AM, Benet LZ. Pharmacokinetic studies of the disposition of acetaminophen in the sheep maternal-placental-fetal unit. J Pharmacol Exp Ther. 1986;238(1):198-205.

20. Vanhatalo S, van Nieuwenhuizen O. Fetal pain? Brain Dev. 2000;22(3):145-150.

21. Smith RP, Gitau R, Glover V, Fisk NM. Pain and stress in the human fetus. Eur J Obstet Gynecol Reprod Biol. 2000;92(1):161-165.

22. Mrzljak L, Uylings HB, Kostovic I, Van Eden CG. Prenatal development of neurons in the human prefrontal cortex: I. A qualitative Golgi study. J Comp Neurol. 1988;271(3):355-386.

23. Gynaecologists TRCoOa. Fetal-awareness-report of a working party. RCOG Press London. 1997.

24. Klimach VJ, Cooke RW. Maturation of the neonatal somatosensory evoked response in preterm infants. Dev Med Child Neurol. 1988;30(2):208-214.

25. Giannakoulopoulos X, Teixeira J, Fisk N, Glover V. Human fetal and maternal noradrenaline responses to invasive procedures. Pediatr Res. 1999;45(4 Pt 1):494-499.

26. Fisk NM, Gitau R, Teixeira JM, Giannakoulopoulos X, Cameron AD, Glover VA. Effect of direct fetal opioid analgesia on fetal hormonal and hemodynamic stress response to intrauterine needling. Anesthesiology. 2001;95(4):828-835.

27. Rawlinson P. Commission of Inquiry into Fetal Sentience. Christian Action Research and Education. 1996;Available at: www.rcog.org.uk.

28. Als H. Developmental care in the newborn intensive care unit. Curr Opin Pediatr. 1998;10(2):138-142.

29. Hepper P. Fetal Psychology: an embryonic science. In: Nijhuis JG (ed), Fetal behaviour. Developmental and perinatal aspects. Oxford University Press Oxford, New York and Tokyo. 1992:130-156.

30. Arabin B, Bos R, Rijlaarsdam R, Mohnhaupt A, van Eyck J. The onset of inter-human contacts: longitudinal ultrasound observations in early twin pregnancies. Ultrasound Obstet Gynecol. 1996;8(3):166-173.

31. Tame JD, Abrams LM, Ding XY, Yen A, Giussani DA, Nathanielsz PW. Level of postoperative analgesia is a critical factor in regulation of myometrial contractility after laparotomy in the pregnant baboon: implications for human fetal surgery. Am J Obstet Gynecol. 1999;180(5):1196-1201.

32. Stevens B, Koren G. Evidence-based pain management for infants. Curr Opin Pediatr. 1998;10(2):203-207.

33. de Bont ES, Martens A, van Raan J, et al. Tumor necrosis factor-alpha, interleukin-1 beta, and interleukin-6 plasma levels in neonatal sepsis. Pediatr Res. 1993;33(4 Pt 1):380-383.

34. Morselli PL, Rovei V. Placental transfer of pethidine and norpethidine and their pharmacokinetics in the newborn. Eur J Clin Pharmacol. 1980;18(1):25-30.

35. van den Anker JN, Schoemaker RC, Hop WC, et al. Ceftazidime pharmacokinetics in preterm infants: effects of renal function and gestational age. Clin Pharmacol Ther. 1995;58(6):650-659.

36. McIntosh N. Pain in the newborn, a possible new starting point. Eur J Pediatr. 1997;156(3):173-177.

37. Anand KJS, Sippell WG, Aynsley-Green A. Randomised trial of fentanyl anaesthesia in preterm babies undergoing surgery: effects on the stress response. Lancet. 1987;1(8524):62-66.

38. Wood CM, Rushforth JA, Hartley R, Dean H, Wild J, Levene MI. Randomised double blind trial of morphine versus diamorphine for sedation of preterm neonates. Arch Dis Child Fetal Neonatal Ed. 1998;79(1):F34-39.

39. Sabatino G, Quartulli L, Di Fabio S, Ramenghi LA. Hemodynamic effects of intravenous morphine infusion in ventilated preterm babies. Early Hum Dev. 1997;47(3):263-270.

40. Hartley R, Green M, Quinn M, Levene MI. Pharmacokinetics of morphine infusion in premature neonates. Arch Dis Child. 1993;69(1 Spec No):55-58.

41. Rutter N, Evans N. Cardiovascular effects of an intravenous bolus of morphine in the ventilated preterm infant. Arch Dis Child Fetal Neonatal Ed. 2000;83(2):F101-103.

42. van Lingen RA. Pharmacokinetics and pharmacodynamics of morphine and its metabolites: The effect of two different dose regimes in ventilated preterm infants. Rotterdam: Erasmus University; 2000.

43. Goldstein RF, Brazy JE. Narcotic sedation stabilizes arterial blood pressure fluctuations in sick premature infants. J Perinatol. 1991;11(4):365-371.

44. Lynn AM, Nespeca MK, Opheim KE, Slattery JT. Respiratory effects of intravenous morphine infusions in neonates, infants, and children after cardiac surgery. Anesth Analg. 1993;77(4):695-701.

45. Anand KJS. Systemic analgesic therapy. Anand KJS, Stevens BJ, McGrath PJ. Pain in Neonates 2nd Revised and Enlarged Edition,Elsevier Amsterdam. 2000;Vol 10:Appendix: page 180.

46. Lucas A, Bloom SR, Aynsley-Green A. Gut hormones and 'minimal enteral feeding'. Acta Paediatr Scand. 1986;75(5):719-723.

47. Inturrisi CE. Clinical pharmacology of opioids for pain. Clin J Pain. 2002;18(4 Suppl):S3-13.

48. Suresh S, Anand KJS. Opioid tolerance in neonates: mechanisms, diagnosis, assessment, and management. Semin Perinatol. 1998;22(5):425-433.

49. Stein C, Machelska H, Binder W, Schafer M. Peripheral opioid analgesia. Curr Opin Pharmacol. 2001;1(1):62-65.

50. Suri A, Estes KS, Geisslinger G, Derendorf H. Pharmacokinetic-pharmacodynamic relationships for analgesics. Int J Clin Pharmacol Ther. 1997;35(8):307-323.

51. Olkkola KT, Hamunen K. Pharmacokinetics and pharmacodynamics of analgesic drugs. In: Anand KJS, Stevens B, McGrath P, eds. Pain in Neonates 2nd Revised and Enlarged Edition. Amsterdam: Elsevier; 2000:135-158.

52. Uhl GR, Sora I, Wang Z. The mu opiate receptor as a candidate gene for pain: polymorphisms, variations in expression, nociception, and opiate responses. Proc Natl Acad Sci U S A. 1999;96(14):7752-7755.

53. Kieffer BL. Opioids: first lessons from knockout mice. Trends Pharmacol Sci. 1999;20(1):19-26.

54. Pasternak GW. Pharmacological mechanisms of opioid analgesics. Clin Neuropharmacol. 1993;16(1):1-18.

55. Loh HH, Liu HC, Cavalli A, Yang W, Chen YF, Wei LN. mu Opioid receptor knockout in mice: effects on ligand-induced analgesia and morphine lethality. Brain Res Mol Brain Res. 1998;54(2):321-326.

56. Matthes HW, Maldonado R, Simonin F, et al. Loss of morphine-induced analgesia, reward effect and withdrawal symptoms in mice lacking the mu-opioid-receptor gene. Nature. 1996;383(6603):819-823.

57. Sora I, Takahashi N, Funada M, et al. Opiate receptor knockout mice define mu receptor roles in endogenous nociceptive responses and morphine-induced analgesia. Proc Natl Acad Sci U S A. 1997;94(4):1544-1549.

58. Rahman W, Dashwood MR, Fitzgerald M, Aynsley-Green A, Dickenson AH. Postnatal development of multiple opioid receptors in the spinal cord and development of spinal morphine analgesia. Brain Res Dev Brain Res. 1998;108(1-2):239-254.

59. Coffman BL, Rios GR, King CD, Tephly TR. Human UGT2B7 catalyzes morphine glucuronidation. Drug Metab Dispos. 1997;25(1):1-4.

60. Osborne PB, Chieng B, Christie MJ. Morphine-6 beta-glucuronide has a higher efficacy than morphine as a mu-opioid receptor agonist in the rat locus coeruleus. Br J Pharmacol. 2000;131(7):1422-1428.

61. Murthy BR, Pollack GM, Brouwer KL. Contribution of morphine-6-glucuronide to antinociception following intravenous administration of morphine to healthy volunteers. J Clin Pharmacol. 2002;42(5):569-576.

62. Osborne R, Thompson P, Joel S, Trew D, Patel N, Slevin M. The analgesic activity of morphine-6-glucuronide. Br J Clin Pharmacol. 1992;34(2):130-138.

63. Thompson PI, Joel SP, John L, Wedzicha JA, Maclean M, Slevin ML. Respiratory depression following morphine and morphine-6-glucuronide in normal subjects. Br J Clin Pharmacol. 1995;40(2):145-152.

64. Gong QL, Hedner J, Bjorkman R, Hedner T. Morphine-3-glucuronide may functionally antagonize morphine-6-glucuronide induced antinociception and ventilatory depression in the rat. Pain. 1992;48(2):249-255.

65. Smith MT, Watt JA, Cramond T. Morphine-3-glucuronide--a potent antagonist of morphine analgesia. Life Sci. 1990;47(6):579-585.

66. Fisher MB, Vandenbranden M, Findlay K, et al. Tissue distribution and interindividual variation in human UDP-glucuronosyltransferase activity: relationship between UGT1A1 promoter genotype and variability in a liver bank. Pharmacogenetics. 2000;10(8):727-739.

67. Choonara I, Ekbom Y, Lindstrom B, Rane A. Morphine sulphation in children. Br J Clin Pharmacol. 1990;30(6):897-900.

68. McRorie TI, Lynn AM, Nespeca MK, Opheim KE, Slattery JT. The maturation of morphine clearance and metabolism. Am J Dis Child. 1992;146(8):972-976.

69. Koren G, Maurice L. Pediatric uses of opioids. Pediatr Clin North Am. 1989;36(5):1141-1156.

70. Choonara IA, McKay P, Hain R, Rane A. Morphine metabolism in children. Br J Clin Pharmacol. 1989;28(5):599-604.

71. Lamacraft G, Cooper MG, Cavalletto BP. Subcutaneous cannulae for morphine boluses in children: assessment of a technique. J Pain Symptom Manage. 1997;13(1):43-49.

72. Lundeberg S, Beck O, Olsson GL, Boreus LO. Rectal administration of morphine in children. Pharmacokinetic evaluation after a single-dose. Acta Anaesthesiol Scand. 1996;40(4):445-451.

73. Reich A, Beland B, van Aken H. Intravenous Narcotics and Analgesic Agents. In: Bissonnette B, Dalens BJ, eds. Pediatric Anesthesia. New York: McGraw-Hill:259-277.

74. Berde CB, Lehn BM, Yee JD, Sethna NF, Russo D. Patient-controlled analgesia in children and adolescents: a randomized, prospective comparison with intramuscular administration of morphine for postoperative analgesia. J Pediatr. 1991;118(3):460-466.

75. Beasley SW, Tibballs J. Efficacy and safety of continuous morphine infusion for postoperative analgesia in the paediatric surgical ward. Aust N Z J Surg. 1987;57(4):233-237.

76. Millar AJ, Rode H, Cywes S. Continuous morphine infusion for postoperative pain in children. S Afr Med J. 1987;72(6):396-398.

77. Farrington EA, McGuinness GA, Johnson GF, Erenberg A, Leff RD. Continuous intravenous morphine infusion in postoperative newborn infants. Am J Perinatol. 1993;10(1):84-87.

78. Esmail Z, Montgomery C, Courtrn C, Hamilton D, Kestle J. Efficacy and complications of morphine infusions in postoperative paediatric patients. Paediatr Anaesth. 1999;9(4):321-327.

79. Quinn MW, Wild J, Dean HG, et al. Randomised double-blind controlled trial of effect of morphine on catecholamine concentrations in ventilated pre-term babies. Lancet. 1993;342(8867):324-327.

80. Chay PC, Duffy BJ, Walker JS. Pharmacokinetic-pharmacodynamic relationships of morphine in neonates. Clin Pharmacol Ther. 1992;51(3):334-342.

81. Kart T, Christrup LL, Rasmussen M. Recommended use of morphine in neonates, infants and children based on a literature review: Part 2--Clinical use. Paediatr Anaesth. 1997;7(2):93-101.

82. Lynn A, Nespeca MK, Bratton SL, Strauss SG, Shen DD. Clearance of morphine in postoperative infants during intravenous infusion: the influence of age and surgery. Anesth Analg. 1998;86(5):958-963.

83. Anderson BJ, Persson M, Anderson M. Rationalising intravenous morphine prescriptions in children. Acute Pain. 1999;2:59-67.

84. Inturrisi CE, Colburn WA. Application of pharmacokinetic-pharmacodynamic modeling to analgesia. In: Foley KM, Inturrisi CE, eds. Advances in Pain Research and Therapy. Opioid Analgesics in the Management of Clinical Pain. New York: Raven Press; 1986:441-452.

85. Kart T, Christrup LL, Rasmussen M. Recommended use of morphine in neonates, infants and children based on a literature review: Part 1--Pharmacokinetics. Paediatr Anaesth. 1997;7(1):5-11.

86. Anderson BJ, McKee AD, Holford NH. Size, myths and the clinical pharmacokinetics of analgesia in paediatric patients. Clin Pharmacokinet. 1997;33(5):313-327.

87. van Lingen R, Simons S, Anderson B, Tibboel D. The Effects of Analgesia in the Vulnerable Infant During Transition from the Intrauterine to the Extrauterine Environment. Clin Perinatol. September 2002;29:511-534.

88. van Lingen RA, Simons SH, Anderson BJ, Tibboel D. The effects of analgesia in the vulnerable infant during the perinatal period. Clin Perinatol. 2002;29(3):511-534.

89. Pacifici GM, Sawe J, Kager L, Rane A. Morphine glucuronidation in human fetal and adult liver. Eur J Clin Pharmacol. 1982;22(6):553-558.

90. Pacifici GM, Franchi M, Giuliani L, Rane A. Development of the glucuronyltransferase and sulphotransferase towards 2-naphthol in human fetus. Dev Pharmacol Ther. 1989;14(2):108-114.

91. Pokela ML, Olkkola KT, Seppala T, Koivisto M. Age-related morphine kinetics in infants. Dev Pharmacol Ther. 1993;20(1-2):26-34.

92. Dagan O, Klein J, Bohn D, Koren G. Effects of extracorporeal membrane oxygenation on morphine pharmacokinetics in infants. Crit Care Med. 1994;22(7):1099-1101.

93. Peters JWB, Simons SHP, Allegaert KM, Uges DRA, Tibboel D. Morphine Profiles During Ecmo, the Effect of Concomitant Medication. European Society for Paediatric Research. 2002;Annual Scientific Meeting:abstract 201.

94. Bhat R, Chari G, Gulati A, Aldana O, Velamati R, Bhargava H. Pharmacokinetics of a single dose of morphine in preterm infants during the first week of life. J Pediatr. 1990;117(3):477-481.

95. Olsen GD. Morphine binding to human plasma proteins. Clin Pharmacol Ther. 1975;17(1):31-35.

96. Benet LZ, Hoener BA. Changes in plasma protein binding have little clinical relevance. Clin Pharmacol Ther. 2002;71(3):115-121.

97. Nichols DJ, Yaster M, Lynn AM, al e. Disposition and respiratory effects of intrathecal morphine in children. Anesthesiology. 1993;79:733-738.

98. Anand KJS. Systemic analgesic therapy. Anand KJS, Stevens BJ, McGrath PJ. Pain in Neonates 2nd Revised and Enlarged Edition,Elsevier Amsterdam. 2000;Vol 10:159-188.

99. Anderson BJ, Ralph CJ, Stewart AW, Barber C, Holford NH. The dose-effect relationship for morphine and vomiting after day-stay tonsillectomy in children. Anaesth Intensive Care. 2000;28(2):155-160.

100. William DG, Hatch DJ, Howard RF. Codeine phosphate in paediatric medicine. Br J Anaesth. 2001;86(3):413-421.

101. Chen ZR, Somogyi AA, Bochner F. Polymorphic O-demethylation of codeine. Lancet. 1988;2(8616):914-915.

102. Sindrup SH, Brosen K. The pharmacogenetics of codeine hypoalgesia. Pharmacogenetics. 1995;5(6):335-346.

103. Williams DG, Patel A, Howard RF. Pharmacogenetics of codeine metabolism in an urban population of children and its implications for analgesic reliability. Br J Anaesth. 2002;89(6):839-845.

104. Eckhardt K, Li S, Ammon S, Schanzle G, Mikus G, Eichelbaum M. Same incidence of adverse drug events after codeine administration irrespective of the genetically determined differences in morphine formation. Pain. 1998;76(1-2):27-33.

105. Parke TJ, Nandi PR, Bird KJ, Jewkes DA. Profound hypotension following intravenous codeine phosphate. Three case reports and some recommendations. Anaesthesia. 1992;47(10):852-854.

106. McEwan A, Sigston PE, Andrews KA, et al. A comparison of rectal and intramuscular codeine phosphate in children following neurosurgery. Paediatr Anaesth. 2000;10(2):189-193.

107. Tobias JD, Lowe S, Hersey S, Rasmussen GE, Werkhaven J. Analgesia after bilateral myringotomy and placement of pressure equalization tubes in children: acetaminophen versus acetaminophen with codeine. Anesth Analg. 1995;81(3):496-500.

108. St Charles CS, Matt BH, Hamilton MM, Katz BP. A comparison of ibuprofen versus acetaminophen with codeine in the young tonsillectomy patient. Otolaryngol Head Neck Surg. 1997;117(1):76-82.

109. Quiding H, Olsson GL, Boreus LO, Bondesson U. Infants and young children metabolise codeine to morphine. A study after single and repeated rectal administration. Br J Clin Pharmacol. 1992;33(1):45-49.

110. Magnani B, Evans R. Codeine intoxication in the neonate. Pediatrics. 1999;104(6):e75.

111. Cunliffe M. Codeine phosphate in children: time for re-evaluation? Br J Anaesth. 2001;86(3):329-331.

112. Poulsen L, Brosen K, Arendt-Nielsen L, Gram LF, Elbaek K, Sindrup SH. Codeine and morphine in extensive and poor metabolizers of sparteine: pharmacokinetics, analgesic effect and side effects. Eur J Clin Pharmacol. 1996;51(3-4):289-295.

113. Rischitelli DG, Karbowicz SH. Safety and efficacy of controlled-release oxycodone: a systematic literature review. Pharmacotherapy. 2002;22(7):898-904.

114. Suresh S, Anand KJS. Opioid tolerance in neonates: a state of the art review. Paediatric Anaesthesia. 2001;11:511-521.

115. Tobias JD. Tolerance, withdrawal, and physical dependency after long-term sedation and analgesia of children in the pediatric intensive care unit. Crit Care Med. 2000;28(6):2122-2132.

116. Berde CB, Beyer JE, Bournaki MC, Levin CR, Sethna NF. Comparison of morphine and methadone for prevention of postoperative pain in 3- to 7-year-old children. J Pediatr. 1991;119(1 (Pt 1)):136-141.

117. Shir Y, Shenkman Z, Shavelson V, Davidson EM, Rosen G. Oral methadone for the treatment of severe pain in hospitalized children: a report of five cases. Clin J Pain. 1998;14(4):350-353.

118. Chana SK, Anand KJS. Can we use methadone for analgesia in neonates? Arch Dis Child Fetal Neonatal Ed. 2001;85(2):F79-81.

119. Berkowitz BA. The relationship of pharmacokinetics to pharmacological activity: morphine, methadone and naloxone. Clin Pharmacokinet. 1976;1(3):219-230.

120. Collins JJ, Geake J, Grier HE, et al. Patient-controlled analgesia for mucositis pain in children: a three-period crossover study comparing morphine and hydromorphone. J Pediatr. 1996;129(5):722-728.

121. Cone EJ, Phelps BA, Gorodetzky CW. Urinary excretion of hydromorphone and metabolites in humans, rats, dogs, guinea pigs, and rabbits. J Pharm Sci. 1977;66(12):1709-1713.

122. Babul N, Darke AC. Putative role of hydromorphone metabolites in myoclonus. Pain. 1992;51(2):260-261.

123. Hagen N, Thirlwell MP, Dhaliwal HS, Babul N, Harsanyi Z, Darke AC. Steady-state pharmacokinetics of hydromorphone and hydromorphone-3-glucuronide in cancer patients after immediate and controlled-release hydromorphone. J Clin Pharmacol. 1995;35(1):37-44.

124. Quigley C. A Systematic Review of Hydromorphone in Acute and Chronic Pain. J Pain Symptom Manage. 2003;25(2):169-178.

125. Goodarzi M. Comparison of epidural morphine, hydromorphone and fentanyl for postoperative pain control in children undergoing orthopaedic surgery. Paediatr Anaesth. 1999;9(5):419-422.

126. Babul N, Darke AC, Hain R. Hydromorphone and metabolite pharmacokinetics in children. J Pain Symptom Manage. 1995;10(5):335-337.

127. Jaffe JH, Martine WR. Opioid analgesics and antagonists. In: Goodman Gilman A, Rall TW, Nies AS, Taylor P, eds. The pharmacological basis of therapeutics. New York: Pergamon Press; 1990:485-531.

128. Latta KS, Ginsberg B, Barkin RL. Meperidine: a critical review. Am J Ther. 2002;9(1):53-68.

129. Vetter TR. Pediatric patient-controlled analgesia with morphine versus meperidine. J Pain Symptom Manage. 1992;7(4):204-208.

130. Berde CB, Sethna NF. Analgesics for the treatment of pain in children. N Engl J Med. 2002;347(14):1094-1103.

131. Ngan Kee WD. Intrathecal pethidine: pharmacology and clinical applications. Anaesth Intensive Care. 1998;26(2):137-146.

132. Yaster M, Koehler RC, Traystman RJ. Effects of fentanyl on peripheral and cerebral hemodynamics in neonatal lambs. Anesthesiology. 1987;66(4):524-530.

133. Hickey PR, Hansen DD, Wessel DL, Lang P, Jonas RA, Elixson EM. Blunting of stress responses in the pulmonary circulation of infants by fentanyl. Anesth Analg. 1985;64(12):1137-1142.

134. Barrington KJ, Byrne PJ. Premedication for neonatal intubation. Am J Perinatol. 1998;15(4):213-216.

135. Saarenmaa E, Huttunen P, Leppaluoto J, Meretoja O, Fellman V. Advantages of fentanyl over morphine in analgesia for ventilated newborn infants after birth: A randomized trial. J Pediatr. 1999;134(2):144-150.

136. Arnold JH, Truog RD, Scavone JM, Fenton T. Changes in the pharmacodynamic response to fentanyl in neonates during continuous infusion. J Pediatr. 1991;119(4):639-643.

137. Franck LS, Vilardi J, Durand D, Powers R. Opioid withdrawal in neonates after continuous infusions of morphine or fentanyl during extracorporeal membrane oxygenation. Am J Crit Care. 1998;7(5):364-369.

138. Tateishi T, Krivoruk Y, Ueng YF, Wood AJ, Guengerich FP, Wood M. Identification of human liver cytochrome P-450 3A4 as the enzyme responsible for fentanyl and sufentanil N-dealkylation. Anesth Analg. 1996;82(1):167-172.

139. Labroo RB, Paine MF, Thummel KE, Kharasch ED. Fentanyl metabolism by human hepatic and intestinal cytochrome P450 3A4: implications for interindividual variability in disposition, efficacy, and drug interactions. Drug Metab Dispos. 1997;25(9):1072-1080.

140. Jacqz-Aigrain E, Burtin P. Clinical pharmacokinetics of sedatives in neonates. Clin Pharmacokinet. 1996;31(6):423-443.

141. Guinsburg R, Kopelman BI, Anand KJS, de Almeida MF, Peres Cde A, Miyoshi MH. Physiological, hormonal, and behavioral responses to a single fentanyl dose in intubated and ventilated preterm neonates. J Pediatr. 1998;132(6):954-959.

142. Barrier G, Attia J, Mayer MN, Amiel-Tison C, Shnider SM. Measurement of post-operative pain and narcotic administration in infants using a new clinical scoring system. Intensive Care Med. 1989;15(Suppl 1):S37-39.

143. Gauntlett IS, Fisher DM, Hertzka RE, Kuhls E, Spellman MJ, Rudolph C. Pharmacokinetics of fentanyl in neonatal humans and lambs: effects of age. Anesthesiology. 1988;69(5):683-687.

144. Taddio A. Opioid analgesia for infants in the neonatal intensive care unit. Clin Perinatol. 2002;29(3):493-509.

145. Collins JJ, Dunkel IJ, Gupta SK, et al. Transdermal fentanyl in children with cancer pain: feasibility, tolerability, and pharmacokinetic correlates. J Pediatr. 1999;134(3):319-323.

146. Hunt A, Goldman A, Devine T, Phillips M. Transdermal fentanyl for pain relief in a paediatric palliative care population. Palliat Med. 2001;15(5):405-412.

147. Lehmann KA, Zech D. Transdermal fentanyl: clinical pharmacology. J Pain Symptom Manage. 1992;7(3 Suppl):S8-16.

148. Sebel PS, Barrett CW, Kirk CJ, Heykants J. Transdermal absorption of fentanyl and sufentanil in man. Eur J Clin Pharmacol. 1987;32(5):529-531.

149. Schechter NL, Weisman SJ, Rosenblum M, Bernstein B, Conard PL. The use of oral transmucosal fentanyl citrate for painful procedures in children. Pediatrics. 1995;95(3):335-339.

150. Koehntop DE, Rodman JH, Brundage DM, Hegland MG, Buckley JJ. Pharmacokinetics of fentanyl in neonates. Anesth Analg. 1986;65(3):227-232.

151. Hughes MA, Glass PS, Jacobs JR. Context-sensitive half-time in multicompartment pharmacokinetic models for intravenous anesthetic drugs. Anesthesiology. 1992;76(3):334-341.

152. Johnson KL, Erickson JP, Holley FO, al e. Fentanyl pharmacokinetics in the paediatric population. Anesthesiology. 1984;61:A441.

153. Billmire DA, Neale HW, Gregory RO. Use of i.v. fentanyl in the outpatient treatment of pediatric facial trauma. J Trauma. 1985;25(11):1079-1080.

154. Hertzka RE, Gauntlett IS, Fisher DM, Spellman MJ. Fentanyl-induced ventilatory depression: effects of age. Anesthesiology. 1989;70(2):213-218.

155. Touw DJ. Clinical implications of genetic polymorphisms and drug interactions mediated by cytochrome P-450 enzymes. Drug Metabol Drug Interact. 1997;14(2):55-82.

156. Tanaka E. Clinically important pharmacokinetic drug-drug interactions: role of cytochrome P450 enzymes. J Clin Pharm Ther. 1998;23(6):403-416.

157. Feierman DE. The effect of paracetamol (acetaminophen) on fentanyl metabolism in vitro. Acta Anaesthesiol Scand. 2000;44(5):560-563.

158. Roses AD. Pharmacogenetics and the practice of medicine Pharmacogenetics and future drug development and delivery. Nature. 2000;405(6788):857-865.

159. Roses AD. Pharmacogenetics and future drug development and delivery. Lancet. 2000;355(9212):1358-1361.

160. Saarenmaa E, Huttunen P, Leppaluoto J, Fellman V. Alfentanil as procedural pain relief in newborn infants. Arch Dis Child Fetal Neonatal Ed. 1996;75(2):F103-107.

161. Pokela ML. Effect of opioid-induced analgesia on beta-endorphin, cortisol and glucose responses in neonates with cardiorespiratory problems. Biol Neonate. 1993;64(6):360-367.

162. Pokela ML, Koivisto M. Physiological changes, plasma beta-endorphin and cortisol responses to tracheal intubation in neonates. Acta Paediatr. 1994;83(2):151-156.

163. Davis PJ, Cook DR. Clinical pharmacokinetics of the newer intravenous anaesthetic agents. Clin Pharmacokinet. 1986;11(1):18-35.

164. Meuldermans W, Woestenborghs R, Noorduin H, Camu F, van Steenberge A, Heykants J. Protein binding of the analgesics alfentanil and sufentanil in maternal and neonatal plasma. Eur J Clin Pharmacol. 1986;30(2):217-219.

165. Wilson AS, Stiller RL, Davis PJ, et al. Fentanyl and alfentanil plasma protein binding in preterm and term neonates. Anesth Analg. 1997;84(2):315-318.

166. Meistelman C, Saint-Maurice C, Lepaul M, Levron JC, Loose JP, Mac Gee K. A comparison of alfentanil pharmacokinetics in children and adults. Anesthesiology. 1987;66(1):13-16.

167. Marlow N, Weindling AM, Van Peer A, Heykants J. Alfentanil pharmacokinetics in preterm infants. Arch Dis Child. 1990;65(4 Spec No):349-351.

168. Killian A, Davis PJ, Stiller RL, Cicco R, Cook DR, Guthrie RD. Influence of gestational age on pharmacokinetics of alfentanil in neonates. Dev Pharmacol Ther. 1990;15(2):82-85.

169. Davis PJ, Stiller RL, Cook DR, Brandom BW, Davis JE, Scierka AM. Effects of cholestatic hepatic disease and chronic renal failure on alfentanil pharmacokinetics in children. Anesth Analg. 1989;68(5):579-583.

170. Pokela ML, Ryhanen PT, Koivisto ME, Olkkola KT, Saukkonen AL. Alfentanil-induced rigidity in newborn infants. Anesth Analg. 1992;75(2):252-257.

171. Thompson JP, Rowbotham DJ. Remifentanil--an opioid for the 21st century. Br J Anaesth. 1996;76(3):341-343.

172. Patel SS, Spencer CM. Remifentanil. Drugs. 1996;52(3):417-427; discussion 428.

173. Duthie DJ. Remifentanil and tramadol. Br J Anaesth. 1998;81(1):51-57.

174. Egan TD. Remifentanil pharmacokinetics and pharmacodynamics. A preliminary appraisal. Clin Pharmacokinet. 1995;29(2):80-94.

175. Dershwitz M, Hoke JF, Rosow CE, et al. Pharmacokinetics and pharmacodynamics of remifentanil in volunteer subjects with severe liver disease. Anesthesiology. 1996;84(4):812-820.

176. Ross AK, Davis PJ, Dear Gd GL, et al. Pharmacokinetics of remifentanil in anesthetized pediatric patients undergoing elective surgery or diagnostic procedures. Anesth Analg. 2001;93(6):1393-1401, table of contents.

177. Davis PJ, Lerman J, Suresh S, et al. A randomized multicenter study of remifentanil compared with alfentanil, isoflurane, or propofol in anesthetized pediatric patients undergoing elective strabismus surgery. Anesth Analg. 1997;84(5):982-989.

178. Prys-Roberts C, Lerman J, Murat I, et al. Comparison of remifentanil versus regional anaesthesia in children anaesthetised with isoflurane/nitrous oxide. International Remifentanil Paediatric Anaesthesia Study group. Anaesthesia. 2000;55(9):870-876.

179. Donmez A, Kizilkan A, Berksun H, Varan B, Tokel K. One center's experience with remifentanil infusions for pediatric cardiac catheterization. J Cardiothorac Vasc Anesth. 2001;15(6):736-739.

180. German JW, Aneja R, Heard C, Dias M. Continuous remifentanil for pediatric neurosurgery patients. Pediatr Neurosurg. 2000;33(5):227-229.

181. Litman RS. Conscious sedation with remifentanil during painful medical procedures. J Pain Symptom Manage. 2000;19(6):468-471.

182. Chiaretti A, Pietrini D, Piastra M, et al. Safety and efficacy of remifentanil in craniosynostosis repair in children less than 1 year old. Pediatr Neurosurg. 2000;33(2):83-88.

183. Davis PJ, Galinkin J, McGowan FX, et al. A randomized multicenter study of remifentanil compared with halothane in neonates and infants undergoing pyloromyotomy. I. Emergence and recovery profiles. Anesth Analg. 2001;93(6):1380-1386, table of contents.

NEONATAL PAIN ASSESSMENT INSTRUMENTS

Monique van Dijk, Sinno H.P. Simons, Dick Tibboel

Introduction

This chapter examines various aspects of pain assessment in neonates, and gives an overview of the pain instruments currently available for use in neonates. Furthermore, differences in pain response between preterm and term neonates are accentuated. Finally, the different applications of pain measurement instruments – either research or clinical practice – and the psychometric standards are discussed.

The studies of Dr Anand and colleagues[1-3] on pain and analgesic use in preterm neonates in the late 1980s were instrumental in the growing awareness that premature neonates are not only able to experience pain but are more vulnerable to pain than older infants and adults are. A contemporary survey in 1988 among English anesthetists showed that while 80% of the respondents considered neonates capable of experiencing pain, still only 52% administered opioids to neonates after surgery.[4] Apart from expressing fear that opioids administration would lead to ventilatory depression, the respondents indicated that objective methods of determining pain intensity in neonates were lacking. From then on, efforts were made to improve pain management in this vulnerable age group and to develop valid and reliable pain assessment indices. Despite clinicians' awareness that daily performed procedures also account for a considerable quantity of pain, we have recently shown that most non-surgical newborns still do not receive adequate analgesic therapy. As this might partly be explained by shortcomings in the available instruments for measuring pain in these patients, we aimed to review these instruments.

Pain assessment instruments in the making

A variety of pain assessment instruments have been developed, based either on behavioral indicators of pain only or on a combination of behavioral and physiological indicators. While the combined instruments are multidimensional by nature, the others tend to focus on one behavioral aspect, for instance facial expression or body movement. The instruments have been reviewed extensively.[5-10] Table 1 lists the greater part of published multidimensional instruments for (premature) neonates.[11-28] Facial expression is the one indicator that is used in all of these sixteen instruments. Other frequently used indicators are body movement and/or (muscle) tone (14 out of 16), cry/vocalization (12 out of 16) and behavioral state or sleep pattern (10 out of 16).

Table 1   Content of multidimensional pain instruments (in chronological order) for (premature) neonates

Name (study)	Type of pain	Facial Expression	Body movement	Posture/tone	Cry/vocal	Behavioral state/sleep pattern	Physiological items	Consolability	Additional items
NIPS (Lawrence, 1993)	Procedural	√	√√		√	√	√		
BPS (Pokela, 1994)	Procedural	√	√	√				√	
PAT (Hodgkinson, 1994)	Postoperative	√	√	√	√	√	√√√√		Nurse's perception
CRIES (Krechel, 1995)	Postoperative	√			√	√	√√		
PIPP (Stevens, 1996)	Procedural/ postoperative	√√√				√	√√		Gestational age
LIDS (Horgan, 1996)	Postoperative	√	√√	√	√√	√			Spontaneous excitability
DSVNI (Horgon, 1996)	Procedural	√	√				√√√√√		
DAN (Carbajal, 1997)	Procedural	√	√		√				
SUN (Blauer, 1998)	Procedural	√	√	√	√	√	√		
COMFORT behavior (van Dijk, 2000)	Procedural/ postoperative	√	√	√	√	√			
CHIPPS (Buttner, 2000)	Postoperative	√	√√	√	√				
EDIN (Debillon, 2001)	Postoperative/ Prolonged pain	√	√			√		√	contact with nurses
BPNS (Cignacco, 2002)	Procedural	√		√	√	√	√	√	
PAIN (Hudson-Barr, 2002)	Procedural	√		√	√		√√√		
COVERS (Geiss, 2003)	Procedural	√	√		√		√√		
N-PASS (Hummel, 2003)	Postoperative /Sedation	√		√	√	√	√√√√		
Frequency of the indicators		16/16	14/16		12/16	10/16	10/16	3/16	

Note: multiple tick marks indicate number of items

Abbreviations: NIPS, Neonatal Infant Pain Scale; BPS, Behavioral Pain Score; PAT, Pain Assessment Tool; CRIES, Crying, requires Increased oxygen, Increased vital signs, Expression, Sleeplessness; PIPP, Premature Infant Pain Profile; LIDS, Liverpool Infant Distress Scale; DSVNI, Distress Score for Ventilated Newborn Infants; DAN, Douleur Aiguë Nouveau-né; SUN, Scale for Use in Newborns; CHIPPS, Children's and Infants' Postoperative Pain Scale; EDIN, Echelle Douleur Inconfort Nouveau-Né; BPNS, Bernese Pain Scale for Neonates; PAIN, Pain Assessment in Neonates; N-PASS, Neonatal-Pain Agitation and Sedation Scale.

Facial expression

Facial expression is generally considered the most sensitive indicator of pain in neonates.[29-31] Total facial activity and a cluster of specific facial actions (brow bulge, eye squeeze, nasolabial furrow and open mouth) have been shown to be significantly associated with pain.[31,32] One drawback of instruments based on facial expression is that they have primarily been tested during or directly after short painful procedures; another drawback is the phenomenon that neonates also show considerable variability in facial expression during non-painful episodes.[33] Furthermore, assessment of facial expression may in practice be hampered by limited view of the neonate's face, due to tapes or eye patches during photo-therapy. An example of a unidimensional instrument focussing on facial expression is the Neonatal Facial Coding System (NFCS), which assesses ten discrete facial actions, either from videotaped material[31] or from bedside observation.[34] Peters et al. established that reduction to five facial actions leaves the NFCS still valid for postoperative pain assessment in neonates.[35]

Total facial activity in most instruments is described in terms of 'relaxed' (score = 0) versus 'grimace' (score 1 or 2). Despite the general recognition of the sensitivity of facial expression for neonatal pain, only one multidimensional instrument, the Premature Infant Pain Profile (PIPP), includes specific facial aspects: brow bulge, eye squeeze and nasolabial furrow, giving much weight to facial expression.

Body movement and (muscle) tone

Pain assessment based on body movement may focus on activity of arms and legs, or more subtly, on the presence of clenched fists /toes when pain is felt.

An overall impression can be gained from posture or tone, to be assessed by mere observation or by touching the neonate's arm or leg. It has been suggested that tone or posture is more tense when pain is present. The unidimensional instrument Infant Body Coding System (IBCS) is based on body movement only.[29]

Grunau et al. were among the first to further determine specific pain responses in preterm neonates.[36,37] To this aim they used the Newborn Individualized Developmental Care and Assessment Program (NIDCAP®) observations. Within the NIDCAP system, movements reflect either stress or stability behaviors. Coding of facial and body movements is detailed. While twitches and startles were found not to be stress cues, finger splay and extension of extremities seemed useful as pain cues in preterm neonates.[36-38] This suggests that pain instruments which incorporate body movements in a global manner may be too imprecise in preterms. Furthermore, the immobile painful child may present a misleading picture if its body movements are used for pain assessment. The EDIN is the

only instrument that recognizes this aspect by scoring both permanent agitation and infrequent movements as indicators for pain.[25]

Crying
Cry features have been extensively studied using spectrographic apparatus. Pain-specific cry features suggested are a short latency from stimulus, longer duration of the first cry cycle, higher fundamental frequency, greater intensity in the upper ranges, etc.[32] A recent study among preterm neonates suggested that cry duration in this age group is not sensitive for pain, because they often do not cry in response to pain.[39] Pain instruments (see table 1) assess crying by either scoring intensity (whimpering, moaning vs. crying) or by scoring frequency (intermittent vs. long lasting). Some pain instruments, such as the NIPS[11] and N-PASS[28] take crying in intubated infants into account, by scoring a crying face without vocalization.

Behavioral state/sleep pattern
Although behavioral state is rather a modifying factor than an indicator of pain, it is incorporated in 10 out of the 16 instruments. However, they show different interpretations of this indicator. For instance, the PIPP includes behavioral state because sleeping infants exhibit fewer sustainable responses.[40,41] Sleeping infants, therefore, score higher on this PIPP item than those awake, which corrects for the less vigorous responses to acute pain when asleep. All other pain instruments do the opposite, and provide higher scores for those who are more awake – or who are unable to sleep. The underlying idea is that infants in pain are thought to have more difficulty to fall asleep. The EDIN for prolonged pain, focuses on sleeping pattern during the hours prior to the pain assessment.[25]

Consolability
Consolability, which assesses if an infant is consolable and how long it takes to calm the child in response to handling, is included in three of the sixteen neonatal pain instruments listed in Table 1.[12,25,26] A fourth one, the CRIES, includes a score for 'inconsolable' crying.[14] Consolability seems to be a subjective and vague concept as there is no standard procedure how to console a neonate.

Physiological parameters
Ten instruments include physiological indicators, such as heart rate, blood pressure, oxygen saturation, change in skin color, and breathing (frequency or irregularity).

Several of these use decrease in oxygen saturation, (or requirement for oxygen to maintain saturation > 95%) as an indicator for pain.[13-15,18,27,28] Some instruments, for example the DSVNI, have been designed for neonates receiving ventilatory support such as, others, such as the CRIES, for spontaneously breathing neonates; a number of instruments can be used in both conditions.

Heart rate is represented in pain instruments by either increase in beats per minute (bpm) or by percentage of increase.

A drawback of the physiological indicators is the fact that deviations may also be caused by the underlying illness, making these indicators less specific for pain.[42-44] Furthermore, daily medical interventions aim at keeping heart rate, blood pressure, and oxygen saturation at acceptable levels without treating pain. A mechanically ventilated neonate who has decreased oxygen saturation levels is treated by adjusting the ventilator and not by increasing analgesic treatment. With regard to the postoperative setting, several publications have confirmed that physiological parameters are not specific for pain assessment in this situation.[22,43,45]

All in all, there is limited information concerning the contribution of physiological items in neonatal pain assessment.[46]

Gestational age

Pain assessment in the premature neonate has been given increasing attention as well in the past ten years, due to the fact that more premature infants survive. Premature neonates are defined as those with gestational age < 37 weeks, but authors sometimes refer to extreme low birth weight babies (ELBW < 1000g) or very low birth weight babies (VLBW < 1500 g).

It has been suggested that a still immature central nervous system makes prematurely born neonates less sensitive to pain compared to full term born infants.[29,47-49] This is a misconception probably resulting from the fact that pain responses in preterm neonates are generally considered to be less robust than those in full term neonates. They show less facial expression, fewer body movements and do not always cry during painful procedures.[29,48] Johnston et al (1996) demonstrated increasingly more behavioral responses to heelstick in the same infants across 8 weeks of development.[47]

Barr (1998) stated that infants lacking energy due to the severity of their illness consequently are less capable of signaling pain e.g. by crying.[50] As their descending pain inhibitory fibers in the spinal cord are not yet fully developed, premature infants actually

seem to be more vulnerable to pain. Two pain instruments, PIPP and N-PASS, correct for less reactivity in the more premature neonates**.**

Applications of pain assessment

Pain assessment may be performed for research purposes or for daily bedside use. In any case, attention must be paid to psychometric and methodological issues.[51] Interobserver agreement between caregivers or between researchers should be improved through training and be established using an adequate interobserver agreement coefficient, such as the intraclass correlation coefficient or Cohen's Kappa.[52,53]

For both applications, it is important to determine occasions and frequency of scoring: before and after procedures, before and after the administration of analgesics and sedatives, or at set uneventful times as well? Some methodological issues, however, seem to be more important when pain assessment is applied for research purposes.

Research

When analgesic treatments are compared by means of pain instruments it is also important to determine in advance the minimum clinically significant change in pain scores before and after treatment, seeing that a significant difference between pain scores does not necessarily have clinical relevance.[54] Adult patients were shown to experience a substantive effect of treatment at about 30% pain relief.[55,56] Studies in children are less consistent. One study found a minimum clinically significant difference in VAS pain score for children aged 8 to 15 years (on a 100-mm VAS scale) to be 10 mm (95% confidence interval 7 to 12 mm).[57] A comparable study using the Colored Analogue Scale (CAS) in 121 children aged from 5 to 16 years showed a minimum significant change when CAS scores decreased with a median of 2.0 cm (IQR 1 to 3).[58] Corresponding figures have not yet been determined for patient groups dependent on observation rather than on self report, such as neonates.

Clinical practice

For pain assessment to be useful, it is vital to standardize pain assessment and to have a written policy, for instance in the shape of an algorithm, defining the actions to be taken when pain scores are too high.[59,60] Nurses will wholeheartedly assess pain only when they know that it will affect treatment. This consequently requires the availability of cut-off scores guiding pain treatment. Cut-off scores may be individualized, as proposed by the

PIPP,[7] in which for each infant a desirable individual baseline score is available for comparison with later pain scores. Although the advantage of this approach is that differences in temperament are taken into account,[10] neonates who are already painful at baseline seem to be disadvantaged. Another option is to determine cut-off scores in a large sample of comparable patients.[61] Table 2 shows the minimum and maximum scores for each pain instrument with suggested cut-off scores if available. For pain scales with scores ranging from 0 and 10, cut-off scores are below the midpoint, either at 3 or 4.

Table 2   Total score ranges, cut-off scores, if available, for the sixteen pain instruments

	Total score	**Cut-off score for analgesic treatment**
NIPS	0-7	$\geq 3^{66}$
BPS	0-12	-
PAT	0-20	≥ 6
CRIES	0-10	
PIPP	< 28 wks GA 3-21	≥ 12
	28 - 31.6 wks GA 2-20	
	32 - 35.6 wks GA 1-19	
	36 wks and more GA 0-18	
LIDS	0-40	-*
DSVNI	0-8 (behavioral items only)	-
DAN	0-10	≥ 3
SUN	0-28	-
COMFORT scale	6-30	≥ 17
CHIPPS	0-10	≥ 4
EDIN	0-15	≥ 7 severe pain
BPNS	0-21 (behavioral)	-
	0-27 (behavioral and physiological)	
PAIN	0-10	-
COVERS	?	-
N-PASS	0-10	≥ 4
	0-13 when GA < 28 wks	
	0-12 when GA 28-31 wks	
	0-11 when GA 32-35 wks	

Abbreviations: GA, gestational age.
* LIDS scores within an infant may be compared over time to detect inacceptable increases for that specific individual (personal communication)

Many instruments for use in daily care have been developed by clinicians or nurses, the very persons who know what indicators are useful. However, pain measurement instruments must also meet certain psychometric requirements, which are often neglected in instrument development. The following section goes into some of the issues involved.

Psychometric standards

Instrument development is the process through which an instrument is planned, constructed, evaluated, and modified. This process preferably makes use of a variety of skills including consideration of content requiring the experience of nurses and physicians working with hospitalized neonates. Next to this, psychometric expertise is required to guarantee optimal choice of item format and psychometric evaluation of the instrument. Other skills required are language expertise to phrase items simply and understandably, tailored to its users.

Unfortunately, lack of expertise seems to results in flaws, as shown by the examples described below. First, although the weighting of different indicators may vary, it should be based on theoretical or statistical grounds. Some instruments use several items for one aspect. For instance, the NIPS assess both arms and legs movement without a motivation for this emphasis on body movements. The PAIN uses three physiological items which together determine 50% of the total score, but the designers omit to justify the contribution of these items in the original publication.[27] Other instruments give varying scores for items (0 to 1 versus 0 to 2 for others) without motivation. Secondly, indefinite qualifiers of time may be used, such as '*restless sleep, awakens frequently*'[28] or *long lasting* crying.[19] Thirdly, response categories may be multiple. In the PIPP for example, the behavioral state score of 2 implies both 'active sleep', 'eyes closed' and 'facial movements'. What if only one or two of these phenomena are seen? The wording in instruments may also be inappropriate and inapplicable to neonates. For instance '*no complaints*' as a response category[19] or '*refuses to have contact with the nurse*'[25] seem rather misplaced for this age group.

Furthermore, the COMFORT scale, for instance, includes both frequency and extent of physical movements without the possibility to score all options. In contrast, the LIDS splits up crying into two items, one scoring quantity and one scoring quality of cry. We feel that response categories containing both quantity and quality should be avoided. Lastly, procedural issues concerning pain assessment are often lacking or incomplete.

Preferred duration of observation, for instance, is often left unmentioned. For the sake of comparison observations should be made during a predetermined time span. A few instruments such as the PIPP and COMFORT scale indeed prescribe duration, 30 seconds and two minutes, respectively.

Concluding remarks

A number of pain assessment instruments have been developed for neonates. Behavioral indicators such as facial expression, cry, body movements and posture are used in slightly different ways within these instruments. The usefulness of physiological indicators has not been tested sufficiently yet. Most caregivers acknowledge the limitations of pain assessment in neonates. In a subsequent chapter we examine the usefulness of physiological indicators during acute painful procedures. Our critique on current pain assessment instruments leaves room for improvement in behavioral pain assessment. However, the question remains, will we ever be able to discriminate between pain, distress, hunger or other sources of discomfort? The available pain instruments do not seem to provide for this discrimination. In the future, more advanced neurophysiological brain imaging will be useful to visualize which areas of the brain are active when premature neonates are experiencing pain. One ray of hope is the fact that comfort is more easily determined in premature neonates. In the Netherlands we have a special expression relating to comfort, rendered as: 'Costa Brava posture', i.e. when a preemie lies in the incubator in a relaxed fashion with the arms stretched along its head as though sunbathing on a beach ion the Costa Brava in Spain.

References

1. Anand KJS, Aynsley-Green A. Metabolic and Endocrine Effects of Surgical Ligation of Patent ductus arteriosus in the Human Preterm Neonate: Are There Implications for Further Improvement of Postoperative Outcome? Modern Problems in Paediatrics 1985;23:143-157.

2. Anand KJS, Sippell WG, Aynsley-Green A. Randomised trial of fentanyl anaesthesia in preterm babies undergoing surgery: effects on the stress response. Lancet 1987;1(8524):243-248.

3. Anand KJS, Hickey PR. Halothane-morphine compared with high-dose sufentanil for anesthesia and postoperative analgesia in neonatal cardiac surgery. New England Journal of Medicine 1992;326(1):1-9.

4. Purcell-Jones G, Dormon F, Sumner E. Paediatric anaesthetists' perceptions of neonatal and infant pain. Pain 1988;33:181-187.

5. Stevens BJ. Composite measures of pain. In: Finley GA, McGrath PJ, eds. Measurement of pain in infants and children. Progress in pain research and management. Seattle: IASP Press, 1998: 161-178.

6. Stevens BJ, Franck LS. Assessment and management of pain in neonates. Paediatr Drugs 2001;3(7):539-58.

7. Stevens BJ, Johnston CC, Gibbins S. Pain assessment in neonates. In: Anand KJS, Stevens BJ, McGrath PJ, eds. Pain in neonates, 2nd revised and enlarged edition. Amsterdam: Elsevier Science, 2000: 101-134.

8. Franck LS, Greenberg CS, Stevens B. Pain assessment in infants and children. Pediatr Clin North Am 2000;47(3):487-512.

9. Abu-Saad HH, Bours GJ, Stevens B, Hamers JP. Assessment of pain in the neonate. Semin Perinatol 1998;22(5):402-16.

10. Wong CM, McIntosh N, Menon G, Franck LS. The pain (and stress) in infants in a neonatal intensive care unit. Pain in infants, children, and adolescents, 2003.

11. Lawrence J, Alcock D, McGrath PJ, Kay J, Brock MacMurray S, Dulberg C. The development of a tool to assess neonatal pain. Neonatal Network 1993;12(6):59-66.

12. Pokela M. Pain relief can reduce hypoxemia in distressed neonates during routine treatment procedures. Pediatrics 1994;93(3):379-383.

13. Hodgkinson K, Bear M, J. T, Van Blaricum S. Measuring pain in neonates: evaluating an instrument and developing a common language. Australian Journal of Advanced Nursing 1994;12(1):17-22.

14. Krechel SW, Bildner J. CRIES: a new neonatal postoperative pain measurement score. Initial testing of validity and reliability. Paediatric Anaesthesia 1995;5(1):53-61.

15. Stevens BJ, Johnston CC, Petryshen P, Taddio A. Premature Infant Pain Profile: development and initial validation. Clinical Journal of Pain 1996;12(1):13-22.

16. Ballantyne M, Stevens B, McAllister M, Dionne K, Jack A. Validation of the premature infant pain profile in the clinical setting. Clin J Pain 1999;15(4):297-303.

17. Horgan M, Choonara IA. Measuring pain in neonates: an objective score. Paediatric Nursing 1996;8(10):24-27.

18. Sparshott MM. The development of a clinical distress scale for ventilated newborn infants. Journal of Neonatal Nursing 1996;April(2):5-11.

19. Carbajal R, Paupe A, Hoenn E, Lenclen R, Olivier-Martin M. [APN: evaluation behavioral scale of acute pain in newborn infants]. Arch Pediatr 1997;4(7):623-8.

20. Blauer T, Gerstmann D. A simultaneous comparison of three neonatal pain scales during common NICU procedures. Clinical Journal of Pain 1998;14(1):39-47.

21. Ambuel B, Hamlett KW, Marx CM, Blumer JL. Assessing distress in pediatric intensive care environments: the COMFORT scale. Journal of Pediatric Psychology 1992;17(1):95-109.

22. van Dijk M, de Boer JB, Koot HM, Tibboel D, Passchier J, Duivenvoorden HJ. The reliability and validity of the COMFORT scale as a postoperative pain instrument in 0 to 3-year-old infants. Pain 2000;84(2-3):367-77.

23. Buttner W, Finke W, Hilleke M, Reckert S, Vsianska L, Brambrink A. [Development of an observational scale for assessment of postoperative pain in infants]. [German]. Anasthesiol.Intensivmed.Notfallmed.Schmerzther. 1998;33(6):353-361.

24. Buttner W, Finke W. Analysis of behavioural and physiological parameters for the assessment of postoperative analgesic demand in newborns, infants and young children: a comprehensive report on seven consecutive studies. Paediatr Anaesth 2000;10(3):303-18.

25. Debillon T, Zupan V, Ravault N, Magny JF, Dehan M. Development and initial validation of the EDIN scale, a new tool for assessing prolonged pain in preterm infants. Arch Dis Child Fetal Neonatal Ed 2001;85(1):F36-41.

26. Cignacco E, Hamers JP, Gessler P. First validation of the 'Bernese pian scale for neonates'. 10th World Congress on pain 2002, San Diego, USA.

27. Hudson-Barr D, Capper-Michel B, Lambert S, Mizell Palermo T, Morbeto K, Lombardo S. Validation of the Pain Assessment in Neonates (PAIN) Scale with the Neonatal Infant Pain Scale. Neonatal Network 2002;21(6):15-21.

28. Hummel PA, Puchalski ML, Creech SD, Weiss MG. N-PASS: Neonatal pain, Agitation, and Sedation Scale - Reliability and Validity. Pediatric Academic Societies' Annual Meeting 2003, Seattle.

29. Craig KD, Whitfield MF, Grunau RVE, Linton J, Hadjistavropoulos HD. Pain in the preterm neonate: behavioural and physiological indices. Pain 1993;52(3):287-299.

30. Craig KD. The facial display of pain. In: Finley GA, McGrath PJ, eds. Measurement of pain in infants and children. Progress in pain research and management. Seattle: IASP Press, 1998: 103-122.

31. Grunau RVE, Craig KD. Facial activity as a measure of neonatal pain expression. In: Tyler DC, Krane EJ, eds. Advances in pain research and therapy. New York: Raven Press, 1990: 147-155.

32. Grunau RVE, Johnston CC, Craig KD. Neonatal facial and cry responses to invasive and non-invasive procedures. Pain 1990;42(3):295-305.

33. Barr RG. "Is this infant in pain?": Caveats from the clinical setting. American Pain Society Journal 1992;1:187-190.

34. Grunau RE, Oberlander T, Holsti L, Whitfield MF. Bedside application of the Neonatal Facial Coding System in pain assessment of premature neonates. Pain 1998;76(3):277-86.

35. Peters JW, Koot HM, Grunau RE, et al. Neonatal Facial Coding System for Assessing Postoperative Pain in Infants: Item Reduction is Valid and Feasible. Clin J Pain 2003;19(6):353-363.

36. Grunau RE, Holsti L, Whitfield MF, Ling E. Are twitches, startles, and body movements pain indicators in extremely low birth weight infants? Clin J Pain 2000;16(1):37-45.

37. Morison SJ, Holsti L, Grunau RE, et al. Are there developmentally distinct motor indicators of pain in preterm infants? Early Hum Dev 2003;72(2):131-46.

38. Holsti L, Grunau R, Oberlander T, Papsdorf M. Can the NIDCAP® Measure Acute Pain in Preterm Infants in the NICU? Pediatric Academic Societies' Annual Meeting 2003, Seattle.

39. Johnston CC, Sherrard A, Stevens B, Franck L, Stremler R, Jack A. Do cry features reflect pain intensity in preterm neonates? A preliminary study. Biol Neonate 1999;76(2):120-4.

40. Grunau RVE, Craig KD. Pain expression in neonates:facial action and cry. Pain 1987;28:395-410.

41. Stevens BJ, Johnston CC, Horton L. Factors that influence the behavioral pain responses of premature infants. Pain 1994;59(1):101-109.

42. Franck LS, Miaskowski C. Measurement of neonatal responses to painful stimuli: a research review. Journal of Pain and Symptom Management 1997;14(6):343-378.

43. van Dijk M, de Boer JB, Koot HM, et al. The association between physiological and behavioral pain measures in 0- to 3-year-old infants after major surgery. J Pain Symptom Manage 2001;22(1):600-9.

44. Sweet SD, McGrath PJ. Physiological measures of pain. In: Finley GA, McGrath PJ, eds. Measurement of pain in infants and children. Progress in pain research and management. Seattle: IASP Press, 1998: 59-81.

45. Buchholz M, Karl HW, Pomietto M, Lynn AM. Pain scores in infants: a modified infant pain scale versus visual analogue. Journal of Pain and Symptom Management 1998;15(2):117-124.

46. Pereira AL, Guinsburg R, de Almeida MF, Monteiro AC, dos Santos AM, Kopelman BI. Validity of behavioral and physiologic parameters for acute pain assessment of term newborn infants. Rev Paul Med 1999;117(2):72-80.

47. Johnston CC, Stevens B, Yang F, Horton L. Developmental changes in response to heelstick in preterm infants: a prospective cohort study. Developmental Medicine and Child Neurology 1996;38:438-445.

48. Johnston CC, Stevens BJ, Yang F, Horton L. Differential response to pain by very premature neonates. Pain 1995;61:471-479.

49. Xia C, Yang L, Zhao P, Zhang X. Response to pain by different gestational age neonates. J Huazhong Univ Sci Technolog Med Sci 2002;22(1):84-6.

50. Barr RG. Reflections on measuring pain in infants: dissocation in responsive systems and "honest signalling". Archives of Disease in Childhood Fetal and Neonatal Edition 1998;79:F152-F156.

51. Association AER, Association AP, Education NCoMi. Standards for Educational and Psychological Testing. Washington: American Educational Research Association, 1999.

52. Shrout PE, Fleiss J, L. Intraclass Correlations: Uses in Assessing Rater reliability. Psychological Bulletin 1979;86(2):420-428.

53. Cohen J. Weighted kappa: Nominal scale agreement provision for scaled disagreement or partial credit. Psychological Bulletin 1968;70(4).

54. McQuay HJ, Barden J, Moore RA. Clinically important changes-what's important and whose change is it anyway? J Pain Symptom Manage 2003;25(5):395-6.

55. Farrar JT, Berlin JA, Strom BL. Clinically important changes in acute pain outcome measures: a validation study. J Pain Symptom Manage 2003;25(5):406-11.

56. Farrar JT, Portenoy RK, Berlin JA, Kinman JL, Strom BL. Defining the clinically important difference in pain outcome measures. Pain 2000;88(3):287-94.

57. Powell CV, Kelly AM, Williams A. Determining the minimum clinically significant difference in visual analog pain score for children. Ann Emerg Med 2001;37(1):28-31.

58. Bulloch B, Tenenbein M. Assessment of clinically significant changes in acute pain in children. Acad Emerg Med 2002;9(3):199-202.

59. Gallo AM. The fifth vital sign: implementation of the Neonatal Infant Pain Scale. J Obstet Gynecol Neonatal Nurs 2003;32(2):199-206.

60. Hester NO, Foster RL, Jordan-Marsh M, Ely E, Vojir CP, Miller KL. Putting Pain Measurement into Clinical Practice. In: Finley GA, McGrath PJ, eds. Measurement of Pain in Infants and Children: Progress in Pain Research and Management. Seattle: IASP press, 1998.

61. van Dijk M, Peters JW, Tibboel D. Sensitivity to change of a pediatric postoperative pain instrument: considerations from clinical practice. 6th International Symposium of Paediatric Pain 2003, Sydney.

ROUTINE MORPHINE INFUSION IN PRETERM NEWBORNS WHO RECEIVED VENTILATORY SUPPORT: A RANDOMIZED CONTROLLED TRIAL

Routine morphine infusion in preterm newborns who received ventilatory support: a randomized controlled trial
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Abstract

Context

Newborns admitted to Neonatal Intensive Care Units (NICUs) undergo a variety of painful procedures and stressful events. Because the effect of continuous morphine infusion in preterm neonates has not been investigated systematically there is confusion regarding whether morphine should be used routinely in this setting.

Objective

To evaluate the effects of continuous intravenous morphine infusion on pain responses, incidence of intraventricular hemorrhage (IVH), and poor neurologic outcome (severe IVH, periventricular leukomalacia, or death).

Design, Setting, and Patients

A randomized, double-blind, placebo-controlled trial conducted between December 2000 and October 2002 in 2 level III NICUs in the Netherlands of 150 newborns who had received ventilatory support (inclusion criteria: postnatal age younger than 3 days and ventilation for less than 8 hours; exclusion criteria: severe asphyxia, severe IVH, major congenital malformations, and administration of neuromuscular blockers).

Interventions

Intravenous morphine (100 μg/kg + 10 μg/kg per hour) or placebo infusion was given for 7 days (or less because of clinical necessity in several cases).

Main outcome measures

The analgesic effect of morphine, as assessed using validated scales; the effect of morphine on the incidence of IVH; and poor neurologic outcome.

Results

The analgesic effect did not differ between the morphine and placebo groups, judging from the following median (interquartile range) pain scores: Premature Infant Pain Profile, 10.1 (8.2-11.6) vs 10.0 (8.2-12.0) ($P = .94$); Neonatal Infant Pain Scale, 4.8 (3.7-6.0) vs 4.8 (3.2-6.0) ($P = .58$); and visual analog scale, 2.8 (2.0-3.9) vs 2.6 (1.8-4.3) ($P = .14$), respectively. Routine morphine infusion decreased the incidence of IVH (23% vs 40%; $P = .04$), but did not influence poor neurologic outcome (10% vs 16%; $P = .66$).

In addition, analyses were adjusted for the use of additional 'open label' morphine (27% of morphine-group vs 40% of placebo group; *P* = .10).

Conclusions
Lack of a measurable analgesic effect and absence of a beneficial effect on poor neurologic outcome do not support the routine use of morphine infusions as a standard of care in preterm newborns who have received ventilatory support. Follow-up is needed to evaluate the long-term effects of morphine infusions on the neurobehavioral outcomes of prematurity.

Introduction

Morphine has been one of the most frequently used drugs to relieve pain in many age groups. Nevertheless, debate remains about whether morphine and analgesic therapy should serve as standard of care for preterm newborns who have received ventilatory support,[1] despite the recognition that all preterm neonates feel pain.

Lack of a 'gold standard' to assess neonatal pain, fear of adverse effects, and uncertainty about the long-term effects of opioids in the neurodevelopmental outcome of newborns, contribute to this clinical conundrum. Although numerous neonatal pain instruments are available they have been based and validated on models of acute pain.[2] It is difficult, therefore, to measure the analgesic effect of morphine in neonates. Suggested adverse effects of morphine are hypotension,[3-6] seizures,[7] bradycardia, decreased gastrointestinal motility,[8] intestinal obstruction, urinary retention and respiratory depression.[9,10] Although a few long-term effects of neonatal morphine exposure have been suggested from animal studies,[11-13] these effects seem to be minimal at 5-6 years in a cohort of former preterm infants.[14] On the other hand, morphine administration may decrease morbidity, such as intraventricular hemorrhage (IVH) and periventricular leucomalacia (PVL).[15] We hypothesized that continuous morphine infusions may improve outcomes and diminish pain responses of non-surgical neonates who have received ventilatory support in stressful conditions. Furthermore, repeated pain exposure may cause hypersensitivity and lower pain threshold in preterm neonates,[16-20] and morphine administration might protect preterm neonates from the harmful effects of pain on their short- and long-term outcomes.[21,22]

Consensus statements on the analgesic treatment of neonatal pain[23,24] have suggested the use of continuous opioid infusions for preterm neonates who have received ventilatory support. Studies[25-28] investigating intravenous opioids in neonates who have received ventilatory support do not provide conclusive evidence. Therefore, double-blind randomized controlled trials have been suggested as a means to resolve the uncertainty over whether and when to administer analgesics to critically ill neonates.[1,29]

Based on the protocol of a multicenter trial (NEOPAIN study), we performed a randomized double blind placebo controlled trial to evaluate the effect of continuous, intravenous morphine infusion on pain responses, the incidence of IVH, and poor neurologic outcomes (severe IVH, PVL or death) in preterm neonates who had received ventilatory support.
We tested the hypothesis that continuous morphine administration in neonates who had received ventilatory support would reduce both the degree of pain experienced and the incidence of poor neurologic outcome and IVH (all grades).

Methods

Patients
All neonates admitted to the Neonatal Intensive Care Unit (NICU) who required mechanical ventilation were eligible for inclusion. Other inclusion criteria were: postnatal age younger than 3 days, artificial ventilation for less than 8 hours, and indwelling (peripheral or umbilical) arterial catheter. Excluded were neonates with severe asphyxia (Apgar-score after 5 minutes of < 4 or cord blood pH < 7.0),[30] severe IVH (grade III or IVH plus apparent periventricular hemorrhagic infarction), major congenital malformations and facial malformations (eg cleft lip and palate), neurologic disorders, or receiving continuous or intermittent neuromuscular blockers.

Patients were recruited from 2 level III NICUs in the Netherlands: Erasmus MC- Sophia Rotterdam (center 1), a university hospital, and the Isala Clinics in Zwolle, a non-university hospital (center 2). Seventy-four percent of neonates admitted to the NICUs were born in the study hospital. The local ethics committees of the participating centers approved the study protocol.

The parents of eligible patients were asked to give written informed consent within 8 hours after endotracheal intubation. If possible, parents were informed about the study before the birth of their child. If consent was refused, information about morphine use of the patient involved was collected retrospectively and compared with information on the participants. Data from non-enrolled patients were not incorporated into other outcome analyses or pooled with that from any other patients. Enrolled patients were randomly allocated to receive a loading dose (100 μg/kg) followed by a continuous infusion (10 μg/kg per hour) of either morphine hydrochlorate or placebo (sodium chloride), both dissolved in 5% glucose. To prevent possible overdosing, the study medication loading dose was not given if a pre-intubation morphine loading dose had been given less than 3 hours before the start of the study. The use of masked study medication was continued for 7 days or less, as required by the patient's clinical condition. After 7 days, study-medication was weaned and stopped or replaced by open label morphine infusion.

If patients from either group were judged to be in pain or distress during masked study medication use, they were given additional morphine based on decisions of the attending physician (independent of the study). Allowed additional doses were 50 μg/kg followed by 5-10 μg/kg per hour of continuous open-label morphine.

Outcomes
Primary outcomes were defined as the analgesic effects of morphine, assessed by validated pain measurement instruments at baseline, before study medication, and 30 minutes after the loading dose, and twice daily at a standardized time point before, during, and after endotracheal suctioning. At each time-point, we videotaped the infants for 2 minutes with 2 cameras: one obtaining a whole-body image and the other focused on the patient's face. Simultaneously, the caregiving nurse applied the visual analog scale (VAS) at bedside. The VAS is a horizontal continuous ten-centimeter line with the anchors 'no pain' on the left side and 'extreme pain' on the right side. Observers estimate the level of pain from 0 to 10 by making a mark on the line. All nurses had been trained to assess neonatal pain. The videotapes were analyzed afterward using the Neonatal Infant Pain Scale (NIPS)[31] and the VAS during all moments and the Premature Infant Pain Profile (PIPP)[32] during suctioning. Videotapes were assessed by 2 researchers (N.J. and S.H.P.S.) with acceptable inter-rater reliability (intraclass correlation coefficient of 0.70 and 0.73 for the NIPS and PIPP, respectively, and 0.67 for the VAS score).

Secondary outcome measures were poor neurologic outcome defined as severe IVH, PVL or death within 28 days and the incidence of all grades of IVH. Other clinical outcome measures were also compared between the morphine and placebo groups, including duration of artificial ventilation, length of NICU-stay, incidence of co-morbidity, and number of painful procedures. Regarding duration of artificial ventilation we distinguished between the first ventilation period (including further periods of ventilation if the infant was extubated in between for < 24 hours) and the second ventilation period (all further periods of artificial ventilation, after extubation > 24 hours). During the first 14 days of a patient's NICU admission, we recorded all painful procedures.

A power-analysis showed that 75 patients per group were needed to achieve a medium effect size (Cohen $d = 0.55$), with an α error of .05 (2-tailed) and a power of 90%. Neonates had an equal probability of being assigned to either condition. The randomization code was developed using a computer random-number generator to select random permuted blocks. These blocks of 10 were stratified into 5 groups of gestational age ranges (< 27 weeks, 27-30.6 weeks, 31-33.6 weeks, 34-36.6 weeks and ≥ 37 weeks) to obtain a balanced number of infants within each stratum.

Using the computer-generated randomization list, independent pharmacists placed ampules of either 1 ml morphine-hydrochloride or 1 ml of placebo into boxes. These boxes were numbered with the study numbers and stored with increasing numbers for the different gestational age groups in a locked closet accessible only to the researchers. At a patient's enrollment, the next box in line for the specific group was taken out by one of the researchers. All research and clinical staff, as well as the parents of the infants, were blinded to treatment.

Statistical analyses
Data were analyzed using SPSS statistical software version 10.1 (SPSS Inc, Chicago, Ill). Non-parametric tests were used and results are shown as medians and percentiles when variables deviated from the normal distribution. Background characteristics between the 2 treatment conditions were compared using non-parametric Mann Whitney tests or Fisher's Exact tests (in case of low incidences). Characteristics of the non-participating patients were compared with data from study infants using Kruskal-Wallis tests.

Pain-scores

Multiple regression analyses were performed with VAS-bedside and NIPS (scored 30 minutes after study medication loading dose) as outcome variables, predicted by treatment condition, having received a morphine dose before intubation, gestational age, Clinical risk index for babies (CRIB) score, center, sex and postnatal age in hours corrected by the pain scored before the bolus was given. Pain scores were log 10 transformed to approximate a normal distribution.

Across all assessments, mean PIPP, NIPS, and VAS scores, scored during endotracheal suctioning, were calculated for each patient and used as outcome variables in multiple regression analyses. Summary statistics (mean scores for each patient) were used to increase reliability and to take repeated measures into account during analyses. Predictors were treatment condition, mean amount of additional morphine, center, sex, and duration in study. The importance of the predictors is shown by unstandardized coefficients.

Clinical outcome

Logistic regression analyses were used with poor neurologic outcome (death within 28 days, IVH grade III or IVH plus apparent periventricular hemorrhagic infarction and/or PVL) and IVH (all grades) as outcome variables; treatment condition and additional morphine use as predictor variables; and center, gestational age, sex, CRIB score, deviation from mean birthweight for gestational age,[33] prenatal corticosteroid use, pre-eclampsia and/or HELLP (Hemolysis, Elevated Liver Enzymes, Low Platelets) syndrome, and the use of indomethacin as covariates.

Collinearity for the logistic regression analyses was checked, by performing a multiple regression analysis instead of the logistic regression analyses to calculate the variance inflation factors, which were all well below 2.0. The same was true for the multiple regression analyses. The risk of overfitting was controlled by using a ratio of 1:10 at least for the number of explanatory variables and sample size. To assess overfitting more precisely, the patients in these 2 groups were split into deciles. To cross-validate, the training sample was composed of 9 of the 10 deciles; the validation sample contained the remaining decile. The predicted values for the remaining decile were obtained by the parameters of the logistic regression analysis performed on 9 of the other deciles. This procedure was repeated 10 times because each decile functioned as a validation sample. The overall mean obtained from the 10 mean values and the pooled SD derived from the 10 SDs of the validation samples for each condition separately were compared with the

overall mean and SD of the predicted values of the total sample. A high level of agreement between the overall solution and the cross-validation samples indicates high stability. Stepwise procedures were used.

Co-morbidity (eg, chronic lung disease, necrotizing enterocolitis, duration of artificial ventilation) was compared using the Mann-Whitney U and Fisher's Exact tests. Missing values were excluded listwise during all analyses in the sense that all cases that had any values missing on any of the variables used in the analyses were excluded. In all analyses, the intention-to-treat principle was used and involved all included infants who were randomly assigned to the morphine and placebo groups.

Results

A total of 210 infants were eligible between December 2000 and October 2002; the parents of 60 newborns refused informed consent and 150 were randomized (see Figure 1).
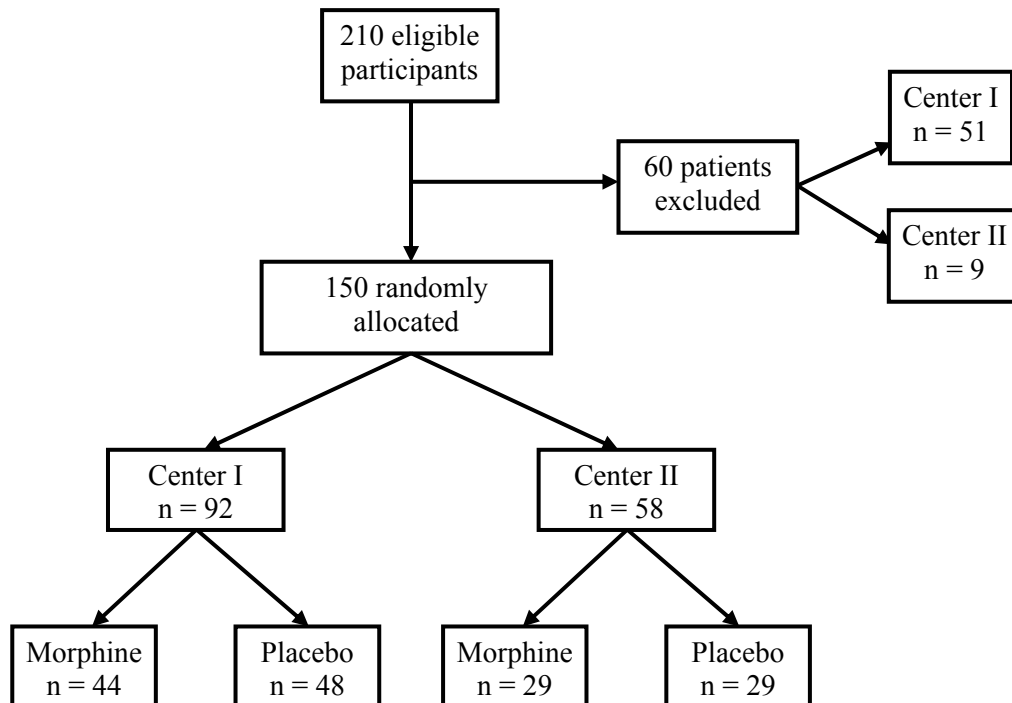
Figure 1   Flow diagram

The percentage of nonenrolled patients was 36% in center 1 (n = 51) and 13% in center 2 (n = 9). Seventy-three patients were allocated to receive continuous morphine infusion (44 in center 1, 29 in center 2), and 77 patients were assigned to receive placebo (48 in center 1, and 29 in center 2). Median duration of study medication infusion was 48 hours (interquartile range [IQR]: 19 to 96 hours). Use of the medication was stopped for the following reasons: extubation (n = 106), 7 days in study (n = 24), hypotension (n = 6), continuous use of neuromuscular blockers (n = 5), death (n = 4), surgery (n = 2), the need for too much additional morphine (n = 2), and overdosing (n = 1).

Patient characteristics for both treatment groups are shown in table 1. All patient characteristics were comparable between the groups. Demographic characteristics of the non-participants also showed no significant difference compared with the participating infants. Painful procedures were counted for a median duration of 6 days (IQR: 3 to 10 days). The number of daily painful procedures was similar in the morphine group (median 13; IQR: 10-16) and placebo group (median 13; IQR: 9-16) (Mann-Whitney U test 2479, P = .66).

Table 1   Background demographic and clinical characteristics for both conditions.

Condition:	Morphine n = 73	Placebo n = 77	P
Background			
Gender (boys / girls)	42 / 31	44 / 33	0.96[§]
In/outborn	57 / 16	54 / 23	0.27[§]
Gestational age*	29.1 (27.4 to 31.6)	29.2 (27.3 to 31.4)	0.70[§]
Birthweight (g)*	1130 (850 to 1680)	1230 (915 to 1560)	0.99[§]
Postnatal age (hrs)*	9 (5 to 13)	8 (5 to 12)	0.57[§]
Apgar 1 min.*	6 (4 to 8)	6 (4 to 8)	0.44[§]
5 min.*	8 (7 to 9)	8 (7 to 9)	0.32[§]
CRIB [a]*	2 (1 to 6)	3 (1 to 7)	0.57[§]
Primary diagnoses			
IRDS [b]	53	65	0.08[§]
Wet Lung	2	2	>0.99[#]
Pneumonia	2	2	>0.99[#]
Meconium aspiration	1	1	>0.99[#]
Primary infection	13	16	0.37[§]

data are shown as median (25th and 75th percentile), § Mann-Whitney U test, Asymp. Significance (2-sided), [#] Fisher's Exact Test, Exact significance (2-sided), [a] Clinical Risk Index for Babies, [b] Idiopathic respiratory distress syndrome.

Pain-scores

At baseline, median NIPS scores in the morphine and placebo groups were 0.0 (IQR: 0.0-0.0) and 0.0 (IQR: 0.0-0.8) and median VAS scores were 0.6 (IQR: 0.3-2.2) and 0.7 (IQR: 0.3-1.5), respectively. Thirty minutes after study medication administration, median NIPS scores in the morphine and placebo groups were 0.0 (IQR: 0.0-0.0) and 0.0 (IQR: 0.0-1.0), and median VAS scores were 0.6 (IQR: 0.3-1.6) and 0.6 (IQR: 0.2-1.4), respectively.

During suctioning, median PIPP scores in the morphine and placebo groups were 10.1 (IQR: 8.2-11.6) and 10.0 (IQR: 8.2-12.0) (*P* = .94), median NIPS scores were 4.8 (IQR: 3.7-6.0) and 4.8 (IQR: 3.2-6.0) (*P* = .58), and median VAS scores were 2.8 (IQR: 2.0-3.9) and 2.6 (IQR: 1.8-4.3) (*P* = .14), respectively (table 2). There were no significant differences between groups for pain scores. Of the 2530 VAS scores, only 293 values indicated moderate pain[34] by exceeding 4 (69% were scored during suctioning), with 146 and 147 values noted in the morphine and placebo groups, respectively. Table 2 shows pain-scores at the different time-points for the morphine and placebo treated patients. The mean SDs of pain scores for those patients who underwent multiple procedures were: 2.5 for the PIPP, 2.2 for the NIPS, and 2.2 for VAS scores.

Multiple regression analyses revealed that VAS_{pain} and NIPS scores after the loading dose of study medication did not significantly differ between the 2 groups (unstandardized regression coefficient [B] = -0.019; 95% confidence interval [CI]: -0.071 to 0.032; *P* = .46 and B = 0.031; 95%CI: -0.053 to 0.12; *P* = .47) and were not influenced by withholding the loading dose (B = -0.014; 95% CI: -0.075 to 0.047; *P* = .65 and B = 0.022; 95% CI: -0.13 to 0.080; *P* = .67). These pain scores were significantly predicted, however, by the pain scores before bolus administration (B = 0.65; 95% CI: 0.53 to 0.78; *P* < .001 and B = 0.54; 95%CI: 0.34 to 0.73; *P* < .001). VAS scores were higher in girls compared with boys (B = -0.057, 95% CI: -0.11 to –0.005; *P* = .03), and higher in center 2 compared with center 1 (B = -0.065; 95% CI: -0.12 to –0.010; *P* = .02). Pain-scores tended to be higher when no morphine prior to intubation was given (B = -0.054; 95% CI: -0.11 to 0.002; *P* = .06 and B = -0.11; 95% CI: -0.20 to 0.018; *P* = .02).

The PIPP, NIPS and VAS scores were not predicted in multiple regression analyses by treatment group or by the amount of additional morphine used (table 3). Mean NIPS and VAS scores decreased with increasing length of study, and VAS scores were lower in center 1 compared with center 2. Spearman ρ correlation coefficients between the different pain scores were 0.44 (NIPS vs PIPP, *P* < .001), 0.31 (NIPS vs VAS, *P* < .001) and 0.22 (PIPP vs VAS, *P* = .02).

Table 2   The median values of pain scores* for the morphine and placebo treated neonates at different moments.

Pain score:	NIPS[1]		PIPP[2]		VAS	
Moment:	Morphine-group	Placebo-group	Morphine-group	Placebo-group	Morphine-group	Placebo-group
Baseline	0.0 (0.0 to 0.0)	0.0 (0.0 to 0.8)	-	-	0.6 (0.3 to 2.2)	0.7 (0.3 to 1.5)
30 minutes after start	0.0 (0.0 to 0.0)	0.0 (0.0 to 1.0)	-	-	0.6 (0.3 to 1.6)	0.6 (0.2 to 1.4)
Before suctioning	0.5 (0.0 to 1.0)	1.0 (0.0 to 1.0)	-	-	0.8 (0.5 to 1.3)	0.9 (0.6 to 1.6)
During suctioning	4.8 (3.7 to 6.0)	4.8 (3.2 to 6.0)	10.1 (8.2 to 11.6)	10.0 (8.2 to 12.0)	2.8 (2.0 to 3.9)	2.6 (1.8 to 4.3)
30 minutes after suctioning	0.0 (0.0 to 1.0)	0.0 (0.0 to 1.0)	-	-	0.9 (0.6 to 1.4)	0.9 (0.6 to 1.4)

Results are show as medians (25-75th percentiles). All pain scores were not significantly different between the 2 conditions.
* *pain scores were averaged in case of repeated measures.*
[1]Neonatal Infants Pain Scale, [2]Premature Infant Pain Profile, [3]Visual Analog Scale

Table 3   Results of multiple regression analyses with mean PIPP, NIPS and VAS$_{pain}$ scores as outcome variables

Outcome variable:	PIPP[1]			NIPS[2]			VAS$_{pain}$[3]		
	B*	95% CI of B	P	B*	95% CI of B	P	B*	95% CI of B	p
Condition	0.038	-0.97 to 1.04	0.94	-0.16	-0.74 to 0.41	0.58	-0.43	-1.00 to 0.15	0.14
Amount of extra morphine	-0.066	-0.25 to 0.12	0.48	0.011	-0.096 to 0.12	0.83	0.030	-0.078 to 0.14	0.59
Center	0.56	-0.51 to 1.62	0.30	0.31	-0.29 to 0.91	0.31	-1.40	-2.00 to -0.80	**<0.001**
Gender	-0.11	-1.12 to 0.89	0.82	0.29	-0.29 to 0.86	0.32	0.30	-0.28 to 0.87	0.31
Total length of study	-0.0022	-0.013 to 0.009	0.69	-0.0070	-0.013 to -0.001	**0.03**	-0.0067	-0.013 to 0.00	**0.04**
		R = 0.14, R_{adj}^2 = 0.026			R = 0.22, R_{adj}^2 = 0.012			R = 0.46, R_{adj}^2 = 0.19	

* *Unstandardized regression coefficients*

Clinical outcome

Table 4 lists the clinical outcomes and incidences of morbidity and mortality for the 2 groups. Overall, 11 infants died within 28 days, and 48 were diagnosed as having IVH, 10 of which had the severe type (grade III or IVH plus apparent periventricular hemorrhagic infarction). Four infants had PVL. Logistic regression analysis showed that the incidence of poor neurologic outcome was not related to treatment group or to additional morphine use (Table 5). It was, however, associated with lower gestational ages ($P = .005$) and higher CRIB scores ($P = .004$) and was more apparent in boys compared with girls ($P = .003$).

The incidence of IVH (all grades), also evaluated with logistic regression analysis, was significantly higher in the placebo group compared with the morphine group (adjusted odds ratio 2.36; 95% CI: 1.05 to 5.28; $P = .04$). Furthermore the incidence of IVH was higher in those born small for gestational age ($P = .05$), and in infants born outside the study hospital ($P = .04$). Median duration of the first period of artificial ventilation, median total duration of ventilation and median length of NICU stay did not significantly differ between groups ($P = .72$, $P = .81$ and $P = .92$, respectively).

Table 4   Clinical outcome for both conditions

Condition:		**Morphine** n = 73	**Placebo** n = 77	**P**
28th day survival		95 %	91 %	$
PVL[a]		3 %	3 %	$
IVH[b]	Severe[c]	4 %	9 %	$
	Overall	23 %	40 %	$
Chronic lung disease		23 %	23 %	0.95[§]
Secondary infection-sepsis		38 %	44 %	0.47[§]
NEC[d]		10 %	9 %	> 0.99[#]
PDA[e]		36 %	36 %	0.92[§]
Infusion study medication (hrs)*		55 (23 to 96)	42 (18 to 96)	0.35[§]
Artificial ventilation (hrs)				
	First period *	73 (35 to 172)	72 (27 to 154)	0.72[§]
	Total *	77 (36 to 184)	82 (32 to 221)	0.81[§]
NICU[f] stay (hrs)*		336 (156 to 804)	312 (144 to 1068)	0.92[§]

* data are shown as median (25th and 75th percentile), § Mann-Whitney U test, Asymp. Significance (2-sided), # Fisher's Exact Test, Exact significance (2-sided), $ outcome measures were analyzed using logistic regression analyses, table 5, [a] Periventricular Leucomalacia, [b] Intraventricular Hemorrhage, [c] grade III IVH or IVH + apparent periventricular hemorrhagic infarction, [d] Necrotizing Enterocolitis, [e] Patent Ductus Arteriosus, [f] Neonatal Intensive Care Unit.

Table 5 Results of logistic regression analyses with poor neurologic outcome[2] and IVH[1] (all grades) as dependent variables.

Outcome variable:	Poor neurologic outcome			IVH[1] all grades		
	OR*	95% CI of OR	P	OR*	95% CI of OR	P
Condition	1.35	0.40 to 4.57	0.63	2.36	1.05 to 5.28	0.04
Amount of extra morphine	1.04	0.84 to 1.29	0.73	1.13	0.97 to 1.31	0.11
Center	0.33	0.70 to 1.58	0.17	1.04	0.41 to 2.65	0.94
Gender	0.074	0.013 to 0.42	0.003	1.01	0.44 to 2.28	0.99
Gestational age	0.93	0.88 to 0.98	0.005	0.96	0.94 to 0.99	0.06
Dev. of mean birth weight[3]	1.34	0.83 to 2.18	0.23	1.44	1.00 to 2.05	0.05
CRIB[4]-score	1.42	1.12 to 1.80	0.004	1.05	0.91 to 1.22	0.49
In/outborn	1.49	0.20 to 11.0	0.70	3.87	1.07 to 14.0	0.04
Prenatal corticosteroids	1.42	0.31 to 6.42	0.65	1.96	0.75 to 5.14	0.17
Pre-eclampsia and/or HELLP[5]	1.20	0.21 to 6.82	0.84	0.79	0.27 to 2.33	0.67
Indomethacin for PDA[6]	2.51	0.59 to 10.6	0.21	1.08	0.40 to 2.90	0.87
Nagelkerke R^2		0.40			0.30	
Hosmer&Lemeshow;		χ^2: 7.6; df 8; P = 0.47			χ^2: 12.5; df 8; P = 0.13	

* = Estimated odds-ratio; [1] Intraventricular Hemorrhage; [2] Poor neurologic outcome = severe IVH (grade III or IVH + apparent periventricular hemorrhagic infarction), periventricular leucomalacia or death at 28th day; [3] For each infant the deviation of birthweight from the mean for gestational age was calculated as a measure of small for gestational age; [4] Clinical Risk Index for Babies; [5] Hemolysis, Elevated Liver Enzymes, Low Platelets; [6] Patent Ductus Arteriosus.

Morphine use

Open-label morphine was administered to 20 infants (27%) in the morphine group and 31 patients (40%) in the placebo group ($\chi^2 = 2.76$, $P = .10$) (table 6), with comparable median dosages of 3.0 μg/kg per hour (IQR: 1.3 to 6.8 μg/kg per hour) and 4.3 μg/kg per hour (IQR: 1.6 to 7.7 μg/kg per hour) in the morphine and the placebo groups, respectively (Mann-Whitney U test: 282.5, $P = .60$). Of the 60 eligible but nonenrolled patients, 55% received morphine with a median dose of 3.6 μg/kg per hour (IQR: 1.7 to 6.7 μg/kg per hour). These infants received 'additional' morphine more frequently than the study infants (Kruskal-Wallis test: $\chi^2 = 10.4$, $P = .005$). Among the 2 centers, nonenrolled patients received morphine more frequently in Center 2 (Mann- Whitney U test: 94.0; $P = .03$).

Table 6   Use of morphine in morphine, placebo and non-participating group.

Condition	Morphine	Placebo	Non-participants	p
Overall	n = 73	n = 77	n = 60	
Masked morphine (µg/kg/hr)	10.0	0.0	0.0	
Additional 'open label' morphine*	0.0 (0.0 to 0.6)	0.0 (0.0 to 3.1)	0.8 (0.0 to 4.6)	0.005
Total amount*	10.0 (10.0 to 10.6)	0.0 (0.0 to 3.1)	0.8 (0.0 to 4.6)	< 0.001
Condition	**Morphine**	**Placebo**	**Non-participants**	**p**
Patients receiving additional morphine	n = 20 (27%)	n = 31 (40%)	n = 33 (55%)	
Amount of additional morphine*	3.0 (1.3 to 6.8)	4.3 (1.6 to 7.7)	3.6 (1.7 to 6.7)	0.80

* median amounts (25th and 75th percentile) in µg/kg/hr

Comment

We hypothesized that continuous morphine infusion in preterm neonates would reduce pain experience and incidences of poor neurologic outcome and IVH. However, pain measurements validated for this age group did not reveal any analgesic effects of morphine. Although routine morphine infusions did not affect poor neurologic outcomes or any other clinical outcome measure, pre-emptive morphine analgesia significantly decreased the incidence of IVH. These findings suggest that routine morphine infusion in preterm newborns who have received ventilatory support neither improves pain relief nor protects against poor neurologic outcome. The impact of decreased IVH in the morphine-treated neonates, however, should be evaluated with their long-term neurobehavioral outcomes.

Overall, we found that pain scores did not significantly differ between the 2 randomized groups. Although the results of pain scores should be viewed with some caution, the PIPP and NIPS have both been validated for the assessment of procedural pain in preterm neonates.[31,32,35,36] The sensitivity and specificity of these methods for measuring acute or chronic pain in preterm infants remains unknown. The VAS has not been specifically validated for this group of patients but appears to reflect the intensity of pain.[34] In this study, the VAS was applied by experienced NICU nurses who were specifically trained for assessing neonatal pain. Measuring the effect of morphine on the pain experienced by preterm neonates remains difficult because of the lack of a 'gold standard' to assess neonatal pain. The absence of a measurable analgesic effect of morphine, as established by these validated pain scores, may be explained by several reasons.

Our patients seemed to experience only minor pain. Most patients showed no evidence of pain before or 30 minutes after the loading dose. Taking the limited time-span from birth to study enrollment (median: 8 hrs; IQR: 5 to 12 hrs) into consideration, the low pain scores may be explained by release of endorphins, resulting from birth[37-39] and postnatal stress.[40] Since severe pain was mostly absent, it need not be relieved by morphine.

Pain scores were obtained during an invasive, presumably noxious procedure. Endotracheal suctioning was the only repetitively, frequently, and routinely performed invasive procedure during our study. Heel lances were not performed routinely because all patients had arterial catheters. Furthermore, previous studies have shown that tracheal suctioning is related to increased pain scores[15,41,42] and stress responses[43] and is considered noxious and painful.[44-47] In our study, tracheal suctioning was associated with a median PIPP score of 10, NIPS score of 4.8, and VAS score of 2.7, indicating mild to moderate pain. These physiologic and behavioral responses are indicators of neonatal pain, but they are also influenced by factors such as gestational age, severity of illness, and time from the previous painful procedure.[48] Previous studies using these measures have reported large inter-individual variability.[49]

The low correlation between the different pain scores also underlines the difficulty of pain assessment in this group of patients, as was recently reviewed by our group.[2,34] However, multivariate analyses, adjusting for these covariates did not show any statistically or clinically significant decrease in pain scores resulting from continuous morphine administration. The explained variance of these analyses was low, the result of low variability of pain scores. The few previous studies on this subject present conflicting findings. The decrease in pain that resulted from higher morphine doses compared with the ones used in our study during endotracheal suctioning and heel lances[15,50] was not confirmed in another study using morphine doses of the same magnitude.[51] The samples sizes in our study were considerably larger and the amounts of morphine used in our study conformed to internationally recommended doses.[52]

Despite the low pain scores a number of infants were given additional morphine (27% in the morphine group and 40% in the placebo group). Because this study aimed to evaluate the effect of routine continuous morphine infusion in newborns who received ventilatory support on primary and secondary outcome measures, placebo-treated infants received open-label morphine if deemed to be in pain. By reflecting variations among patients that occur in real clinical practice, this study is a pragmatic trial that aimed to inform choices

between treatments (routine morphine administration or no routine morphine infusion). In pragmatic trials, the treatment response is the total difference between 2 treatments, including both treatment and associated placebo effects, since this will best reflect the likely clinical response in practice. Because the intention-to-treat principle was used in our study, patients in both groups receiving open-label morphine were not dropped out but included in the analyses. In daily practice, a newborn in pain who receives ventilatory support needs to receive analgesic treatment, independent of any routine morphine administration. If an infant was in pain, morphine was given. In this way, our study was a realistic reflection of 2 different strategies of daily NICU practice.

By randomizing patients and blinding physicians, parents, and researchers, clinical bias was minimized. The attending physicians and nurses obviously considered these infants to be uncomfortable and in need of extra pain relief, although this was not reflected in their pain scores. The use of extra morphine was not significantly different between the randomized groups, as reported previously.[15] The nonparticipating infants received open-label morphine somewhat more frequently than those in the study group, suggesting that participation in this trial was not a causative factor for additional morphine prescription. Furthermore, additional morphine could be used only according to the protocol. Therefore, physicians were allowed to administer additional doses of 50 μg/kg followed by 5-10 μg/kg per hour continuous open-label morphine. The non-participants, however, often received 'standard' morphine boluses of 100 μg/kg. Additional morphine use in non-participants differed between the 2 centers perhaps due to different prescribing policies or to different patient characteristics.

Our results are indicative of non-standardized pain management under which lack of decision rules results in prescribing analgesics on the basis of personal clinical experience. This is not only the case in our centers, but also representative of clinical practice in most NICUs worldwide.[53] Implementation of pain scores (ie, using cut-off points for prescribing additional analgesics that are integrated in clinical algorithms or flowcharts) may be required for rationalizing the use of opioid analgesics in the NICU. The development of new techniques, such as functional magnetic resonance imaging and positron emission tomographic scans, might be useful in the near future to further objectify the analgesic effects of opioids in newborns, but they are not applicable in daily NICU care.

Morphine use might decrease the fluctuations in cerebral blood volume and intracranial pressure caused by neonatal reactions to pain and painful procedures. Morphine may thus protect against the development of venous hemorrhage in the germinal matrix or brain parenchyma, or against the extension of a small previous IVH.[54,55] High pain scores were not related to the incidence of IVH or poor neurologic outcome. Oberlander et al.[56] also found that parenchymal brain injury did not cause a difference in pain response in premature neonates. Significantly fewer neonates in the morphine treated group were found to have IVH compared with the placebo group. This effect of morphine can be partly explained by a decreased incidence of low grades IVH. The impact of routine morphine administration, by reduction of low-grade IVH, on long term outcome is hard to predict. Both PVL and IVH were diagnosed and staged from cranial ultrasounds by staff neonatologists, using standard criteria.[57,58] It is hard to determine the neurobehavioral outcome in infants with IVH, because other confounding criteria, such as co-morbidity, are involved. Mortality and major neurological sequelae are generally related to the degree of hemorrhage[59-63] and, to a greater extent, to the degree of associated parenchymal injury.[58] Infants with IVH grade I and II, without venous infarction, seem to have little increased risk of adverse outcome compared with those without IVH.[58,60,64-66] When we studied the impact of morphine infusion on poor neurologic outcomes (eg, death, PVL, IVH grade III, or IVH and apparent periventricular hemorrhagic infarction), there were no differences between the 2 groups.

The neurologic condition of our patients, however, needs to be re-evaluated at older ages. A study by Quinn et al. also showed comparable clinical outcomes between placebo and morphine treated neonates.[67] A pilot study by Anand et al.,[15] with a slightly different study design, showed decreased poor neurologic outcomes on account of morphine compared with midazolam hydrochloride and placebo. Relatively small groups, numbering approximately 20, in those studies, as well as differences in morphine dose-regimen, might explain the differing results. Further results of that study should conclusively show whether routine use of morphine reduces the incidences of IVH and poor neurologic outcome.

Conclusion

Overall, our results show a lack of measurable analgesic effects and absence of a beneficial effect on poor neurological outcome from routine morphine infusion in preterm

neonates. Future research is needed to establish cut-off points and an algorithm for the administration of analgesic agents in this specific age group of children, which should be included in consensus statements.[23,24] Furthermore, better understanding of individual differences in responses to morphine and pain is necessary to improve neonatal pain management.

Our findings suggest that morphine infusion in preterm newborns who receive ventilatory support should not be used as a standard of care. The long-term consequences of reduced IVH incidence in the morphine treated neonates should be evaluated at predetermined time points at older ages, using validated assessment instruments for neurodevelopmental outcome.

Acknowledgements

References

1. Kennedy KA, Tyson JE. Narcotic analgesia for ventilated newborns: are placebo-controlled trials ethical and necessary? J Pediatr. 1999;134(2):127-129.

2. van Dijk M, Tibboel D. Review of Pain Assessment Measures for Postoperative Pain in Vulnerable Infants. Clin Perinatol. September 2002;29:469-491.

3. Rutter N, Evans N. Cardiovascular effects of an intravenous bolus of morphine in the ventilated preterm infant. Arch Dis Child Fetal Neonatal Ed. 2000;83(2):F101-103.

4. Wood CM, Rushforth JA, Hartley R, Dean H, Wild J, Levene MI. Randomised double blind trial of morphine versus diamorphine for sedation of preterm neonates. Arch Dis Child Fetal Neonatal Ed. 1998;79(1):F34-39.

5. Sabatino G, Quartulli L, Di Fabio S, Ramenghi LA. Hemodynamic effects of intravenous morphine infusion in ventilated preterm babies. Early Hum Dev. 1997;47(3):263-270.

6. Hartley R, Green M, Quinn M, Levene MI. Pharmacokinetics of morphine infusion in premature neonates. Arch Dis Child. 1993;69(1 Spec No):55-58.

7. Koren G, Butt W, Pape K, Chinyanga H. Morphine-induced seizures in newborn infants. Vet Hum Toxicol. 1985;27(6):519-520.

8. Saarenmaa E, Neuvonen PJ, Rosenberg P, Fellman V. Morphine clearance and effects in newborn infants in relation to gestational age. Clin Pharmacol Ther. 2000;68(2):160-166.

9. Way WL, Costley EC, Way EL. Respiratory sensitivity of the newborn infant to meperidine and morphine. Clin Pharmacol Ther. 1965;6:454-461.

10. Lynn AM, Nespeca MK, Opheim KE, Slattery JT. Respiratory effects of intravenous morphine infusions in neonates, infants, and children after cardiac surgery. Anesth Analg. 1993;77(4):695-701.

11. Handelmann GE, Dow-Edwards D. Modulation of brain development by morphine: effects on central motor systems and behavior. Peptides. 1985;6(Suppl 2):29-34.

12. Tempel A. Visualization of mu opiate receptor downregulation following morphine treatment in neonatal rat brain. Brain Res Dev Brain Res. 1991;64(1-2):19-26.

13. Bhutta AT, Rovnaghi C, Simpson PM, Gossett JM, Scalzo FM, Anand KJS. Interactions of inflammatory pain and morphine in infant rats: long-term behavioral effects. Physiol Behav. 2001;73(1-2):51-58.

14. MacGregor R, Evans D, Sugden D, Gaussen T, Levene M. Outcome at 5-6 years of prematurely born children who received morphine as neonates. Arch Dis Child Fetal Neonatal Ed. 1998;79(1):F40-43.

15. Anand KJS, 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. 1999;153(4):331-338.

16. Fitzgerald M, Millard C, MacIntosh N. Hyperalgesia in premature infants [letter]. Lancet. 1988;1(8580):292.

17. Fitzgerald M, Millard C, McIntosh N. Cutaneous hypersensitivity following peripheral tissue damage in newborn infants and its reversal with topical anaesthesia. Pain. 1989;39(1):31-36.

18. Andrews K, Fitzgerald M. The cutaneous withdrawal reflex in human neonates: sensitization, receptive fields, and the effects of contralateral stimulation. Pain. 1994;56(1):95-101.

19. Anand KJS, Coskun V, Thrivikraman KV, Nemeroff CB, Plotsky PM. Long-term behavioral effects of repetitive pain in neonatal rat pups. Physiol Behav. 1999;66(4):627-637.

20. Grunau RE, Oberlander TF, Whitfield MF, Fitzgerald C, Lee SK. Demographic and therapeutic determinants of pain reactivity in very low birth weight neonates at 32 Weeks' postconceptional Age. Pediatrics. 2001;107(1):105-112.

21. Porter FL, Grunau RE, Anand KJS. Long-term effects of pain in infants. J Dev Behav Pediatr. 1999;20(4):253-261.

22. Peters JW, Koot HM, de Boer JB, et al. Major surgery within the first 3 months of life and subsequent biobehavioral pain responses to immunization at later age: a case comparison study. Pediatrics. 2003;111(1):129-135.

23. Anand KJS. Consensus statement for the prevention and management of pain in the newborn. Arch Pediatr Adolesc Med. 2001;155(2):173-180.

24. Prevention and management of pain and stress in the neonate. American Academy of Pediatrics. Committee on Fetus and Newborn. Committee on Drugs. Section on Anesthesiology. Section on Surgery. Canadian Paediatric Society. Fetus and Newborn Committee. Pediatrics. 2000;105(2):454-461.

25. Franck LS, Miaskowski C. The use of intravenous opioids to provide analgesia in critically ill, premature neonates: a research critique. J Pain Symptom Manage. 1998;15(1):41-69.

26. Kart T, Christrup LL, Rasmussen M. Recommended use of morphine in neonates, infants and children based on a literature review: Part 1--Pharmacokinetics. Paediatr Anaesth. 1997;7(1):5-11.

27. Kart T, Christrup LL, Rasmussen M. Recommended use of morphine in neonates, infants and children based on a literature review: Part 2--Clinical use. Paediatr Anaesth. 1997;7(2):93-101.

28. Berde CB, Sethna NF. Analgesics for the treatment of pain in children. N Engl J Med. 2002;347(14):1094-1103.

29. Ambalavanan N, Carlo WA. Analgesia for ventilated neonates: where do we stand? J Pediatr. 1999;135(4):403-405.

30. Use and abuse of the Apgar score. Committee on Fetus and Newborn, American Academy of Pediatrics, and Committee on Obstetric Practice, American College of Obstetricians and Gynecologists. Pediatrics. 1996;98(1):141-142.

31. Lawrence J, Alcock D, McGrath P, Kay J, MacMurray SB, Dulberg C. The development of a tool to assess neonatal pain. Neonatal Netw. 1993;12(6):59-66.

32. Stevens B, Johnston C, Petryshen P, Taddio A. Premature Infant Pain Profile: development and initial validation. Clin J Pain. 1996;12(1):13-22.

33. Usher R, McLean F. Intrauterine growth of live-born Caucasian infants at sea level: standards obtained from measurements in 7 dimensions of infants born between 25 and 44 weeks of gestation. J Pediatr. 1969;74(6):901-910.

34. van Dijk M, Koot HM, Saad HH, Tibboel D, Passchier J. Observational visual analog scale in pediatric pain assessment: useful tool or good riddance? Clin J Pain. 2002;18(5):310-316.

35. Blauer T, Gerstmann D. A simultaneous comparison of three neonatal pain scales during common NICU procedures. Clin J Pain. 1998;14(1):39-47.

36. Ballantyne M, Stevens B, McAllister M, Dionne K, Jack A. Validation of the premature infant pain profile in the clinical setting. Clin J Pain. 1999;15(4):297-303.

37. Raisanen I, Paatero H, Salminen K, Laatikainen T. Beta-endorphin in maternal and umbilical cord plasma at elective cesarean section and in spontaneous labor. Obstet Gynecol. 1986;67(3):384-387.

38. Pohjavuori M, Rovamo L, Laatikainen T, Kariniemi V, Pettersson J. Stress of delivery and plasma endorphins and catecholamines in the newborn infant. Biol Res Pregnancy Perinatol. 1986;7(1):1-5.

39. Bacigalupo G, Langner K, Schmidt S, Saling E. Plasma immunoreactive beta-endorphin, ACTH and cortisol concentrations in mothers and their neonates immediately after delivery--their relationship to the duration of labor. J Perinat Med. 1987;15(1):45-52.

40. Pokela ML, Koivisto M. Physiological changes, plasma beta-endorphin and cortisol responses to tracheal intubation in neonates. Acta Paediatr. 1994;83(2):151-156.

41. Saarenmaa E, Huttunen P, Leppaluoto J, Fellman V. Alfentanil as procedural pain relief in newborn infants. Arch Dis Child Fetal Neonatal Ed. 1996;75(2):F103-107.

42. Grunau RE, Holsti L, Whitfield MF, Ling E. Are twitches, startles, and body movements pain indicators in extremely low birth weight infants? Clin J Pain. 2000;16(1):37-45.

43. Greisen G, Frederiksen PS, Hertel J, Christensen NJ. Catecholamine response to chest physiotherapy and endotracheal suctioning in preterm infants. Acta Paediatr Scand. 1985;74(4):525-529.

44. Skov L, Ryding J, Pryds O, Greisen G. Changes in cerebral oxygenation and cerebral blood volume during endotracheal suctioning in ventilated neonates. Acta Paediatr. 1992;81(5):389-393.

45. Pokela ML. Pain relief can reduce hypoxemia in distressed neonates during routine treatment procedures. Pediatrics. 1994;93(3):379-383.

46. McCulloch KM, Ji SA, Raju TN. Skin blood flow changes during routine nursery procedures. Early Hum Dev. 1995;41(2):147-156.

47. Evans JC, Vogelpohl DG, Bourguignon CM, Morcott CS. Pain behaviors in LBW infants accompany some "nonpainful" caregiving procedures. Neonatal Netw. 1997;16(3):33-40.

48. 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.

49. Johnston CC, Stevens BJ, Yang F, Horton L. Differential response to pain by very premature neonates. Pain. 1995;61(3):471-479.

50. Scott CS, Riggs KW, Ling EW, et al. Morphine pharmacokinetics and pain assessment in premature newborns. J Pediatr. 1999;135(4):423-429.

51. Quinn MW, Wild J, Dean HG, et al. Randomised double-blind controlled trial of effect of morphine on catecholamine concentrations in ventilated pre-term babies. Lancet. 1993;342(8867):324-327.

52. Anand KJS. Systemic analgesic therapy. Anand KJS, Stevens BJ, McGrath PJ. Pain in Neonates 2nd Revised and Enlarged Edition, Elsevier Amsterdam. 2000;Vol 10:Appendix: page 180.

53. Kahn DJ, Richardson DK, Gray JE, et al. Variation among neonatal intensive care units in narcotic administration. Arch Pediatr Adolesc Med. 1998;152(9):844-851.

54. Perlman JM, McMenamin JB, Volpe JJ. Fluctuating cerebral blood-flow velocity in respiratory-distress syndrome. Relation to the development of intraventricular hemorrhage. N Engl J Med. 1983;309(4):204-209.

55. Ghazi-Birry HS, Brown WR, Moody DM, Challa VR, Block SM, Reboussin DM. Human germinal matrix: venous origin of hemorrhage and vascular characteristics. AJNR Am J Neuroradiol. 1997;18(2):219-229.

56. Oberlander TF, Grunau RE, Fitzgerald C, Whitfield MF. Does parenchymal brain injury affect biobehavioral pain responses in very low birth weight infants at 32 weeks' postconceptional age? Pediatrics. 2002;110(3):570-576.

57. de Vries LS, Eken P, Dubowitz LM. The spectrum of leukomalacia using cranial ultrasound. Behav Brain Res. 1992;49(1):1-6.

58. Volpe JJ. Intracranial Hemorrhage: Germinal Matrix-Intraventricular Hemorrhage of the Premature Infant. Neurology of the Newborn. 4th ed. Philadelphia: Saunders; 2000:428-491.

59. Thorburn RJ, Lipscomb AP, Stewart AL, Reynolds EO, Hope PL, Pape KE. Prediction of death and major handicap in very preterm infants by brain ultrasound. Lancet. 1981;1(8230):1119-1121.

60. Shankaran S, Slovis TL, Bedard MP, Poland RL. Sonographic classification of intracranial hemorrhage. A prognostic indicator of mortality, morbidity, and short-term neurologic outcome. J Pediatr. 1982;100(3):469-475.

61. Smith WL, McGuinness G, Cavanaugh D, Courtney S. Ultrasound screening of premature infants: longitudinal follow-up of intracranial hemorrhage. Radiology. 1983;147(2):445-448.

62. Vohr B, Garcia Coll C, Flanagan P, Oh W. Effects of intraventricular hemorrhage and socioeconomic status on perceptual, cognitive, and neurologic status of low birth weight infants at 5 years of age. J Pediatr. 1992;121(2):280-285.

63. Feingold E, Sheir-Neiss G, Melnychuk J, Bachrach S, Paul D. HRQL and severity of brain ultrasound findings in a cohort of adolescents who were born preterm. J Adolesc Health. 2002;31(3):234-239.

64. Papile LA, Munsick-Bruno G, Schaefer A. Relationship of cerebral intraventricular hemorrhage and early childhood neurologic handicaps. J Pediatr. 1983;103(2):273-277.

65. Stewart AL, Thorburn RJ, Hope PL, Goldsmith M, Lipscomb AP, Reynolds EO. Ultrasound appearance of the brain in very preterm infants and neurodevelopmental outcome at 18 months of age. Arch Dis Child. 1983;58(8):598-604.

66. Dubowitz LM, Dubowitz V, Palmer PG, Miller G, Fawer CL, Levene MI. Correlation of neurologic assessment in the preterm newborn infant with outcome at 1 year. J Pediatr. 1984;105(3):452-456.

67. Quinn MW, de Boer RC, Ansari N, Baumer JH. Stress response and mode of ventilation in preterm infants. Arch Dis Child Fetal Neonatal Ed. 1998;78(3):F195-198.

Chapter 5

RANDOMISED-CONTROLLED-TRIAL EVALUATING EFFECTS OF MORPHINE ON (NOR)EPINEPHRINE PLASMA-CONCENTRATIONS IN NEWBORNS

Abstract

Objective
To determine the effects of continuous morphine infusion in ventilated newborns on (nor)epinephrine plasma concentrations and their relationship with clinical outcome.

Design
Blinded randomised placebo-controlled trial

Setting
Level III Neonatal Intensive Care Units in two centres

Patient
Hundred twenty-six ventilated neonates (Inclusion criteria: postnatal age < 3 days, duration of ventilation < 8 hours, indwelling arterial catheter for clinical purpose. Exclusion criteria: severe asphyxia, severe intraventricular haemorrhage (IVH), major congenital anomalies, neuromuscular blockers).

Interventions
(Nor)epinephrine plasma concentrations were determined in patients during blinded morphine (n = 60) and placebo (n = 66) infusion (100 $\mu g.kg^{-1}$ + 10 $\mu g.kg^{-1}.h^{-1}$).

Results
Plasma concentrations ($nmol.l^{-1}$) at baseline were comparable between the morphine- (epinephrine: 0.22; IQR: 0.31, and norepinephrine: 2.52; IQR: 2.99) and placebo-treated infants (epinephrine: 0.29; IQR: 0.46, and norepinephrine: 2.44; IQR: 3.14). Median epinephrine concentrations during infusion of study medication were 0.12 (IQR: 0.28) and 0.18 (IQR: 0.35); median norepinephrine concentrations were 2.8 (IQR: 3.7) and 3.8 (IQR: 4.0) for the morphine- and placebo-treated infants, respectively. Multivariate analyses showed that norepinephrine ($P = 0.029$), but not epinephrine ($P = 0.18$), concentrations were significantly lower in the morphine group compared to the placebo group. Furthermore, norepinephrine levels were related to NICU stay.

Conclusions
Continuous morphine infusion significantly reduced norepinephrine plasma concentrations in ventilated newborns as compared to placebo treatment. The results of

this study support the idea that routine morphine administration decreases stress responses in ventilated neonates.

What is already known on this topic
As it is very difficult to measure the short- and long-term analgesic effects of morphine in neonates, the use routine of continuous morphine for neonatal pain during intensive care treatment is still under debate.

What this study adds
This study shows that norepinephrine, but not epinephrine, plasma concentrations are sensitive markers of neonatal stress and are decreased by the use of continuous morphine infusions. This decreased stress response seen during morphine administration supports the idea that continuous morphine treatment in ventilated neonates should be part of the standard of care.

Abbreviations
NICU: Neonatal Intensive Care Unit
IVH: intra ventricular haemorrhage
HPLC: High Performance Liquid Chromatography
CRIB: Clinical Risk Index for Babies
IQR: interquartile range
CI: confidence interval

Introduction

As part of their intensive care treatment, premature neonates experience large amounts of painful procedures and continuous stressful respiratory support.[1-5] Although even the most premature neonates are able to feel pain, adequate analgesic treatment by continuous opioid treatment is still limited.[2,5,6] This might be explained by the fact that there is disagreement as to whether the currently available evidence is sufficient to justify prolonged exposure to opioids in this vulnerable group of patients. As it is still a major challenge to quantify the degree of pain relief in neonates, there is still lack of evidence about the analgesic effect of opioids. Observational pain scales, using both physiological and behavioural indicators are validated for prematurely and term born neonates,[7-9] but failed to identify the analgesic effects of opioids.[10]

Preterm neonates are, however, capable of mounting hormonal responses to stress related to birth, illness, intensive care treatment, surgical procedures, and mechanical ventilation, as manifested by high catecholamine plasma concentrations.[11-17] These catecholamine plasma levels were reduced by analgesic treatment in newborns[18,19] and as a consequence might also represent the stress relieving effect of continuous morphine infusion in neonates.

Therefore we hypothesised that routine morphine administration reduces stress-responses of ventilated newborns. To test our hypothesis plasma concentrations of epinephrine and norepinephrine were analysed in ventilated newborns who participated in a blinded randomised placebo-controlled trial evaluating the analgesic effect of routine morphine administration in preterm ventilated newborns.[10] As lower stress responses are associated with improved outcome[15,20] and decreased postoperative mortality in neonates,[13,21] we also aimed to determine if a possible decrease in neonatal stress responses by continuous morphine treatment would be related to beneficial effects on neonatal outcome.

Methods

Patients
Neonatal patients were included from December 2000 to October 2002 in two centres level III NICUs (Centre I: Erasmus MC-Sophia Rotterdam, a university hospital and centre II: The Isala Clinics Zwolle, a non-university hospital). Neonates with gestational age between 25 to 42 weeks requiring mechanical ventilation, with postnatal age < 3 days, endotracheal intubation < 8 hours before start, and with an indwelling arterial catheter were eligible for inclusion. Exclusion criteria were severe asphyxia (Apgar-score after 5 min. < 4 or cord blood pH < 7.0)[22], severe IVH (grade III or IVH + apparent periventricular hemorrhagic infarction),[23] major congenital anomalies and facial malformations (i.e. cleft lip and palate), neurological disorders, and continuous or intermittent neuromuscular blockers.

Procedure
The local ethical committees of the participating centres approved the study protocol. If possible, parents were already informed about the study before birth of their child. Written informed parental consent was obtained for all included patients. Masked study medication consisted of either morphine hydrochloride or placebo (sodium chloride), both

dissolved in glucose 5%. After enrolment patients were randomly allocated to receive a masked loading dose (100 μg.kg^{-1}) followed by a masked continuous infusion (10 μg.kg^{-1}.h^{-1}). To prevent possible overdosing, the study medication loading-dose was not given if, before intubation, a morphine loading-dose had been given less than 3 hours before the start of study. Study medication was continued for a maximum of 7 days; if the patient's clinical condition required so, it was discontinued earlier. After 7 days, study medication was weaned and replaced by real morphine infusion if necessary.

All patients judged to be in pain or distress were given additional morphine during the study on guidance of the attending physician (independent of the study) with allowed doses of 50 μg.kg^{-1} followed by 5-10 μg.kg^{-1}.h^{-1} continuous open-label morphine. Blood-samples for (nor)epinephrine analyses were taken at baseline, i.e. before start of study medication, and at 24, 48 and 96 hours after start of study medication at rest in centre I, and at day 2, 3 and 5 within 5 minutes after endotracheal suctioning in centre II. Blood-samples of 0.6 ml were drawn from the arterial catheter into a heparin micro-container and taken to the laboratory in ice water. The samples were centrifuged (4°C, 10 min, 3,000xg) and plasma was separated and stored at -80ºC. Epinephrine and norepinephrine plasma concentrations were determined using HPLC with fluorimetric detection.[24]

Outcome
Primary outcome measures were the concentrations of epinephrine and norepinephrine measured in arterial blood plasma.
To determine the association between (nor)epinephrine concentrations and the clinical outcome, secondary outcome measures were defined as: development of intraventricular haemorrhage (IVH), poor neurologic outcome (severe IVH [grade III or IVH + apparent periventricular hemorrhagic infarction], periventricular leucomalacia or death within 28 days), total duration of artificial ventilation and total duration of NICU admission, was evaluated.

Randomisation and blinding
A power-analysis showed that 60 patients per group were needed to achieve a medium effect size (Cohen's d = 0.59), with alpha error of 0.05 (2-tailed) and power of 90%. Neonates had an equal probability of being assigned to either condition, using a randomisation code and stratification into five groups of gestational age ranges (< 27, 27-30.6, 31-33.6, 34-36.6 and ≥ 37 weeks) to obtain a balanced number of morphine and placebo participants within each stratum.

Independent pharmacists, using the computer generated randomisation list, placed ampoules of either 1 ml morphine-HCL or 1 ml placebo into boxes numbered with the study numbers. If a new patient was enrolled, the next box in line for the relevant age group was taken. All research and clinical staff, as well as the parents of the participants were blinded to treatment.

Statistical analyses

Multiple regression analyses were used to simultaneously estimate the effect of treatment condition (morphine vs placebo), the amount of additional open-label morphine, gestational age, deviation from mean birthweight, CRIB,[25] sex and participating centre on the epinephrine and norepinephrine plasma concentrations (means per patient during masked medication infusion) corrected for the baseline levels of (nor)epinephrine, prenatal corticosteroid use and dopamine infusion. To achieve normal distributions of epinephrine and norepinephrine levels, outcome variables as well as baseline levels were logarithmically (ln) transformed. After using the enter method, non-significant covariates (Pin < 0.05; Pout > 0.10) were excluded from the analyses to minimise the number covariates in both analyses.

Furthermore, multiple regression analyses were used with duration of artificial ventilation and duration of NICU admission as outcome variables, predicted by epinephrine and norepinephrine concentrations, corrected by the number of samples per infant. Logistic regression analyses were used with the incidences of IVH and poor neurologic outcome as outcome variables, also predicted by epinephrine and norepinephrine concentrations, and corrected for the number of samples per infant. Data were analysed using SPSS statistical software version 10.1 (SPSS Inc, Chicago, Ill). Multicollinearity was tested by determining the Variance Inflation Factors.

Results

During the inclusion period 210 patients were eligible for inclusion, for 150 of whom parental informed consent was obtained. These were randomly allocated to receive morphine or placebo. For practical (i.e. lack of venous/arterial access) and ethical reasons (i.e. less than 3ml/kg of blood sampling allowed for the duration of the study) (nor)epinephrine plasma concentrations could not be determined in 23 patients. One other patient was given intravenous norepinephrine because of persistent hypotension and was

therefore excluded from analysis. Thus (nor)epinephrine concentrations could be determined and analysed for 126 patients (see Figure 1).

Sixty patients were allocated to receive continuous morphine infusion: 31 in centre I, 29 in centre II, and 66 patients to receive placebo: 38 in centre I, and 28 in centre II.
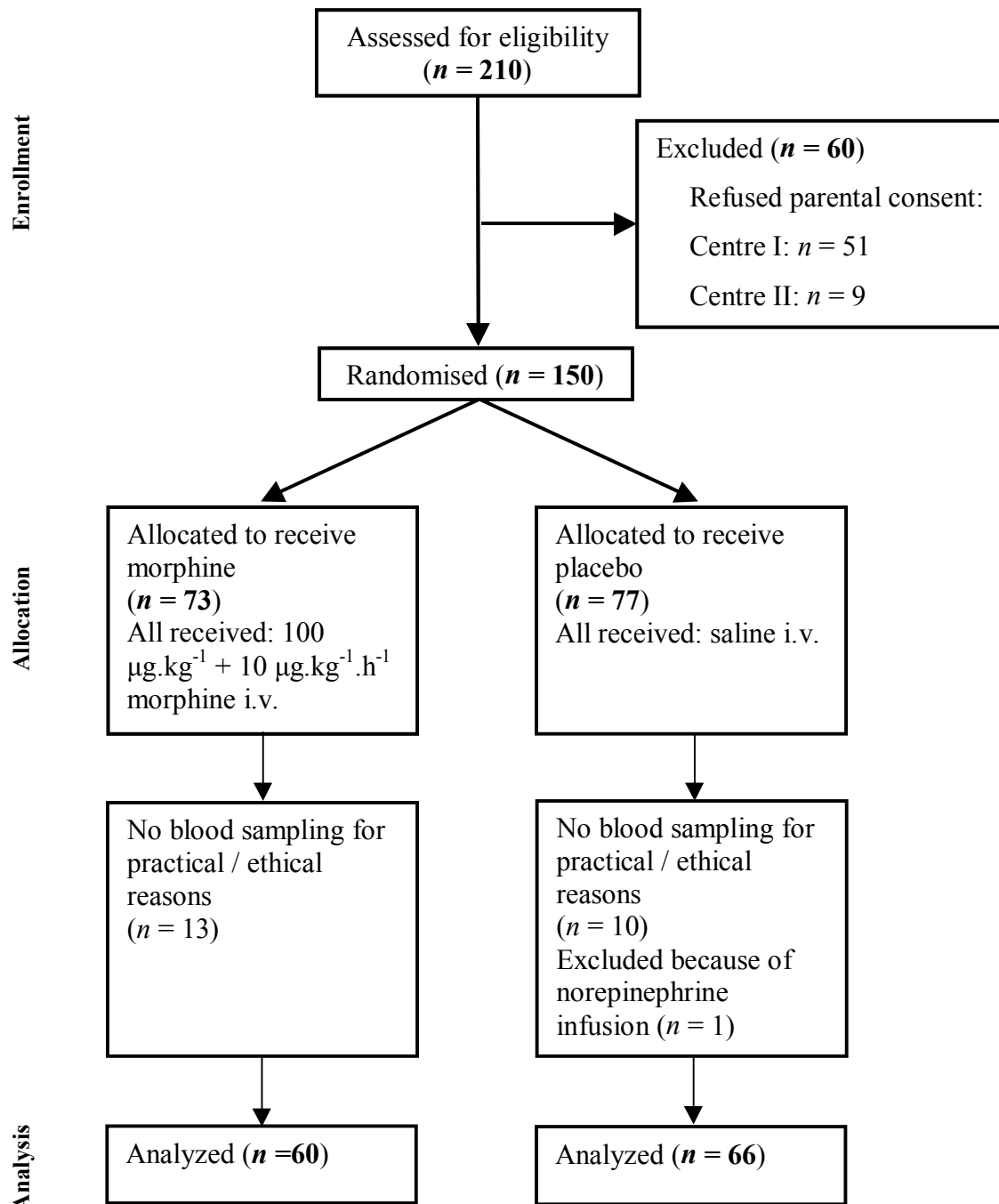
Enrollment

Assessed for eligibility
(*n* = 210)

Excluded (*n* = 60)
  Refused parental consent:
  Centre I: *n* = 51
  Centre II: *n* = 9

Randomised (*n* = 150)

Allocation

Allocated to receive morphine
(*n* = 73)
All received: 100 μg.kg^{-1} + 10 μg.kg^{-1}.h^{-1} morphine i.v.

Allocated to receive placebo
(*n* = 77)
All received: saline i.v.

No blood sampling for practical / ethical reasons
(*n* = 13)

No blood sampling for practical / ethical reasons
(*n* = 10)
Excluded because of norepinephrine infusion (*n* = 1)

Analysis

Analyzed (*n* =60)

Analyzed (*n* = 66)

Figure 1   Flow diagram

Median duration of study medication infusion was 47 hours (IQR:19 to 92). Infusion was stopped for the following reasons: extubation (n = 98), 7 days in study (n = 15), hypotension (n = 4), continuous administration of neuromuscular blockers (n = 4), surgery (n = 2), deceased (n = 1), requiring too much additional morphine (n = 1), and overdosing (n = 1). Patient characteristics for both treatment groups are shown in Table 1; they were all comparable between both conditions. The (nor)epinephrine concentrations were comparable at baseline: median epinephrine plasma concentrations were 0.22 nmol.l^{-1} (IQR:0.31) in the morphine-treated infants and 0.29 nmol.l^{-1} (IQR:0.46) in the placebo-treated infants. Median norepinephrine concentrations were 2.52 nmol.l^{-1} (IQR:2.99) and 2.44 nmol.l^{-1} (IQR:3.14) for the morphine- and placebo-treated infants, respectively. During masked study medication infusion median epinephrine plasma concentrations were 0.12 nmol.l^{-1} (IQR:0.28) and 0.18 nmol.l^{-1} (IQR:0.35) and median norepinephrine concentrations were 2.8 nmol.l^{-1} (IQR:3.7) and 3.8 nmol.l^{-1} (IQR:4.0) for the morphine- and placebo-treated infants, respectively.

Multiple regression analysis with the (mean per infant) epinephrine concentration (ln transformed) during masked study medication as outcome variable showed that epinephrine concentrations were not predicted by treatment condition (B = -0.079; 95% CI:-0.20 to 0.037; P = 0.18) or by the used amounts of additional 'open-label' morphine (B = -0.0091; 95% CI:-0.028 to 0.010; P = 0.34).

Table 1   Background characteristics.

	Condition:	Morphine n = 60	Placebo n = 66
Background:			
Sex (boys / girls)		38 / 22	37 / 29
In/outborn[a]		45 / 15	44 / 22
Gestational age (weeks)*		30.3 (27.5 to 32.1)	29.6 (28.4 to 32.1)
Birthweight (g)*		1380 (1004 to 1840)	1340 (1024 to 1674)
Postnatal age (hrs)*		8.5 (5.0 to 13.0)	8.0 (5.0 to 12.0)
Apgar score	1 min.*	6 (4 to 8)	6 (4 to 8)
	5 min.*	8 (7 to 9)	8 (7 to 9)
% patients with dopamine infusions		23 %	23 %
% patients receiving prenatal corticosteroids		57 %	55 %
CRIB score [b]*		2 (1 to 5)	3 (1 to 6)

* data are shown as median (25th and 75th percentile);
[a] In/outborn: born inside or outside of the participating hospitals;
[b] Clinical Risk Index for Babies.

Table 2 shows the details of the regression analysis. Using the mean norepinephrine concentration (ln transformed) during masked study medication as outcome variable, multiple regression analysis showed that norepinephrine plasma concentrations were significantly lower in the morphine group
(B = -0.25; 95% CI:-0.48 to -0.027; *P* = 0.029) compared to the placebo group. The used amounts of additional 'open label' morphine did not significantly influence norepinephrine concentrations.

This analysis as well is detailed in Table 2. Infants who received dopamine infusions and those who had low birthweights for their gestational age had higher levels of both epinephrine and norepinephrine. Furthermore infants who had received prenatal corticosteroids had significantly higher norepinephrine plasma-concentrations. The (nor)epinephrine levels tended to be higher in centre II compared to centre I.

Table 3 shows clinical outcome measures for the morphine and placebo treated infants. The duration of NICU stay was significantly related to plasma concentrations of norepinephrine (B = 50.2; 95% CI: 20.0 to 80.4; *P* = 0.001) but not of epinephrine (B = -137.5; 95% CI: -300.0 to 25.0; *P* = 0.10). The (nor)epinephrine concentrations did not significantly predict the duration of artificial ventilation, incidence of IVH and poor neurologic outcome.

Table 2   Multiple regression analyses with epinephrine and norepinephrine plasma concentrations (means per patient), ln transformed, as outcome variables.

Outcome variable:	Epinephrine conc. (ln)			Norepinephrine conc. (ln)		
	B*	95% CI of B	P	B*	95% CI of B	p
Condition	-0.079	-0.20 to 0.037	0.18	-0.25	-0.48 to -0.027	**0.029**
Amount of extra Morphine [a]	-0.0091	-0.028 to 0.010	0.34	-0.0081	-0.046 to 0.030	0.67
Deviation birthweight [b]	-0.039	-0.077 to 0.001	**0.046**	-0.091	-0.17 to -0.015	**0.019**
Dopamine infusion	0.23	0.082 to 0.37	**0.003**	0.42	0.13 to 0.71	**0.005**
Prenatal corticosteroids				0.41	0.17 to 0.65	**0.001**
Participating centre	-0.11	-0.24 to 0.013	0.078	-0.23	-0.47 to 0.004	0.054
	$R = 0.50, R_{adj}^2 = 0.20$			$R = 0.64, R_{adj}^2 = 0.36$		

* *Unstandardized regression coefficients;*
[a] *The mean amount of extra morphine for each infant in µg.kg^{-1} per hour during study was used;*
[b] *Birthweight was compared to normal mean birthweight for each patient, as a measure of small for gestational age infants;*
Gestational age (P =0.81 and 0.67), baseline (nor)epinephrine concentrations (ln transformed) (P = 0.21 and 0.13) and prenatal corticosteroid use (only in epinephrine analyses; P = 0.42) were excluded from the analysis (P > 0.1) by using the backward method.

Table 3 Clinical outcome for the morphine and placebo treated infants.

Condition:	Morphine n = 60	Placebo n = 66
Outcome:		
NICU stay (hrs)*	312 (150 to 552)	288 (138 to 906)
Artificial ventilation (hrs)*	67 (28 to 126)	73 (28 to 158)
IVH[1] (all grades)	18 %	38 %
Poor neurologic outcome[2]	5 %	15 %

data are shown as median (25th and 75th percentile);
[1] IVH = Intraventricular haemorrhage;
[2] Poor neurologic outcome = severe IVH [IVH grade III or IVH + apparent periventricular hemorrhagic infarction], periventricular leucomalacia or death within 28 days.

Discussion

In this study we evaluated whether continuous morphine infusion in newborn ventilated infants would reduce stress responses as reflected by plasma concentrations of epinephrine and norepinephrine. Routine morphine infusions were shown to reduce norepinephrine ($P = 0.029$), but not epinephrine ($P = 0.18$), plasma concentrations. The use of additional open label morphine did not influence (nor)epinephrine levels. Previous studies showed lowering of (nor)epinephrine plasma levels by the use of opioids post-operatively.[26,27] Quinn et al. showed in a placebo-controlled trial that high morphine dosages (100 µg.kg^{-1} for 2 h + 25 µg.kg^{-1}.h^{-1}) effectively reduced just epinephrine, but not norepinephrine, concentrations in ventilated neonates after 24 hours compared with placebo treatment.[18] Our and previous studies also showed that increased levels are not only associated with stress but are also influenced by other parameters such as the use of dopamine infusion[28] and prenatal corticosteroids, asphyxia, or being born small for gestational age.[17] Next to different methods of statistical analyses and amounts of used morphine the contrast in results between the different studies might also be explained by variability in patient characteristics and treatment of neonates.

Decreased levels of norepinephrine were related to a shorter NICU stay, but no further relationships between (nor)epinephrine levels and clinical outcome were found. Plasma levels of epinephrine and norepinephrine have previously been associated with poor outcome in newborns.[16,20] Anand et al. showed that high levels of epinephrine and norepinephrine in preterm neonates after patent ductus arteriosus-ligation in the absence

of analgesia were associated with high mortality rates.[21] In another study the same authors showed that a decrease in neonatal stress response was related to improved clinical stability during and after surgical operations.[13] Although these findings would suggest that outcome in premature neonates might be related to catecholamine levels, we were not able to show a relationship between (nor)epinephrine concentrations and neurological outcome. Different circumstances (surgery vs no surgery) and different patient criteria (e.g. in- or exclusion of asphyxiated patients) in previous studies compared to ours might explain this disparity.

Increased neonatal stress has been suggested to change stress responses at older ages.[29] Studies in animals have suggested that acute fetal or neonatal stress can alter the trigger level of the hypothalamic-pituitary-adrenal axis even for life, resulting in changed stress responses at older ages.[30] In concordance with this observation, human adrenomedullary and adrenocortical activity were still increased in 12-year-old children born small for gestational age compared with full-term appropriate for gestational age-matched controls.[31] This mechanism might also partly explain the protective role of analgesics against negative consequences of early pain experience.[32] Extrapolation of these data to the patients in our study could lead to the suggestion that those treated with continuous morphine infusion from the first postnatal day onwards might show decreased stress responses at older ages. Peters et al. showed no difference in cortisol levels and pain response to immunisation between toddlers who received pre-emptive morphine after major surgery within the first 3 months of life compared to controls,[33] and Evans et al. showed no correlation between neonatal catecholamine levels and cognitive or motor impairment at 5-6 years.[16] For our specific study population no further data are yet available. Therefore this highly speculative suggestion is now systematically evaluated in a follow-up study of our cohort of patients at 3 years of age.

Unfortunately we were not able to collect plasma from all patients in our study. Although the amount of blood needed for analysis each time was only 0.6 ml, collection proved to be a problem in particularly the smallest patients. Our finding that gestational age does not significantly influence catecholamine levels might counteract this shortcoming. Although it was previously suggested that catecholamine levels are higher in preterm than in near-term infants,[14] we have shown that they are not related to gestational age but that they are increased in small for gestational age neonates. The results of our study, therefore, probably reflect stress responses in neonates within a wide range of gestational ages. Although newborns with severe asphyxia or otherwise high catecholamine levels[17]

were excluded from our study, we were still able to measure the effects of low morphine doses on norepinephrine plasma concentrations. As neonates in centre II, sampled after suctioning, tended to show higher plasma concentrations of (nor)epinephrine compared to centre I, sampled in rest, the levels of (nor)epinephrine are probably also somewhat increased after acute stressful moments in neonates. A previous study also showed increased norepinephrine concentrations after suctioning.[12]

As a fast analysis method is not yet available, determining catecholamine plasma levels has only limited usefulness for individual neonatal pain management in daily clinical practice. Evaluation of norepinephrine concentrations is, however, an objective method to determine evidence of the stress relieving effects of pharmacological agents. Therefore, we are convinced that stress hormone levels constitute important parameters for future studies evaluating pain and effects of analgesics in particular age groups.

Conclusion

In this blinded randomised placebo-controlled trial we showed that routine administration of morphine in ventilated newborns reduces norepinephrine plasma concentrations suggesting a beneficial effect of routine morphine administration in the neonate. In analogue with our previous report,[10] showing no decrease in pain scores by the use of routine morphine infusions in newborns who have received ventilatory support, we also found no decrease in epinephrine plasma levels. Follow-up of our patients is necessary to evaluate long term stress responses and outcome.

Acknowledgements

References

1. Barker DP, Rutter N. Exposure to invasive procedures in neonatal intensive care unit admissions. Arch Dis Child Fetal Neonatal Ed 1995;72(1):F47-8.

2. Johnston CC, Collinge JM, Henderson SJ, Anand KJS. A cross-sectional survey of pain and pharmacological analgesia in Canadian neonatal intensive care units. Clin J Pain 1997;13(4):308-12.

3. Porter FL, Anand KJS. Epidemiology of pain in neonates. Res Clin Forums. 1998;20:9-18.

4. Benis MM, Suresh GK. Frequency of invasive procedures in very low birth weight (VLBW) infants in the neonatal intensive care unit (NICU), abstract. Pediatric Research 2001;49(2):392A, 2253.

5. Simons SHP, van Dijk M, Anand KJS, Roofthooft D, van Lingen RA, Tibboel D. Do we still hurt newborn babies? A prospective study of procedural pain and analgesia in neonates. Arch Pediatr & Adolesc Med 2003;157:1058-1064

6. Debillon T, Bureau V, Savagner C, Zupan-Simunek V, Carbajal R. Pain management in French neonatal intensive care units. Acta Paediatr 2002;91(7):822-6.

7. Lawrence J, Alcock D, McGrath P, Kay J, MacMurray SB, Dulberg C. The development of a tool to assess neonatal pain. Neonatal Netw 1993;12(6):59-66.

8. Stevens B, Johnston C, Petryshen P, Taddio A. Premature Infant Pain Profile: development and initial validation. Clin J Pain 1996;12(1):13-22.

9. van Dijk M, de Boer JB, Koot HM, Tibboel D, Passchier J, Duivenvoorden HJ. The reliability and validity of the COMFORT scale as a postoperative pain instrument in 0 to 3-year-old infants. Pain 2000;84(2-3):367-77.

10. Simons SHP, van Dijk M, van Lingen RA, Roofthooft DWA, Duivenvoorden HJ, Jongeneel N, et al. Routine morphine infusion in preterm newborns who received ventilatory support: a randomized controlled trial. JAMA 2003;290(18):2419-2427.

11. Lagercrantz H, Bistoletti P. Catecholamine release in the newborn infant at birth. Pediatr Res 1977;11(8):889-93.

12. Greisen G, Frederiksen PS, Hertel J, Christensen NJ. Catecholamine response to chest physiotherapy and endotracheal suctioning in preterm infants. Acta Paediatr Scand 1985;74(4):525-9.

13. Anand KJS, Sippell WG, Aynsley-Green A. Randomised trial of fentanyl anaesthesia in preterm babies undergoing surgery: effects on the stress response. Lancet 1987;1(8524):62-6.

14. Mehandru PL, Assel BG, Nuamah IF, Fanaroff AA, Kalhan SC. Catecholamine response at birth in preterm newborns. Biol Neonate 1993;64(2-3):82-8.

15. Quinn MW, de Boer RC, Ansari N, Baumer JH. Stress response and mode of ventilation in preterm infants. Arch Dis Child Fetal Neonatal Ed 1998;78(3):F195-8.

16. Evans DJ, MacGregor RJ, Dean HG, Levene MI. Neonatal catecholamine levels and neurodevelopmental outcome: a cohort study. Arch Dis Child Fetal Neonatal Ed 2001;84(1):F49-52.

17. Greenough A, Lagercrantz H, Pool J, Dahlin I. Plasma catecholamine levels in preterm infants. Effect of birth asphyxia and Apgar score. Acta Paediatr Scand 1987;76(1):54-9.

18. Quinn MW, Wild J, Dean HG, Hartley R, Rushforth JA, Puntis JW, et al. Randomised double-blind controlled trial of effect of morphine on catecholamine concentrations in ventilated pre-term babies. Lancet 1993;342(8867):324-7.

19. Wood CM, Rushforth JA, Hartley R, Dean H, Wild J, Levene MI. Randomised double blind trial of morphine versus diamorphine for sedation of preterm neonates. Arch Dis Child Fetal Neonatal Ed 1998;79(1):F34-9.

20. Barker DP, Rutter N. Stress, severity of illness, and outcome in ventilated preterm infants. Arch Dis Child Fetal Neonatal Ed 1996;75(3):F187-90.

21. Anand KJS, Hansen DD, Hickey PR. Hormonal-metabolic stress responses in neonates undergoing cardiac surgery. Anesthesiology 1990;73(4):661-70.

22. Use and abuse of the Apgar score. Committee on Fetus and Newborn, American Academy of Pediatrics, and Committee on Obstetric Practice, American College of Obstetricians and Gynecologists. Pediatrics 1996;98(1):141-2.

23. Volpe JJ. Intracranial Hemorrhage: Germinal Matrix-Intraventricular Hemorrhage of the Premature Infant. Neurology of the Newborn. 4th ed. Philadelphia: Saunders, 2000:428-491.

24. van der Hoorn FA, Boomsma F, Man in 't Veld AJ, Schalekamp MA. Determination of catecholamines in human plasma by high performance liquid chromatography: comparison between a new method with fluorescence detection and an established method with electrochemical detection. J Chromatogr 1989;487:17-27.

25. The CRIB (clinical risk index for babies) score: a tool for assessing initial neonatal risk and comparing performance of neonatal intensive care units. The International Neonatal Network. Lancet 1993;342(8865):193-8.

26. Anand KJS, Hickey PR. Halothane-morphine compared with high-dose sufentanil for anesthesia and postoperative analgesia in neonatal cardiac surgery. N Engl J Med 1992;326(1):1-9.

27. Bouwmeester NJ, Anand KJS, van Dijk M, Hop WC, Boomsma F, Tibboel D. Hormonal and metabolic stress responses after major surgery in children aged 0-3 years: a double-blind, randomized trial comparing the effects of continuous versus intermittent morphine. Br J Anaesth 2001;87(3):390-9.

28. Stopfkuchen H, Racke K, Schworer H, Queisser-Luft A, Vogel K. Effects of dopamine infusion on plasma catecholamines in preterm and term newborn infants. Eur J Pediatr 1991;150(7):503-6.

29. Taylor A, Fisk NM, Glover V. Mode of delivery and subsequent stress response. Lancet 2000;355(9198):120.

30. Meaney MJ, Bhatnagar S, Diorio J, Larocque S, Francis D, O'Donnell D, et al. Molecular basis for the development of individual differences in the hypothalamic-pituitary-adrenal stress response. Cell Mol Neurobiol 1993;13(4):321-47.

31. Tenhola S, Martikainen A, Rahiala E, Parviainen M, Halonen P, Voutilainen R. Increased adrenocortical and adrenomedullary hormonal activity in 12-year-old children born small for gestational age. J Pediatr 2002;141(4):477-82.

32. Grunau RE, Oberlander TF, Whitfield MF, Fitzgerald C, Lee SK. Demographic and therapeutic determinants of pain reactivity in very low birth weight neonates at 32 Weeks' postconceptional Age. Pediatrics 2001;107(1):105-12.

33. Peters JW, Koot HM, de Boer JB, Passchier J, Bueno-de-Mesquita JM, de Jong FH, et al. Major surgery within the first 3 months of life and subsequent biobehavioral pain responses to immunization at later age: a case comparison study. Pediatrics 2003;111(1):129-35.

Chapter 6

MORPHINE IN VENTILATED NEONATES: ITS EFFECTS ON ARTERIAL BLOOD PRESSURE

Abstract

Objective
To study the effects of continuous morphine infusion on arterial blood pressure in ventilated neonates.

Design
Blinded randomized placebo-controlled trial.

Setting
Level III Neonatal Intensive Care Unit in two centers.

Patients
144 ventilated neonates (Inclusion criteria: postnatal age < 3 days, ventilation < 8 hours, indwelling arterial line. Exclusion criteria: severe asphyxia, severe IVH, major congenital anomalies, neuromuscular blockers).

Intervention
Arterial blood pressure was measured before the start and during the first 48 hours of masked medication infusion (morphine or placebo; 100 µg.kg^{-1} + 10 µg.kg^{-1}.h^{-1}).

Outcome measures
Hypotension and blood pressure variability.

Results
Mean arterial blood pressures were comparable between the morphine group (median 36 mmHg; IQR 6) and the placebo group (median 38 mmHg; IQR 6) ($P = 0.11$). Although significantly more morphine treated patients (70%) showed hypotension compared to the placebo group (47%) ($P = 0.004$), the use of volume expansion and vasopressor drugs was comparable (morphine group: 44%; placebo group: 48%; $P = 0.87$) showing the limited clinical significance of this side effect. Blood pressure variability was not influenced by routine morphine analgesia ($P = 0.81$) and additional morphine ($P = 0.80$). Patients with and without intraventricular hemorrhage showed comparable blood pressures (Mann Whitney U-tests:1953; $P = 0.14$) and incidences of hypotension (χ^2 test: 1.16; *df*: 1; $P = 0.28$).

Conclusions

Overall arterial blood pressures, use of inotropic therapy and blood pressure variability were not influenced by morphine infusion. Therefore, the clinical impact of hypotension as a side effect of low dose morphine treatment in neonates is minimal.

Abbreviations

NICU: Neonatal intensive care unit

IVH: intraventricular hemorrhage

MAP: mean arterial blood pressure

CRIB: Clinical Risk Index for Babies

ΔMAP: difference between each two consecutive MAP recordings

IQR: Interquartile Range

Introduction

The development of pain assessment tools,[1-3] consensus statements,[4,5] and developmental care strategies[6,7] during the last decades reflects the increasing interest in adequate pain prevention and treatment in newborns admitted to NICUs. Pain treatment policies among the different centers vary widely, however, and the use of analgesics is still limited in many NICUs.[8-11] The restrained use of extensive pharmacological therapy may be explained by potential serious adverse events of analgesic treatment that have not been properly studied. While hemodynamic side effects of pharmacological therapy in the critically ill premature newborn are very undesirable there is still uncertainty about the extent morphine is able to effect blood pressure in the preterm infant.

Intravenous administration of morphine has been shown to relieve pain[12-14] and routine use of morphine might also decrease the incidence of poor neurologic outcome and IVH.[14,15] The development of IVH has been related to blood pressure variability [16,17] which might be associated with the use of morphine, but the latter has never been properly studied. In addition, morphine may also have additional adverse effects, like hypotension[12,18,19] and respiratory depression.[20-23] Respiratory side effects, however, can be easily corrected during mechanical ventilation, and preterm neonates receiving morphine have been shown to accept ventilatory support more easily.[24] Previous studies on hemodynamic effects of morphine in neonates report conflicting findings, ranging

from significant[18] and non-significant decreases[12,25] to no decrease of blood pressure at all,[24,26] and therefore warrant further evaluation.

As part of a blinded randomized placebo controlled trial, evaluating the effects of morphine in ventilated newborns on their pain experience,[15] we conducted a separate in-depth analysis of the effects of continuous morphine infusion on arterial blood pressure. In the current study we tested the hypothesis that continuous morphine infusion would (a) cause hypotension and (b) decrease blood pressure variability.

Methods

Participants

Patients enrolled in this study also participated in a randomized placebo controlled trial evaluating the analgesic effects of continuous morphine in ventilated neonates.[15] In short, all neonates admitted at two level III NICUs (Erasmus MC-Sophia, Rotterdam, and the Isala Clinics, Zwolle) between December 2000 and October 2002 who required mechanical ventilation were eligible for inclusion if postnatal age was < 3 days, intubation and initiation of mechanical ventilation < 8 hours ago, and an indwelling arterial catheter was already in place for clinical purposes. Neonates with severe asphyxia (Apgar score after 5 minutes < 4 or cord blood pH < 7.00),[27] severe IVH (grade III or IVH + apparent periventricular hemorrhagic infarction), major congenital malformations and facial malformations (i.e. cleft lip and palate), neurological disorders, or those receiving continuous or intermittent neuromuscular blockers were excluded. The local ethical committees of the participating centers approved the study protocol.

Procedure / intervention

After parents of eligible patients gave written informed consent, patients were randomly allocated to receive a loading dose (100 $\mu g.kg^{-1}$) followed by a continuous infusion (10 $\mu g.kg^{-1}.h^{-1}$) of either morphine (morphine HCL) or placebo (NaCl), both dissolved in glucose 5%. The loading dose was not given if a pre-intubation morphine loading dose had been administered within 3 hours before the start of study. Masked study medications were continued for a maximum of 7 days. If the attending physician judged patients – from either group – to be in pain or distress, doses of 50 $\mu g.kg^{-1}$ followed by 5-10 $\mu g.kg^{-1}.h^{-1}$ continuous 'real' additional morphine were administered.

After insertion of an indwelling umbilical or peripheral arterial catheter, 'baseline' MAP, arterial systolic and diastolic blood pressure were determined every 2 hours, for a

maximum of 10 hours, before the start of the administration of the study medication. Arterial blood pressures were measured again every two hours for 48 hours after start of study medication. We collected data on all variables likely to influence blood pressure, such as volume expansion or inotropic support (dopamine, dobutamine, etc.), background characteristics (birthweight, gestational age and postnatal age). The CRIB was used as a measure of severity of illness.[28]

Arterial blood pressure was measured by peripheral (Vygon, premicath 27 Gauche, 150mm; Vygon, nutriline 2 French, 300mm) or umbilical (Vygon, 3.5-5.0 French; 40 cm) arterial catheters, using a disposable blood pressure system (Gabarith PMSET, Becton-Dickinson) and 1DT-XX blood pressure transducer.

During the study, the attending physicians defined hypotension using a normative data model for different birthweights (MAP < 95% CI of linear regression model, Versmold et al.)[29] and if necessary applied anti-hypotensive therapy, using a standardized algorithm (Figure 1).
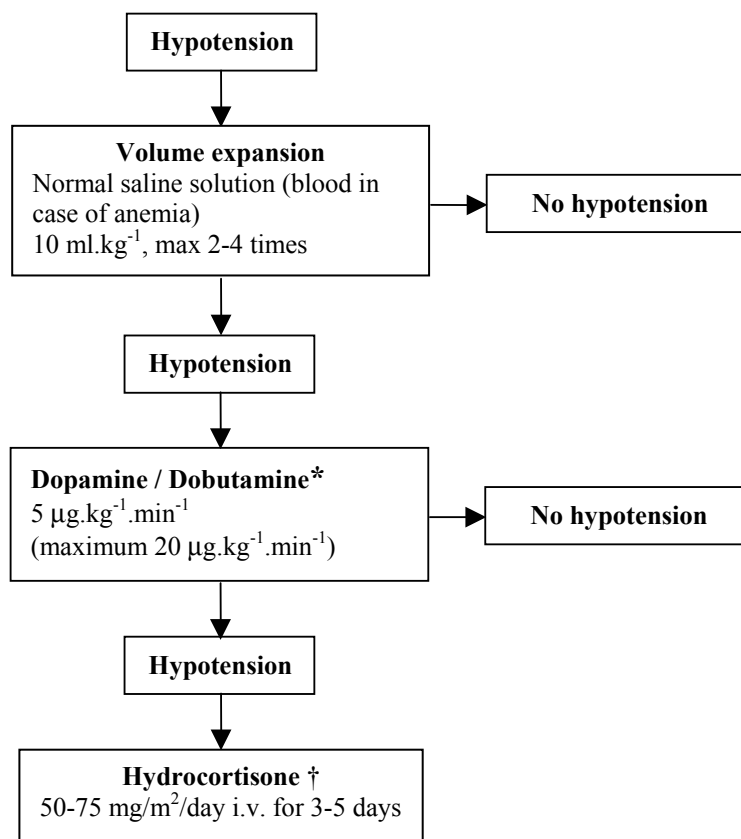
Figure 1   Schedule used in case of hypotension, showing consecutive steps of treatment.
* In term born neonates with asphyxia, congenital heart disease or other illnesses with diminished myocardial contractility dobutamine is the first drug of choice, dopamine is used if dobutamine fails to treat hypotension in these infants. In all other infants dopamine is given before dobutamine.
† In term born neonates norepinephrine (0.1 to 1.0 $\mu g.kg^{-1}.min^{-1}$) is used before hydrocortisone in case of persistent hypotension.

Outcomes

Primary outcome was the hypotensive effect of morphine. This was determined by comparing three different outcome measures between the morphine and placebo treated infants:

1. Overall arterial blood pressures (MAP, diastolic and systolic).
2. Frequency of volume expansion and administration of vasopressor drugs.
3. The numbers of infants having hypotensive MAP measurements, as compared to 'normal' values of blood pressure.

We calculated 'normal' values of MAP for each specific patient at the different postnatal times of measurement using an equation based on previous studies calculating linear regression models for blood pressure in newborns with variable birthweights, gestational and postnatal ages.[29,30] The equation was:

MAP (mmHg) = 29.80 + 5.16 * bodyweight (kg) + 0.12 * postnatal age (hrs)

In this equation bodyweight is estimated by birthweight. The constant value of 29.80 and the regression coefficients 5.16 and 0.12 in this equation are derived from studies of Versmold et al[29] and Leflore et al,[30] respectively. The 95% confidence interval was calculated as MAP ± 9 mmHg.[29] Hypotension was defined as a MAP below the lower value of this interval.

Secondary outcome was variability in arterial blood pressure, calculated from the differences between each two consecutive MAP recordings per patient (= ΔMAP). Blood pressure variability was defined as the interquartile range of ΔMAP per patient.

To determine the clinical significance of the hemodynamic effects of morphine, further outcome of this study was the relationship between these hemodynamic effects and the development of IVH. Therefore the impact of MAPs, hypotension and blood pressure variability on the incidence of IVH was studied.

Sample size, randomization and blinding
A power-analysis showed that 70 patients per group needed to be included to achieve a medium effect size (d = 0.55), with an alpha of 0.05, two-tailed, and a power of 90%. Neonates had an equal probability of being assigned to either condition. The randomization code was developed using a computer random number generator to select

random permuted blocks. These blocks (length 10) were stratified into five groups of gestational age ranges (< 27 weeks, 27-30^6 weeks, 31-33^6 weeks, 34-36^6 weeks and ≥ 37 weeks) to obtain a balanced number of morphine and placebo participants within each stratum. All research and clinical staff, as well as the parents of the participants were blinded to treatment.

Statistical analyses

All infants data were analyzed using the 'intent to treat' principle. All results are shown as median values and their 25th and 75th percentiles when variables deviated from normal distribution. Systolic, MAP and diastolic blood pressure at baseline and during masked medication infusion, as well as hypotension, were compared between the two conditions using the non-parametric, Mann Whitney U-test. Summary statistics (mean blood pressure values for each patient) were used to take repeated measures into account and to increase reliability. The use of inotropic therapy was compared between the groups using the χ^2 test (Yates corrected). Correlations between blood pressure variability and the incidence of IVH were calculated using non-parametric Mann Whitney U-tests. Multiple regression analysis was used with MAP variability as outcome variable, predicted by treatment condition and the amount of used additional 'open label' morphine and with center, CRIB-score, sex, the use of volume expansion or inotropic therapy, gestational age and deviation from mean birthweight as covariates. The model was checked on collinearity and overfitting. All data were analyzed using SPSS version 10.1

Results

In 6 of the 150 patients included in the randomized controlled trial (210 neonates were eligible for inclusion, parents of 60 patients refused) the indwelling arterial lines failed to properly register arterial blood pressure. Thus, 144 patients' blood pressure recordings were included in the analyses (see Figure 2). Of these patients, 71 were allocated to receive continuous morphine infusion: 43 in center I, 28 in center II, and 73 to receive placebo: 44 in center I and 29 in center II. Median duration of study medication infusion was 52 hours (25-75th percentiles: 21 to 97) and was discontinued for the following reasons: extubation (n = 102), 7 days in study (n = 24), deceased (n = 4), continuous neuromuscular blockers (n = 3), overdosing (n = 1), surgery (n = 2), medication stopped because too much additional morphine was needed (n = 2). In 6 patients the, blinded, attending physicians had the impression that masked study medication was morphine and

caused hypotension and for this reason they stopped masked medication in these patients. Two of these patients received placebo (hypotension measured in 0% and 6.7% of recordings, respectively) and 4 received morphine (hypotension measured in 25%, 52%, 64% and 80% of recordings, respectively).

Table 1 lists the background characteristics for the morphine and placebo group. During blood pressure recordings, 15 patients (21%) in the morphine group and 20 patients (27%) in the placebo group received additional open-label morphine (χ^2 test: 0.77; *df*: 1; *P* = 0.38). The median overall dose (masked + open label) of received morphine was 12.4 (11.0 to 16.1) and 3.3 (0 to 6.7) $\mu g.kg^{-1}.h^{-1}$ during the study in the morphine and placebo group respectively.

The hypotensive effect of morphine
Blood-pressure data were collected over a median of 46 hours (25-75th percentiles: 32 to 54) per infant. Both baseline and study values (mean values per patient) of systolic, MAP and diastolic blood pressure did not significantly differ between the two groups (Table 2.).
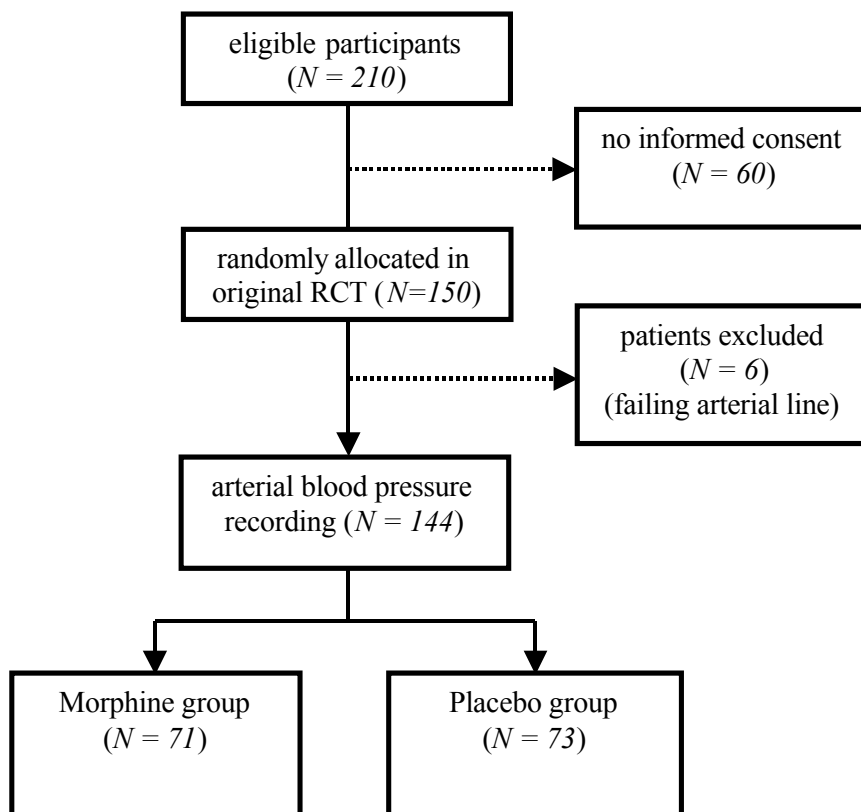
Figure 2   Flow diagram

Table 1   Background demographic and clinical characteristics.

	Condition:	**Morphine** **n = 71**	**Placebo** **n = 73**
Background			
Sex (boys / girls)		40 / 31	43 / 30
In / outborn		55 / 16	52 / 21
Gestational age (weeks)*		29.0 (27.4 to 31.8)	29.1 (27.3 to 31.3)
Birthweight (g)*		1100 (835 to 1655)	1215 (910 to 1511)
Postnatal age (hrs)*		9 (5 to 13)	8 (5 to 12)
Apgar	1 min*	6 (4 to 8)	6 (4 to 8)
	5 min*	8 (7 to 9)	8 (7 to 9)
CRIB *†		2 (1 to 6)	3 (1 to 7)
Infusion study medication (hrs)*		55 (23 to 97)	46 (18 to 101)
% of patients receiving additional 'open label' morphine		21 %	27 %

* data are shown as median (25th and 75th percentile), † Clinical Risk Index for Babies.

Table 2   Systolic, MAP and diastolic blood pressure at baseline and during masked study medication for both conditions.

	Morphine group	**Placebo group**	**P***
Baseline			
Systolic	43 (40 to 51)	43 (39 to 48),	0.43
MAP	33 (31 to 40)	34 (30 to 37)	0.43
Diastolic	27 (24 to 32)	28 (23 to 32)	0.67
During study			
Systolic	44 (42 to 50)	46 (43 to 51)	0.22
MAP	36 (33 to 39)	38 (35 to 41)	0.11
Diastolic	28 (26 to 32)	30 (27 to 34)	0.06

Blood pressures (mmHg) are given as median values (25th-75th percentile)
* Mann Whitney U-tests, significance (2-tailed)

Figure 3 shows median MAPs (and interquartile ranges) at baseline and after start of study medication infusion for both treatment groups. During the infusion of study medication 44% and 48% of the patients in the morphine and placebo group, respectively, received plasma expansion and / or pharmacological treatment of hypotension (Table 3). The difference in the use of these anti-hypotensive treatments between the two conditions failed to reach statistical significance (χ^2 test: 2.46; *df*: 6; $P = 0.87$).
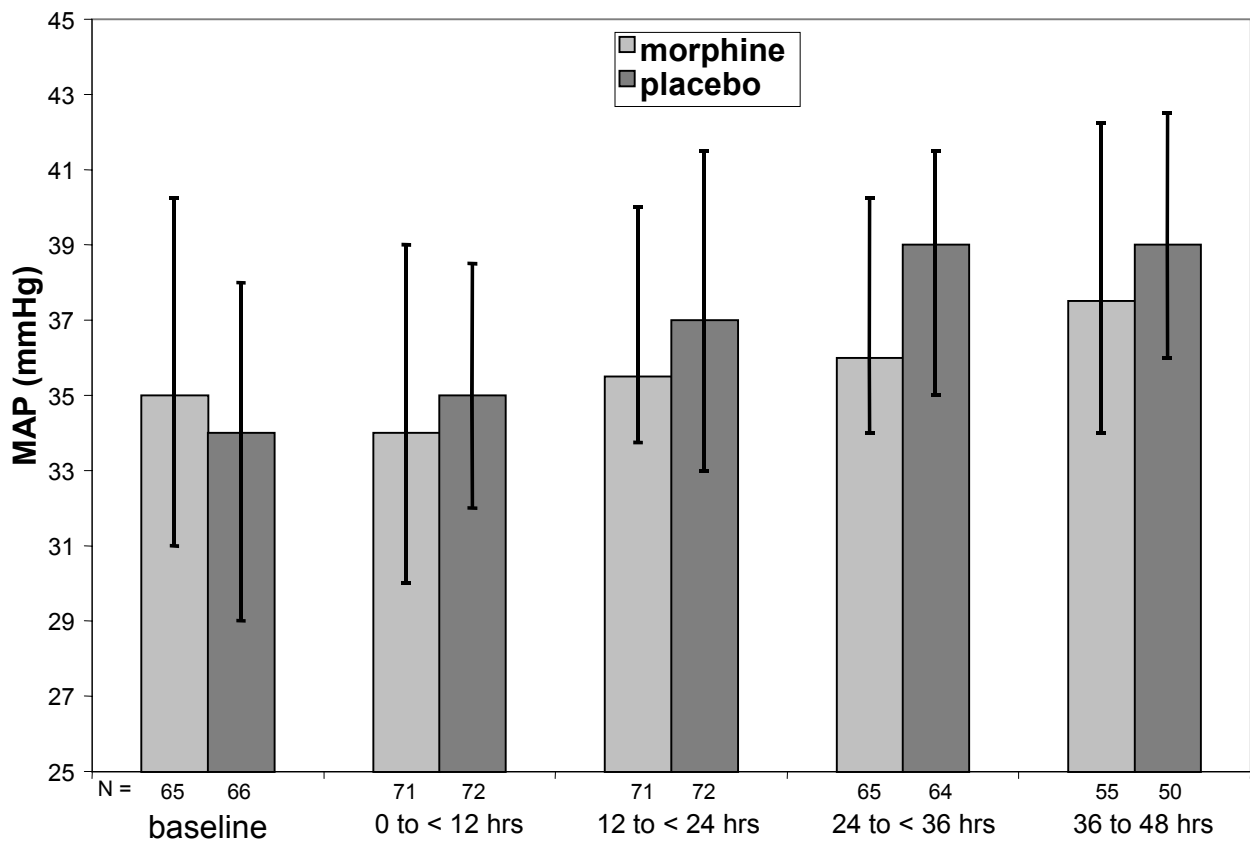
Figure 3   Median MAP (mmHg) and interquartile ranges for both treatment conditions plotted for consecutive 12 hour time-intervals during study. At these intervals, there was no significant difference in MAP between the morphine and placebo treated infants (Mann Whitney U tests: p = 0.43, p = 0.23, p = 0.21, p = 0.17, and p = 0.35 for the different intervals, respectively)

Table 3   Percentages of used volume expansion and inotropic therapy in the morphine and placebo group

Inotropic therapy	Morphine (n = 71)	Placebo (n = 73)
Plasma expanders	20 %	23 %
Dopamine	3 %	5 %
Plasma expanders + dopamine	17 %	14 %
Dopamine + dobutamine	1 %	0 %
Plasma expanders + dopamine + dobutamine	1 %	4 %
Hydrocortisone	1 %	0 %
Norepinephrine	0 %	1 %
Total	44 %	48 %

Fifty patients (70%) in the morphine group compared to 34 patients (47%) of the placebo group did show hypotensive MAP measurements during the study. Although blood pressure between these patients with hypotension did not differ between the morphine (median MAP: 34 mmHg; IQR 4.5) and placebo (median MAP 34 mmHg; IQR 6.0) treated infants (Mann Whitney U-test: 830; $P = 0.88$), the difference in numbers of infants with hypotension between the two conditions reached statistical significance (χ^2 test: 8.42; *df*: 1; $P = 0.004$) (Figure 4.). Taking the use of additional morphine also into consideration, placebo-treated infants with and without additional morphine showed hypotension in 70% (14/20) and 38% (20/53) of the patients, respectively (Fisher's Exact test: $P = 0.018$). Eighty percent (12/15) of the morphine treated infants with additional morphine showed hypotension versus 68% (38/56) of the morphine treated patients who did not receive additional morphine (Fisher's Exact test: $P = 0.53$).
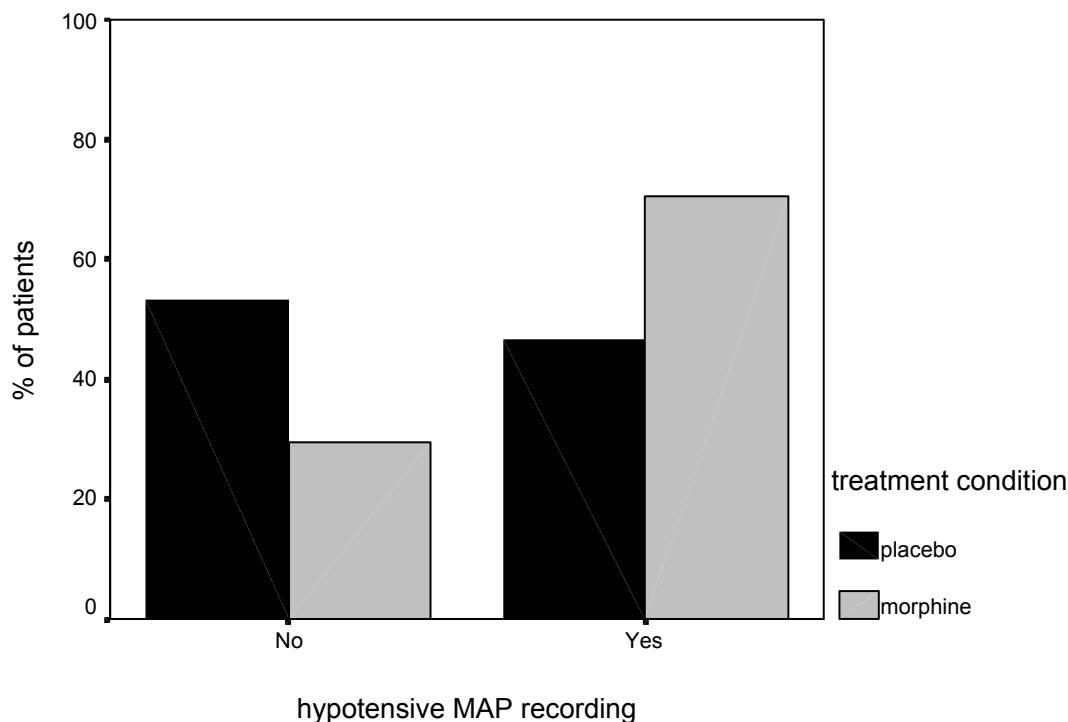
Figure 4   Proportions of patients (%) for the morphine and placebo groups with and without hypotensive mean arterial pressures (= MAP below the lower limit of the 95%CI for normative blood pressure) during masked medication infusion. Significantly more patients in the morphine group were found to have hypotensive MAPs ($\chi^2 = 8.42$; df: 1; p = 0.004).

Blood pressure variability

Fluctuations (median, 25-75th percentile) in MAP during study medication administration were 5.5 (4.5 to 7.0) mmHg in the morphine group and 5.0 (4.0 to 7.0) mmHg in the placebo group, respectively. Multiple regression analyses (Table 4.) revealed that fluctuations in blood pressure were not predicted by treatment group (B = 0.094; 95%CI: -0.67 to 0.85; p = 0.81), nor by the amount of used additional 'open label' morphine (B = 0.019; 95% CI: -0.12 to 0.16; P = 0.80) or by gestational age (B = 0.0090; 95% CI: -0.008 to 0.026; P = 0.30). The use of inotropic therapy significantly predicted higher blood pressure variability (B = 0.90; 95% CI: -0.095 to 1.70; P = 0.03).

Clinical significance

In a previous report of the original trial, in which the present study was embedded, we demonstrated a significantly lower incidence of IVH in the morphine group compared to the placebo group.[15] In the current analyses, blood pressure did not significantly differ between patients with an IVH and those without an IVH (Mann Whitney U-tests:1953; P = 0.14) and the incidence of IVH was not higher in patients with hypotension compared to patients without hypotension (χ^2 test: 1.16; df: 1; P = 0.28). As neonates with an IVH and neonates without an IVH also did not show a significant difference of blood pressure variability (Mann Whitney U-test: 2950; P = 0.51), a relationship between blood pressure variability and the development of an IVH could not be demonstrated.

Table 4   Results of multiple regression analyses with fluctuations in MAP (interquartile range of ΔMAP, mmHg) as outcome variable.

	IQR ΔMAP (mmHg) *		
	B †	95% CI of B	P
Condition	0.094	-0.67 to 0.85	0.81
Amount of open label morphine	0.019	-0.12 to 0.16	0.80
Center	0.25	0.60 to 1.10	0.56
CRIB-score ‡	0.068	-0.076 to 0.21	0.35
Gender	-0.094	-0.88 to 0.69	0.81
Use of inotropics	0.90	0.095 to 1.70	0.03
Gestational age	0.0090	-0.008 to 0.026	0.30
Dev. mean birthweight §	0.033	-0.32 to 0.25	0.82

* IQR ΔMAP = Interquartile Range of ΔMAP (difference two consecutive MAP recordings) , used as a measure of blood pressure variability; † B = Unstandardized regression coefficients; ‡ CRIB = Clinical Risk Index for Babies; § Birthweights were compared to normal values of birthweight for each infants' gestational age.

Discussion

This study aimed to determine the effects of continuous morphine infusion on blood pressure in ventilated newborn infants. While we did not establish significant overall differences in blood pressure or in the use of volume expansion and vasopressor drugs between the morphine and placebo treated infants, we found that significantly more patients in the morphine group (70%) showed hypotension compared to the placebo group (47%) ($P = 0.004$). Routine morphine analgesia as well as the amount of additional used 'open label' morphine did not influence the variability of MAP. From a clinical perspective the lack of a relation between the incidence of IVH and blood pressure variability is a very important observation.

According to the attending physicians masked medication caused hypotension in 6 patients (4%) and was discontinued for this reason. Although blinded to treatment, the physicians obviously assumed that 'morphine' infusion was the cause of the low blood pressures. As two patients actually received placebo infusions, this again illustrates that clinicians still show misconception and uncertainty about the potential side effects of morphine on the neonates' blood pressure. Previous studies investigating these effects are difficult to compare because of different settings and morphine dosage regimens. Morphine has previously been shown to cause significant changes[18] and non-significant changes,[12,25,26] as well as no effect on blood pressure at all.[24,26] In concordance with Quinn et al., who also investigated the effect of continuous morphine infusion on blood pressure in a placebo controlled trial, using much higher morphine doses (100 μg.kg^{-1} for 2 h + 25 μg.kg^{-1}.h^{-1})[12] compared to our study, no overall statistically significant reduction of blood pressure was shown. Continuous morphine infusion obviously does not cause an overall decrease of blood pressure. In the individual neonatal patient, however, morphine might well exert hypotensive effects. Our study indeed showed that 70% of the morphine treated infants showed hypotension during at least some time, versus 47% of the placebo treated infants. Furthermore, patients in the placebo group receiving additional 'open label' morphine showed significantly more hypotension than the placebo treated infants who did receive no morphine at all. In summary this indicates that while hypotension is not a general effect of morphine in neonates, it might occur as a side effect. Probably some infants are more vulnerable to become hypotensive during morphine infusion as compared to others. The clinical significance of hypotension as a side effect of morphine is, however, hard to determine. Although an increased incidence of hypotension by the use of morphine was found, an absolute decrease in mmHg of blood pressure was not detectable.

As the use of volume expanders and vasopressor drugs was not increased in the morphine group, the hypotension induced by morphine did not appear to affect the hemodynamic stability of these infants. Furthermore, we were not able to detect any negative consequences of morphine on clinical outcome measures, such as length of NICU stay, duration of ventilation, and incidences of secondary morbidity.[15] We conclude, therefore, that major clinical consequences of the increased incidence of hypotension are not very likely.

One of the major limitations of our study, as well as of previous studies investigating hemodynamic effects of morphine in newborns, is that effects of prolonged morphine use on blood pressure were not studied. Because the existing models calculating 'normal blood pressures' seem to be reliable during the first postnatal days only, we analyzed blood pressures for the first 48 hours only. Furthermore, as blood pressure data were not continuously analyzed but in 2 hours time-intervals, we might have failed to register some hypotension in the studied infants. However, while all infant's monitors immediately alarmed the attending clinicians in case of hypotension, giving them the opportunity to administer anti-hypotensive therapy, the use of anti-hypotensive therapy did not differ between the 2 treatment conditions. Therefore the analyzed blood pressures seem to realistically reflect actual continuous blood pressures.

To our knowledge no previous study investigated the effect of continuous morphine infusion on blood pressure variability in neonates. Our main trial showed a significant reduction of IVH by the use of routine morphine infusion.[15] It has been suggested that a decrease of blood pressure fluctuations, protecting against the development of venous hemorrhage in the germinal matrix or brain parenchyma,[16,17] might be caused by morphine administration. We were, however, not able to show any relationship between blood pressure variability and IVH, or with the use of morphine. While this suggests that decreased blood pressure fluctuations are not an important part of the protective effect of morphine against neurological damage, it should be noted that we measured peripheral arterial blood pressure and not cerebral blood flow at the time of continuous morphine infusion. As we also studied blood pressure only during the first 48 hours of continuous morphine infusion, future research should focus on longer periods of cerebral blood pressure measurement during continuous morphine administration.

Conclusions

In this randomized placebo-controlled trial we were not able to link the protective effect of morphine on the development of intraventricular hemorrhage with a decrease in blood pressure variability. Furthermore we showed that hypotension was not a general effect of morphine in ventilated neonates, as no significant overall difference in blood pressures between the two treatment conditions was found. Hypotension was suggested as a side-effect of morphine in some neonates, as significantly more patients in the morphine group (70%) showed hypotension compared to the placebo group (47%) ($P = 0.004$). The clinical significance of this hypotension in clinical practice is probably minimal as volume expansion and vasopressor drugs were used with equal frequencies in the morphine and placebo treated infants, but this needs to be evaluated in our patients during long-term follow-up.

Acknowledgements

References

1. Lawrence J, Alcock D, McGrath P, Kay J, MacMurray SB, Dulberg C. The development of a tool to assess neonatal pain. Neonatal Netw 1993;12(6):59-66.

2. Stevens B, Johnston C, Petryshen P, Taddio A. Premature Infant Pain Profile: development and initial validation. Clin J Pain 1996;12(1):13-22.

3. van Dijk M, de Boer JB, Koot HM, Tibboel D, Passchier J, Duivenvoorden HJ. The reliability and validity of the COMFORT scale as a postoperative pain instrument in 0 to 3-year-old infants. Pain 2000;84(2-3):367-77.

4. Prevention and management of pain and stress in the neonate. American Academy of Pediatrics. Committee on Fetus and Newborn. Committee on Drugs. Section on Anesthesiology. Section on Surgery. Canadian Paediatric Society. Fetus and Newborn Committee. Pediatrics 2000;105(2):454-61.

5. Anand KJS. Consensus statement for the prevention and management of pain in the newborn. Arch Pediatr Adolesc Med 2001;155(2):173-80.

6. Als H. A synactive model of neonatal behavioral organization. Phys Occup Ther Pediatr 1986;6:3-55.

7. Als H. Developmental care in the newborn intensive care unit. Curr Opin Pediatr 1998;10(2):138-42.

8. Johnston CC, Collinge JM, Henderson SJ, Anand KJS. A cross-sectional survey of pain and pharmacological analgesia in Canadian neonatal intensive care units. Clin J Pain 1997;13(4):308-12.

9. Kahn DJ, Richardson DK, Gray JE, Bednarek F, Rubin LP, Shah B, et al. Variation among neonatal intensive care units in narcotic administration. Arch Pediatr Adolesc Med 1998;152(9):844-51.

10. Debillon T, Bureau V, Savagner C, Zupan-Simunek V, Carbajal R. Pain management in French neonatal intensive care units. Acta Paediatr 2002;91(7):822-6.

11. Simons SHP, van Dijk M, Anand KJS, Roofthooft DWE, van Lingen RA, Tibboel D. Do we still hurt newborn babies? A prospective study of procedural pain and analgesia in neonates. Arch Pediatr & Adolesc Med 2003;157:1058-1064.

12. Quinn MW, Wild J, Dean HG, Hartley R, Rushforth JA, Puntis JW, et al. Randomised double-blind controlled trial of effect of morphine on catecholamine concentrations in ventilated pre-term babies. Lancet 1993;342(8867):324-7.

13. Scott CS, Riggs KW, Ling EW, Fitzgerald CE, Hill ML, Grunau RV, et al. Morphine pharmacokinetics and pain assessment in premature newborns. J Pediatr 1999;135(4):423-9.

14. Anand KJS, Barton BA, McIntosh N, Lagercrantz H, Pelausa E, Young TE, 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 1999;153(4):331-8.

15. Simons SHP, van Dijk M, van Lingen RA, Roofthooft DWA, Duivenvoorden HJ, Jongeneel N, et al. Lack of analgesic effect of routine morphine infusion in ventilated preterm newborns: a blinded randomized placebo controlled trial. JAMA 2003;290(18):2419-2427.

16. Perlman JM, McMenamin JB, Volpe JJ. Fluctuating cerebral blood-flow velocity in respiratory-distress syndrome. Relation to the development of intraventricular hemorrhage. N Engl J Med 1983;309(4):204-9.

17. Ghazi-Birry HS, Brown WR, Moody DM, Challa VR, Block SM, Reboussin DM. Human germinal matrix: venous origin of hemorrhage and vascular characteristics. AJNR Am J Neuroradiol 1997;18(2):219-29.

18. Wood CM, Rushforth JA, Hartley R, Dean H, Wild J, Levene MI. Randomised double blind trial of morphine versus diamorphine for sedation of preterm neonates. Arch Dis Child Fetal Neonatal Ed 1998;79(1):F34-9.

19. Hartley R, Green M, Quinn M, Levene MI. Pharmacokinetics of morphine infusion in premature neonates. Arch Dis Child 1993;69(1 Spec No):55-8.

20. Way WL, Costley EC, Way EL. Respiratory sensitivity of the newborn infant to meperidine and morphine. Clin Pharmacol Ther 1965;6:454-461.

21. Lynn AM, Nespeca MK, Opheim KE, Slattery JT. Respiratory effects of intravenous morphine infusions in neonates, infants, and children after cardiac surgery. Anesth Analg 1993;77(4):695-701.

22. Barker DP, Simpson J, Pawula M, Barrett DA, Shaw PN, Rutter N. Randomised, double blind trial of two loading dose regimens of diamorphine in ventilated newborn infants. Arch Dis Child Fetal Neonatal Ed 1995;73(1):F22-6.

23. Gill AM, Cousins A, Nunn AJ, Choonara IA. Opiate-induced respiratory depression in pediatric patients. Ann Pharmacother 1996;30(2):125-9.

24. Dyke MP, Kohan R, Evans S. Morphine increases synchronous ventilation in preterm infants. J Paediatr Child Health 1995;31(3):176-9.

25. Sabatino G, Quartulli L, Di Fabio S, Ramenghi LA. Hemodynamic effects of intravenous morphine infusion in ventilated preterm babies. Early Hum Dev 1997;47(3):263-70.

26. Rutter N, Evans N. Cardiovascular effects of an intravenous bolus of morphine in the ventilated preterm infant. Arch Dis Child Fetal Neonatal Ed 2000;83(2):F101-3.

27. Use and abuse of the Apgar score. Committee on Fetus and Newborn, American Academy of Pediatrics, and Committee on Obstetric Practice, American College of Obstetricians and Gynecologists. Pediatrics 1996;98(1):141-2.

28. The CRIB (clinical risk index for babies) score: a tool for assessing initial neonatal risk and comparing performance of neonatal intensive care units. The International Neonatal Network. Lancet 1993;342(8865):193-8.

29. Versmold HT, Kitterman JA, Phibbs RH, Gregory GA, Tooley WH. Aortic blood pressure during the first 12 hours of life in infants with birth weight 610 to 4,220 grams. Pediatrics 1981;67(5):607-13.

30. LeFlore JL, Engle WD, Rosenfeld CR. Determinants of blood pressure in very low birth weight neonates: lack of effect of antenatal steroids. Early Hum Dev 2000;59(1):37-50.

MORPHINE PHARMACOKINETICS
DURING VENOARTERIAL ECMO IN NEONATES

Abstract

Objective

To examine morphine and metabolite plasma concentrations in neonates undergoing venoarterial ECMO and to quantify clearance differences between neonates during ECMO therapy and 0-3 year old infants subjected to noncardiac major surgical procedures.

Design

Observational study

Setting

Level III referral center

Patients

Fourteen neonates (< 7 days old) undergoing ECMO.

Measurements

Morphine and concomitant medications were given according to standard protocol, adapted according to the neonate's clinical condition. Blood samples were collected eight hourly. Measurements: Plasma morphine, M3G and M6G concentrations were determined using HPLC with fluorimetric detection.

Data analysis

Non-linear Mixed Effects Modeling was used. Data from this current study were combined with data of another study describing morphine pharmacokinetic profiles of 0-3 year-old-children after noncardiac major surgery. Parameter estimates were standardized to a 70-kg person using allometric modeling

Results

Formation clearances to M3G and M6G at the start of ECMO on day 1 were lower (3.5 and 0.5 l/h/70kg respectively) than those in postoperative infants (10.6 and 0.6 l/h/70kg respectively). This matured more rapidly (maturation half-lives 25.5 and 13.6 days respectively) than in postoperative infants (56.6 days). Higher ECMO flows were associated with reduced formation clearances. Elimination clearances of M3G and M6G increased from 3.05 and 1.05 l/h/70kg at birth to 20.5 and 7.11 l/h/70kg respectively with

a maturation half-life of 174 days. These elimination clearances were correlated positively with ECMO flow and negatively with dopamine dose. The use of loop diuretics had no effect on metabolite clearance. Hemofiltration cleared M3G and M6G, but not morphine. The volume of distribution increased throughout ECMO to a volume 2.81 times greater than that in postoperative infants.

Conclusions

Formation clearance to M3G and M6G is reduced in critically ill neonates requiring ECMO, but improves rapidly and is similar to that seen in postoperative neonates by 2 weeks. Metabolite elimination clearance is related to creatinine clearance. ECMO flow had a small effect on metabolite clearance. Higher flows were associated with decreased formation clearances, possibly reflecting illness severity. Dopamine infusions use reflected decreased renal clearance. Vecuronium, midazolam, and fentanyl did not affect metabolite clearance.

Introduction

Data concerning morphine pharmacokinetics in neonates supported by extracorporeal membrane oxygenation (ECMO) are few. Dagan et al[1,2] reported increased morphine formation clearance (CL) in neonates after decanulation. The volume of distribution in neonates on ECMO is believed to be increased because the affinity of free morphine to bind to the membrane oxygenator is high;[2] others, however, have refuted this claim.[3] There are few data concerning the effects of age, size, concomitant medication, ECMO flow or duration, or disease processes on morphine pharmacokinetics available in the literature, in contrast to other drugs.[4]

Morphine is metabolized predominantly by glucuronidation into morphine 3-glucuronide (M3G) and morphine 6-glucuronide (M6G).[5,6] M6G has been shown to have analgesic activity exceeding that of morphine. M3G lacks analgesic activity and antagonizes the effects of both morphine and M6G. The maturation time courses of the 3- and 6-glucuronidation enzyme systems have been poorly described until now.[7-9] The assessment of morphine clearance maturation in neonates and infants[10-12] is muddied by size effects.[13] Allometric ¼ size models[14,15] may be more appropriate for scaling pharmacokinetic parameters in children than the per kilogram model.[16,17]

The first aim of this study was to examine morphine and MG3 and MG6 metabolite plasma concentrations in neonates undergoing venoarterial ECMO therapy. A population-

based approach that included size as the primary covariate was used to disentangle age related factors from size related factors. The second aim was to examine clearance differences between neonates during ECMO therapy and 0-3 year old subjected to noncardiac major surgical procedures.[18] Lastly we wished to investigate the effects of age, size, concomitant medication, ECMO flow, duration on ECMO, and underlying disease on clearance in neonates on ECMO.

Patients and methods

The Erasmus MC-Sophia Children's University Hospital Rotterdam serves as a level III referral center for all pediatric surgical subspecialties. It is one of two designated pediatric ECMO centers in the Netherlands. The Medical Ethical Committee approved the study and informed consent was obtained from the parents.

Criteria for ECMO were postgestational age (PGA) greater than 34 weeks, birth weight greater than 2000 g, mechanically ventilation for less than 7 days, an alveolar arterial oxygen difference of less than 80 kPa, and a maximal PaO_2 of less than 10 kPa. Neonates who were expected to die within 24 hours were excluded.

Procedure

Morphine was administered for sedation purposes mainly. The morphine-loading dose (100 mcg/kg) was given before cannulation mainly as analgesic agent, after which an infusion (0-40 mcg/kg/h) was started. Concomitant medication, such as dopamine, furusemide and midazolam were given according to standard protocol and adapted to the neonate's clinical condition. Drug infusion rates (mcg/kg/min) and ECMO flow were recorded every two hours from the start of ECMO. Blood samples (1 ml) were taken every 8 hours during the ECMO run. These samples were taken proximal to the oxygenator in the ECMO circuit and were collected in heparinized tubes. Plasma was separated by centrifugation for 10 min at 5500 rpm, and stored in polypropylene tubes at -80°C.

Assay method

Plasma 0.2 ml was put in a polypropylene tube, mixed with 0.4 ml of 0.01 M ammonium hydrogen carbonate (pH 9.3) and centrifuged. The supernatant was applied on an equilibrated C8 Bound-Elute (Merck, Darmstad, Germany) solid phase extraction column. After 5 minutes the column was washed with 0.01 M ammonium hydrogen carbonate,

dried under vacuum, washed again with hexane and dried again. Morphine and its glucuronides were eluted with 0.5 ml of 0.05 M acetic acid in methanol/water (9/1 v/v) under vacuum for 20 sec. The eluate was evaporated to dryness under nitrogen at room temperature. The residue was reconstituted in 150 ml of 0.05% (w/v) phosphoric acid. An aliquot of 75 ml was injected onto the HPLC column (Merck Lichrocart 250-4 fitted with Merck Lichrocart 4-4 precolumn, both Lichrospher 60, RP select B 5 mm). These analytical columns were eluted after 20 min isocratic and then with a linear increasing gradient of 0 to 60% acetonitrile in 0.2 M potassium dihydrogen phosphate buffer (pH 3.0) in 8 min with a flow of 1.2 ml/min. Detection was with an extremely sensitive spectro-fluorimetric detector L-7480 (Hitachi/Merck) with an 8 ml flow cell, at excitation 210 nm, and emission 350 nm; response time 4 sec. The limit of detection (signal to noise ratio > 3) for morphine, M3G, and M6G was 8, 11, and 19 mg/l plasma respectively and at 100 mg/l (n = 5) the inter-day CV's were 1.1, 6.3, and 2.5% and the bias -5.23, -1.4, and -0.019%, respectively. The extracts were stable in the auto-sampler at 4^0C during the least 22 hrs.[19]

ECMO system applications

The ECMO material circuit consisted of extracorporeal cannulae (pediatric venous cannulae, Medtronic, USA), tubing (Bentley Bypass 70 tubing, Baxter, The Netherlands), membrane (Pediatric Extended Capacity membrane Oxygenator, Medtronic, USA), and heat exchanger (Heat Exchanger Monitoring adapter and Luer-lock, Medtronic, USA). The priming volume of the ECMO system ranges between 300-350 ml.

Modeling

Population parameter estimates

Morphine HCl dose, M3G and M6G were converted to morphine mg equivalents using a molecular weight of 285 for morphine, 322 for morphine HCl, and 461 for the two glucuronide metabolites. Population parameter estimates were obtained using a non-linear mixed effect model (NONMEM). This model accounts for population parameter variability (between and within subjects) and residual variability (random effects) as well as parameter differences predicted by covariates (fixed effects). A proportional variance model modeled the population parameter variability in model parameters. Additive terms characterized the residual unknown variability for plasma morphine, M3G and M6G concentrations. This error model assumes that the residual variability is of the same order of magnitude over the whole range of measurements. The population mean parameters between subject variance and residual variances were estimated using NONMEM version

V release 1.1.[20] Estimation used the first order conditional estimate method with the interaction option and ADVAN 6 with Tol = 5. Convergence criterion was 3 significant digits. A Compaq Digital Fortran Version 6.5 compiler with Intel Pentium III 1 GHz CPU under Windows 2000 was used.

Differential equations were used to describe the pharmacokinetics of morphine and its metabolites. The model is shown in Figure 1.

$$dCM/dt = RATEIN - CM * (CL2M3G + CL2M6G + CLexch + Clfiltr) / VM$$
$$dM3G/dt = CL2M3G * CM - CM3G * (CLM3G + CLexch + Clfiltr) / V3M$$
$$dM6G/dt = CL2M6G * CM - CM6G * (CLM6G + CLexch + Clfiltr) / V6M$$

CL2M3G and CL2M6G are the formation clearances to metabolites M3G and M6G respectively, RATEIN is morphine infusion rate, CM is morphine concentration in plasma, CLexch is clearance attributable to exchange transfusion, CLfiltr is that clearance attributable to hemofiltration, VM is the volume of distribution of morphine, CM3G and CM6G are metabolite concentrations in plasma, CLM3G and CLM6G are metabolite elimination clearances, V3M and V6M are the volume of distributions of the glucuronide metabolites. The glucuronide metabolite volumes of distribution (V3M, V6M) could not be identified with the current study design. These were fixed at 23 and 30 l/70kg, based on studies by Penson et al[21] and Hanna et al[22] in adults.
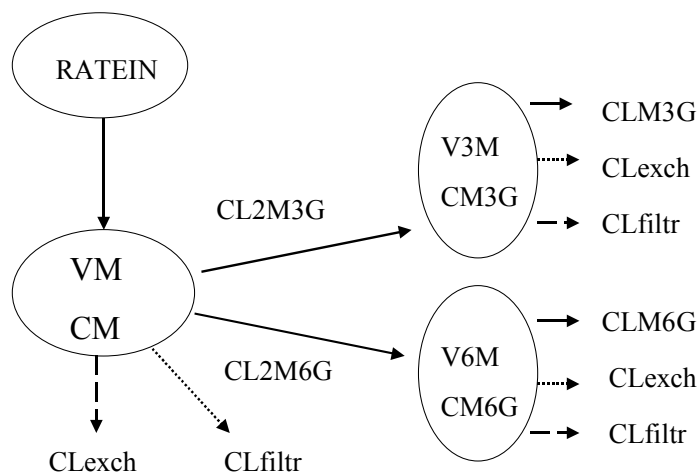
Figure 1   Pharmacokinetic Model. RATEIN is the morphine base infusion rate, VM is the volume of distribution for morphine, CM is morphine plasma concentration, CL2M3G is the formation clearance to M3G, CL2M6G is the formation clearance to M6G, CLM3G is the elimination clearance of M3G, CLM6G is the elimination clearance of M6G, V3M and V6M are the volumes of distribution of glucuronide metabolites, CLexch is clearance due to exchange transfusion CLfiltr is clearance by filtration

Data from this current study were combined with those from another study, which investigated morphine pharmacokinetics in postoperative infants 0-3 years (n = 184) in order to compare morphine pharmacokinetics neonates on ECMO with these postoperative infants.[18]

Covariate Analysis

The parameter values were standardized for a body weight of 70-kg using an allometric model[16,23]

$$P_i = P_{std} \, x \, (W_i \, / \, W_{std})^{\,PWR}$$

Where P_i is the parameter in the ith individual, W_i is the weight in the ith individual and P_{std} is the parameter in an individual with a weight W_{std} of 70 kg. The *PWR* exponent was 0.75 for clearance and 1 for distribution volumes[14,24,25]

Exponential functions were used to describe age-related developmental changes in the formation clearances (CL2M3G, CL2M6G) and elimination clearances (CLM3G, CLM6G) of metabolites

$$FCL2MxG = (1 - \beta cl * EXP(-PNA \text{ in days} * Ln(2)/Tcl))$$
$$FCLMxG = (1 - \beta rf * EXP(-PNA \text{ in days} * Ln(2)/Trf))$$
$$FVM = (1 - \beta vol * EXP(-PNA \text{ in days} * Ln(2)/Tvol))$$

FCL2M*x*G and FCLM*x*G represent the formation and elimination clearances of either M3G or M6G. FVM represents morphine distribution volume as a fraction of standard 70-kg adult values i.e. when age is sufficiently large that the exponential expression tends to zero. βcl, βrf, and βvol are parameters estimating the fraction below 'adult' values of parameters predicted at birth; PNA is postnatal age; Tcl, Trf, and Tvol describe the maturation half-lives of the age-related changes in the parameters CL2M3G, CLM3G, and VM.

Neonates undergoing ECMO had an additional factor applied to βcl, βvol, Tcl, and Tvol in order to determine maturation differences from the group of surgical neonates. We anticipated a higher VM in neonates on ECMO and a further factor was applied to VM in neonates on ECMO therapy. In addition the impact of ECMO pump flow (Flow, l/h/70kg) on metabolite formation clearances was examined by adding a scaling factor (Fpump):

$$FC2MxGecmo = FCL2MxG * EXP(Flow * Fpump)$$

The effect of altered renal function on CLM3G and CLM6G was modeled using an estimate of renal function in infants older than one week of age. Renal function (FRF) was standardized to a 40-year-old adult male with a creatinine clearance of 6 l/h and a serum creatinine of 85.947 mcmol/l.[26] This empirical model used age (PNA) as a covariate to predict creatinine production rate with scaling constant (Kage) for age:

$$FRF = 85.947/creatinine \times EXP(Kage \times PNA/365-40)$$

The impact of ECMO pump flow (Flow, l/h/70kg) and dopamine (Dop, mcg/kg/min) on renal function in children on ECMO was examined using scaling constants (Fflow & Fdop):

$$FRFecmo = FRF \times EXP(Fflow \times Flow) \times EXP(Fdop \times Dop)$$

Serum bilirubin (mcmol/l) is a crude marker of hepatic function in postoperative neonates and its effect on CL2M3G & CL2M6G was modeled with an exponential function with a scaling constant (Kbili):

$$FBILI = EXP(bilirubin \times Kbili)$$

The clearance in a child with specific age, serum creatinine and bilirubin was then predicted by multiplying each of the covariate factors times the population parameter value for a standard 70-kg adult:

$$CL2M3G = CL2M3Gstd * FCL2MxG * FBILI$$
$$CLM3G = CLM3Gstd * FCLMxGecmo * FRFecmo$$

The quality of fit of the pharmacokinetic model to the data was assessed by visual examination of plots of observed versus predicted concentrations. Models were nested and an improvement in the objective function was referred to the Chi-squared distribution to assess significance, e.g. an objective function change (OBJ) of 3.84 is significant at α = 0.05.

Results

Neonates (n = 14) had a median (10th -90th percentile) gestation age of 40 (37-42) weeks and weight 3.2 (2.2–4.4) kg. There were 8 girls and 6 boys. The primary diagnoses of the patients were meconium aspiration syndrome (n = 5), persistent pulmonary hypertension (n = 7), therapy-resistant respiratory insufficiency (n = 1), and sepsis (n = 1). Neonates were given ECMO for a median duration of 207 (107-410) hours. The median morphine infusion was 20 (10-40) mcg/h/kg (see Table 1).

The median amounts and the number of children who had received co-administered drugs as well as the bilirubin and creatinine plasma concentrations are given in Table 1. The ECMO subset consisted of 953 observations. The postoperative subset included 184 children and consisted of 1856 observations. Thus the total analysis used 2809 concentration observations from 198 subjects. Parameter estimates, standardized to a 70-kg, 40-year-old person, are shown in Table 2a. Covariate analysis estimates for the pooled population is shown in Table 2b. Additional covariate analyses for the children receiving ECMO therapy only are shown in Table 2c. The covariance of the pharmacokinetic parameters, expressed as the correlation of population parameter variability, was low (see Table 3). The objective function decreases as additional covariates such as ECMO pump flow or dopamine were added, as shown in Table 4. Figures 2a-2c demonstrate that the individual Bayesian a-posteriori estimated parameters predicted the data well.

Table 1   Data on medication and bilirubin/ creatinine plasma concentrations

	n*	Median	10th –90th percentile
Morphine (µg/kg/h)	14	20	10-40
Midazolam (µg/kg/h)	14	200	100-400
Fentanyl (µg/kg/h)	5	3.0	0.30-7.0
Furusemide (µg/kg/h)	13	90	0-200
Dopamine (µg/kg/min)	14	10	3-15
Vecuronium (µg/kg/h)	6	100	80-200
Bilirubin (µmol/l)		14	8-130
Creatinine (µmol/l)		40	14-93

Note: * number of children who had received this drug

Table 2a   Pharmacokinetic parameter estimates

Parameter	Estimate	CV %
CLT	54.91	-
C2LM3G	52	61.2
CL2M6G	2.91	78.0
CLM3G	20.5	58.4
CLM6G	7.11	73.6
VM	139	61.2
V3M	23 fixed	-
V6M	30 fixed	-
Err morphine (proportional)	0.34	
Err M3G (additive) ng/ml	8.56	
Err M3G (proportional)	0.29	
Err M6G (additive) ng/ml	0.42	
Err M6G (proportional)	0.28	

CLT = population estimate for total morphine CL (l/h/70kg), VM is the volume of distribution for morphine (l/70kg), CL2M3G is formation clearance to M3G (l/h/70kg), CL2M6G is formation clearance to M6G (l/h/70kg), CLM3G is the clearance of M3G (l/h/70kg), CLM6G is the clearance of M6G (l/h/70kg), Err is the residual error
Note: These estimates are standardized to a 70-kg person using an allometric size model; %CV is the coefficient of variation for the population parameter estimate. The metabolite volumes of distribution (V3M, V6M) can not be identified with the current study design and were fixed at 23 and 30 l/70kg, based on studies by Penson et al.[21] and Hanna et al[22] in adults.

Table 2b   Covariate Models and Estimates for Pooled Population Parameters

Parameter	Estimate
βvol	0.944
Tvol	2.54 days
βcl	0.806
Tcl	56.6 days
βrf	0.852
Trf	174 days
Kage	0.0125
Kbili	-0.00153

Table 2c   Covariates for ECMO Children

Parameter	estimate	SE%
Fvol (on VM)	2.81	
Fpump (on formation CL2M3G)	-0.00157	
Fdop (on renal metab)	-0.0277	
Ffiltr (on filtrate clearance metabolites)	1.99	
Fflow (on renal metab)	0.0009	
FCL03 (base maturation F2M3G)	1.19	
FTCL3 (CL2M3G maturation half time)	0.45	
FCL06 (base maturation CL2M3G)	1.08	
FTCL6 (CL2M3G maturation half-time)	0.24	

The difference between individual Bayesian and population prediction is attributable to covariates. Figures 3, 4 and 5 show pharmacokinetic data analysis fits. These demonstrate that the individual Bayesian a-posteriori estimated parameters predicted the data well.

The population mean formation clearances to M3G (see Figure 6a) and M6G (see Figure 6b) at the start of ECMO on day 1 were lower (3.5 and 0.5 l/h/70kg respectively) than those in postoperative surgical neonates (10.6 and 0.6 l/h/70kg respectively), but matured more rapidly (maturation half-lives 25.5 and 13.6 days respectively) than those in the postoperative infants (56.6 days). Higher ECMO flows were associated with reduced formation clearances (Fpump = -0.00157). Population mean elimination clearances of M3G (see Figure 7a) and M6G (see Figure 7b) increased from 3.05 and 1.05 l/h/70kg at birth to 20.5 and 7.11 l/h/70kg, respectively, with a maturation half-life of 174 days (see Figures 4a and 4b, and table 2a). These elimination clearances were correlated positively with ECMO flow (Fflow = 0.0009) and negatively with dopamine dose (Fdop = -0.0277; see Table 2c). The use of loop diuretics had no effect on metabolite clearance.

Table 3   Correlation of population pharmacokinetic parameter variability

	CL2M3G	CL2M6G	CLM3G	CLM6G	VM
CL2M3G	1				
CL2M6G	0.228	1			
CLM3G	0.377	-0.202	1		
CLM6G	-0.200	0.712	0.098	1	
VM	0.671	0.115	0.359	-0.181	1

Table 4   Objective Function changes with addition of covariates

Covariate	ΔOBJ	p-value
Factor volume of distribution (Fvol)	45.938	0.001
Maturation factors on VM	18.32	0.001
Maturation formation clearances	13.993	0.001
Individual formation clearances	18.32	0.001
Pump flow on CL2M3G	54.446	0.001
Pump flow on CL2M6G	7.003	0.01
Pump flow on Clearance metabolites	6.284	0.05
Dopamine on Clearance metabolites	29.69	0.001

Note: ΔOBJ 3.841 = 0.05, ΔOBJ 6.635 = 0.01, ΔOBJ 10.827 = 0.001 for 1degree freedom
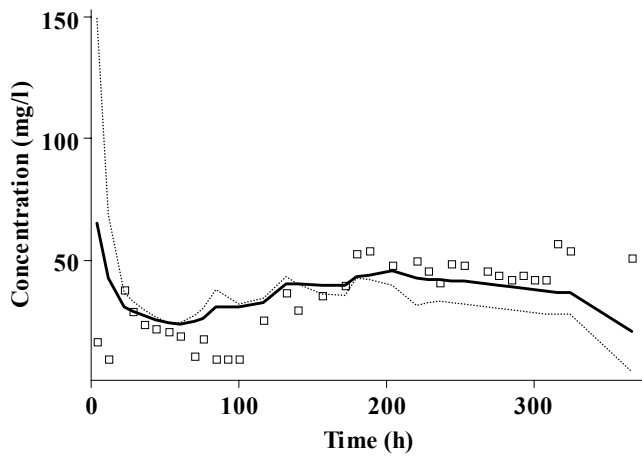
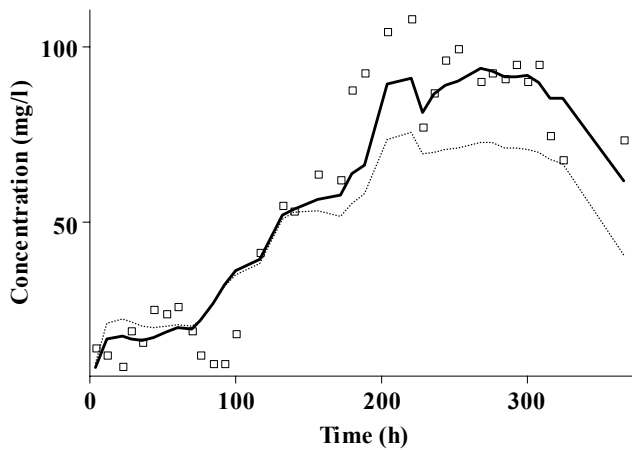**Figure 2a**
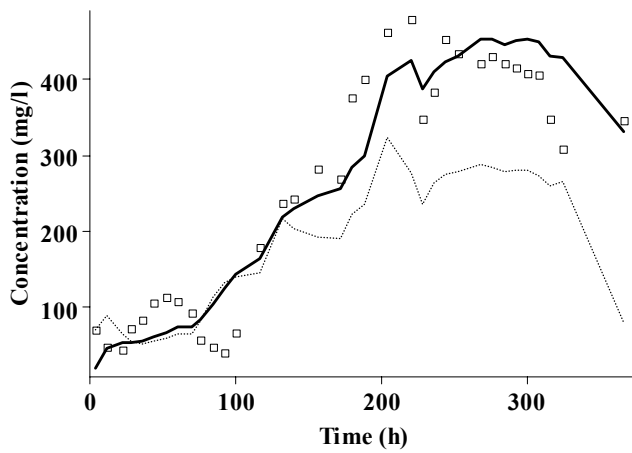
Figure 2b

Figure 2c

Figure 2   Figures 2a, b & c observed concentration (open squares), individual Bayesian prediction (solid line) and population prediction (dashed line) for a typical individual for morphine (2a), M3G (2b) and M6G (2c). The difference between the individual Bayesian prediction and the population prediction is attributable to covariates.
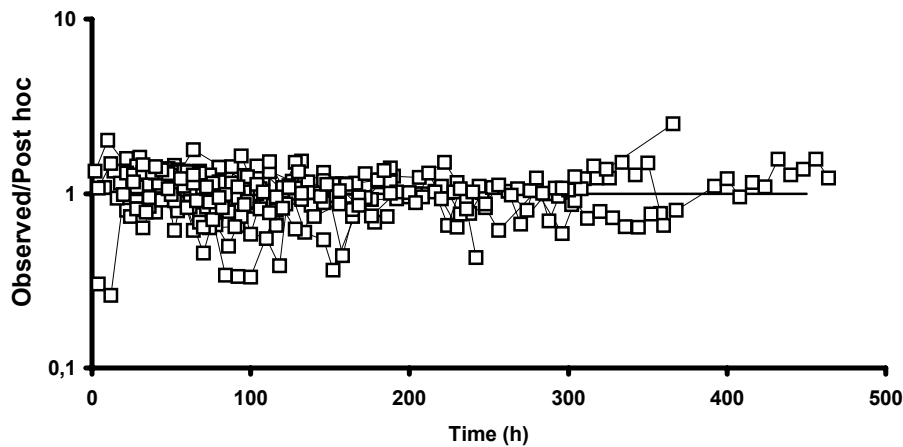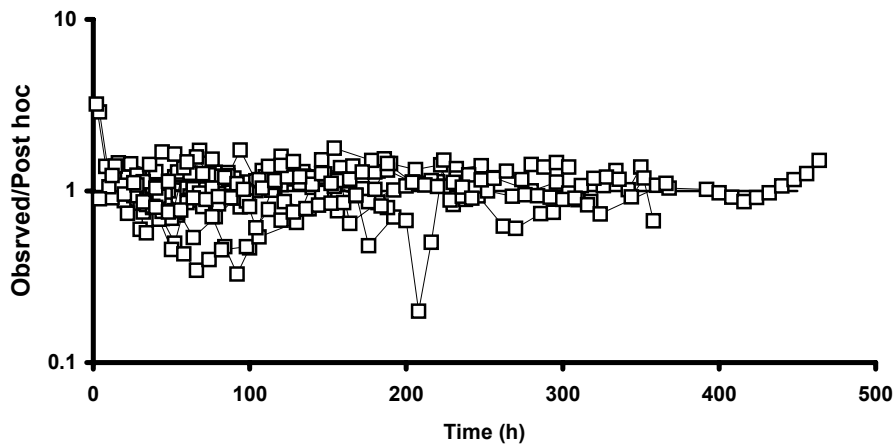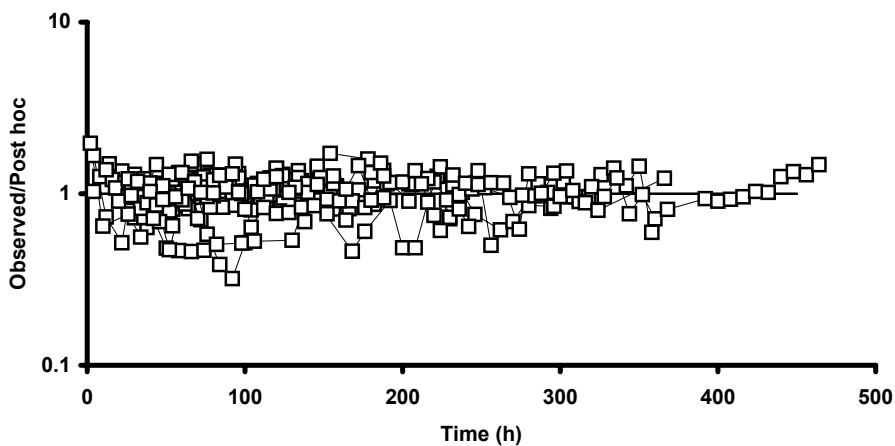
Figure 3a

Figure 3b

Figure 3c

Figure 3 Figures 3a, b & c demonstrate the quality of fit for pharmacokinetic data from children on ECMO over the study time period – a line connects each subject's data. The individual a posteriori Bayesian predictions (post hoc) for plasma concentration of morphine (3a), M3G (3b) and M6G (3c) are compared to those observed. These predictions are based on maximum a posteriori Bayesian estimates of the parameters for each specific individual using their observed data.

Hemofiltration was carried out in two patients and cleared M3G and M6G (Ffiltr = 1.99), but not morphine. The volume of distribution increased throughout ECMO to a volume (Fvol) 2.81 times greater than that in postoperative neonates (see Figure 8). Metabolite formation clearances decreased with increasing serum bilirubin concentration in postoperative infants, but there was no association between formation clearances and bilirubin in children receiving ECMO. We were unable to demonstrate any relationship between clearances and disease, concomitant midazolam infusion, or vecuronium dose. For comparison purposes, parameter estimates for an ECMO treated neonate (day 5) and a postoperative neonate are shown in Table 5.

Table 5   Pharmacokinetic parameter estimates

Parameter	Estimate (CV%) ECMO neonate (day 5)	Estimate (CV%) Postoperative neonate (day 5)
CL Total body	9.45	13.30
CL2 M3G	8.47 (612)	12.57 (91)
CL2M6G	0.95 (78.0)	0.70 (87.0)
CLM3G	3.37 (58.4)	3.30 (65.0)
CLM6G	1.17 (73.6)	1.14 (76.0)
VM	285 (612)	105 (54)
VM3G*	1.15 fixed	1.15 fixed
VM6G*	1.5 fixed	1.5 fixed

CV%: coefficient of variation for the population parameter estimate, CL: Clearance (l.h.70kg^{-1}), Vd: distribution volume (l.70kg^{-1})
*Note: * the metabolite volumes of distribution (Vd M3G, Vd M6G) can not be identified with the current study design and were fixed at 23 and 30 l/70kg, based on studies in adults.*

Discussion

This current study is the first to estimate pharmacokinetic parameters of morphine and its metabolites in neonates receiving ECMO therapy. The use of a population based approach with data from these ECMO patients and postoperative infants allowed covariate analyses and comparison between the two patient groups.

Population mean formation clearance
Neonates on ECMO had initially reduced formation clearances to M3G and M6G in comparison with the postoperative neonates of similar age. We do not have any good explanation for this. It may reflect the severity of illness of the ECMO treated neonates.
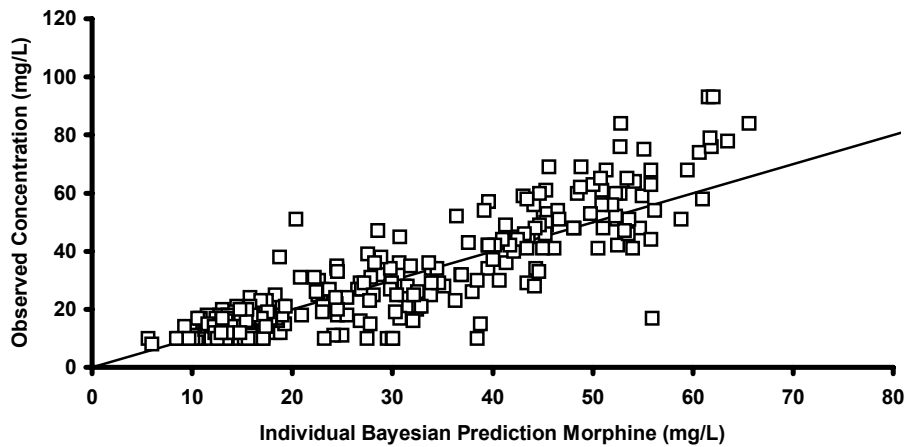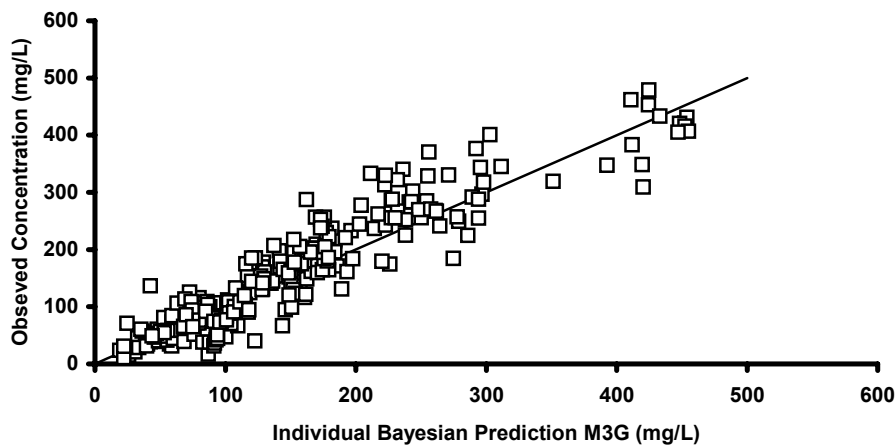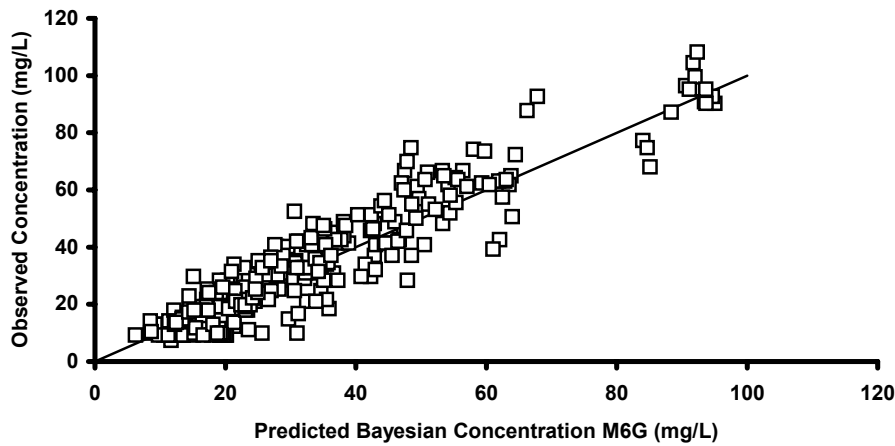
Figure 4a

Figure 4b

Figure 4c

Figure 4 Quality of fit of pharmacokinetic data for children receiving ECMO. Individual Bayesian concentration predictions based on values of the parameters for the specific individual are compared to observed. The line x = y is the line of identity. 4a) Plasma morphine concentration data 4b) plasma M3G data 4c) plasma M6G data
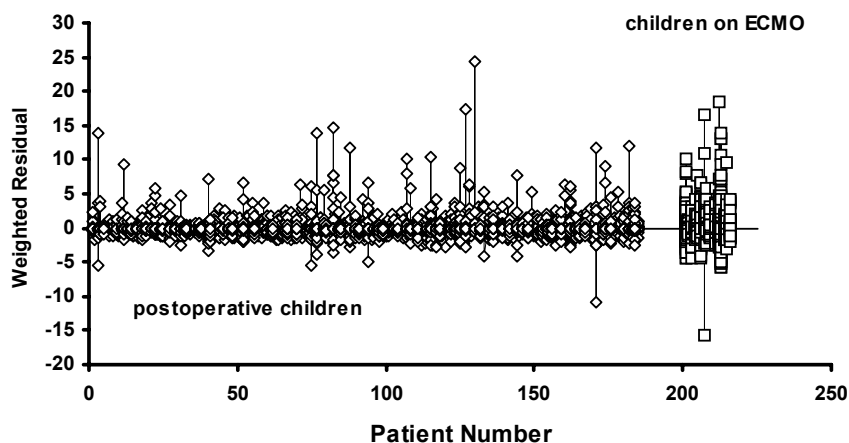
Figure 5   The weighted residuals (WRES) for each subject (postoperative children and those on ECMO) with values for each subject joined by vertical bars are shown.

Studies in adults[27] and in infants[10,28,29] have shown that CL was significant lower in those who needed mechanically support, who suffered from cardiac insufficiency, or who had periods of hypoxia. Hypoxic periods prior to ECMO, due to for example meconium aspiration, may result in liver ischemia and in lower activity of the liver enzymes responsible for morphine glucuronidation.

In addition, we found in the neonates on ECMO that after initiation of ECMO clearance matured more rapidly than in the postoperative neonates. It is generally acknowledged that the metabolism of morphine is age related.[7,30] As the maturation in the ECMO neonates took place over a median period of 9 days, our findings suggest that other mechanisms may interfere; for example, the liver may have been recovering from the ischemia damage prior to ECMO. Postoperative neonates may also suffer from ischemia liver damage, as clearance in this group is also lower than that in healthy controls.[27] The differences in design between the ECMO and postoperative studies, however, do not allow evaluation of this hypothesis. Most of the ECMO neonates (n = 11/14) were followed for a median period of 9 days, while the postoperative infants were followed during the first 24 hours after surgery.

ECMO flows

The morphine metabolites M3G and M6G are water-soluble compounds, enabling renal excretion. The time course of metabolite elimination clearance is similar to that of glomerular filtration rate (GFR), although clearance of morphine glucuronide metabolites is greater.[18] This may be attributable to renal tubular secretion[31-33] and non-renal elimination.[34,35] Renal dysfunction in children on ECMO therapy results from either

nonpulsatile perfusion or low cardiac output prior to ECMO. Our data are corrected for creatinine clearance. Attempts to use the Cockcroft and Gault models[26] to predict creatinine production rate failed. An empirical formula based on age to predict creatinine production, rather than the Cockcroft and Gault models,[36] was used. Creatinine production increased with age (Kage 0.0141) as opposed to adults in whom production decreases with age.[36] The increase in creatinine production is assumed to be a consequence of increasing muscle bulk with age as opposed to the decrease in muscle bulk that occurs with age in adults. M6G elimination clearance in the neonates on ECMO was the same as that in postoperative infants. M3G elimination clearance was reduced, possibly reflecting reduced tubular secretion or non-renal elimination in the ECMO treated neonates. Increased ECMO pump flow resulted in increased elimination clearance, probably due to increased renal blood flow.
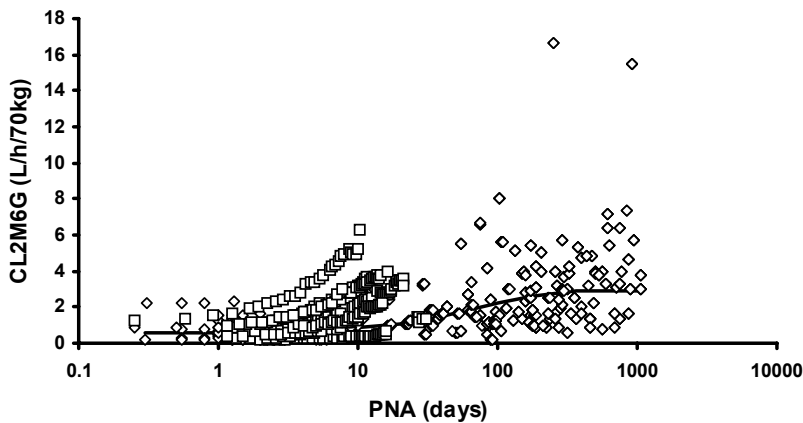

Figure 6a Individual predicted morphine formation clearances to M6G, standardised to a 70-kg person, from the NONMEM post hoc step, are plotted against age. The solid line represents the non-linear relation between clearance and age for postoperative infants (open diamonds). Predictions from neonates on ECMO therapy are shown as open squares.
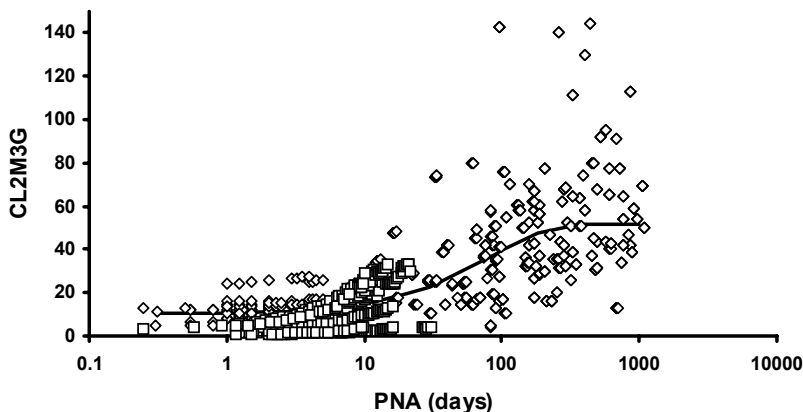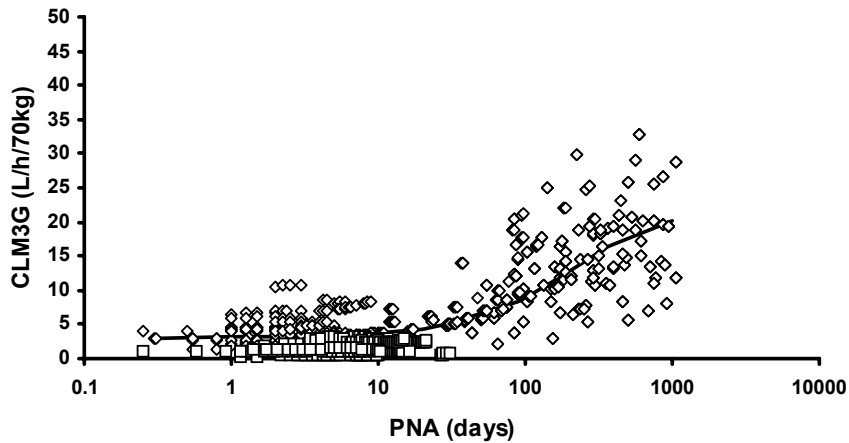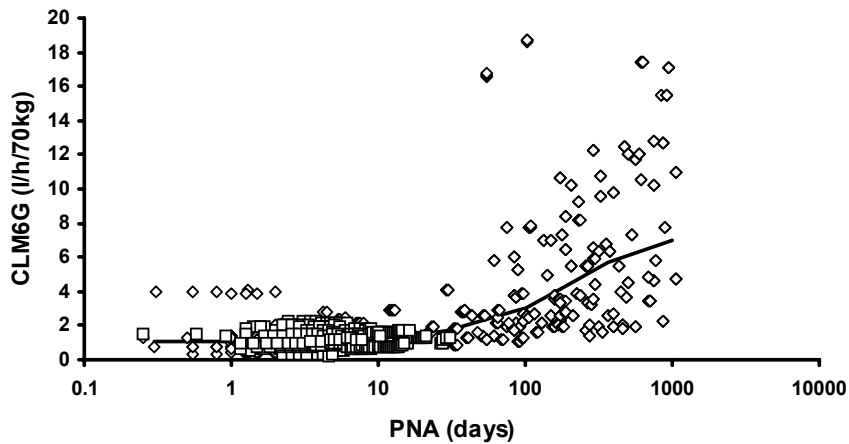

Figure 6b Individual predicted morphine formation clearances to M3G, standardised to a 70-kg person, from the NONMEM post hoc step, are plotted against age. The solid line represents the non-linear relation between clearance and age for postoperative infants (open diamonds). Predictions from neonates on ECMO therapy are shown as open squares.

Figure 7a

Figure 7b

Figure 7 Individual a posteriori Bayesian estimates for postoperative infants (open diamonds) of elimination clearances, CLM3G (7a) and CLM6G (7b), plotted against postnatal age. Neonates receiving ECMO therapy have reduced clearance (open squares)

Hemofiltration

Two children in this study had significant renal complications, necessitating hemofiltration. Previous reports have shown that renal failure significantly reduces the clearance of morphine both in animals[35] and in humans,[37] resulting in increased morphine metabolite concentrations. Hemofiltration was included as a clearance method. As hypothesized, hemofiltration increased renal clearance and thus enhanced M3G and M6G elimination clearance, although this method appeared relatively ineffective. The neonates on ECMO therapy had large volumes of distribution (in excess of 2 l/kg) and a blood volume exchange thus can be expected to have minimal impact.

In addition, we did not found any effect of hemofiltration on morphine clearance. Morphine, in contrast to its' metabolites, is less soluble in water. Normally, small amounts of morphine are eliminated by the kidneys as free unbound morphine.[12] In neonates and infants this depends on the age and plasma concentration of M3G and M6G.[12,31] The fraction free unbound morphine eliminated by hemofiltration may have been too small or too variable to detect effects on morphine plasma concentrations.

Dopamine

We found that dopamine contributed to reduced clearance of the morphine metabolites M3G and M6G. Dopamine in low doses improves urine output, but has no effect on serum creatinine or the incidence of oliguria,[38] and thus seems to have no effect on morphine metabolite clearance. At high doses (> 10 mcg/kg/min), it stimulates the arteriolar α-adrenergic receptors, causing vasoconstriction (including renal vasoconstriction), and hypertension.[39] Dagan et al.[40] reported that high doses of inotrophic support (i.e. dopamine, dobutamine, or epinephrine > 10 mcg/kg/min) lowered the clearance of morphine.

Bilirubin

Serum bilirubin concentration was used as a marker of hepatic function and was related to formation clearances. It proved an unsatisfactory marker of hepatic function for children on ECMO. High serum concentrations were found in the early postnatal period. None of the patients, however, showed clinical signs of icterus or abnormal liver enzyme values, as assessed by other markers of hepatic function (albumin concentration, gamma-glutamyltransferase, aspartate transaminase, alanine aminotransferase). When excluding the bilirubin values corresponding to the first three days on ECMO, bilirubin values returned to normal values (median (10th –90th percentile): 16 (11-37 µmol/l). Bilirubin is a very crude marker of hepatic function, as the serum concentrations depend on both formation and clearance of bilirubin. Bilirubin is metabolized in the liver by another glucuronosyltransferase, UGT1A1, and does not compete for the same metabolic pathway as morphine.[41] In addition, red blood cell destruction by the ECMO circuit and oxygenator causes elevated bilirubin concentrations, whereas hemofiltration clears bilirubin. The priming volume of the ECMO circuit is about 300-350 ml., which is about the same as that of the neonate. This may also have distorted the real bilirubin serum concentration in the early period.
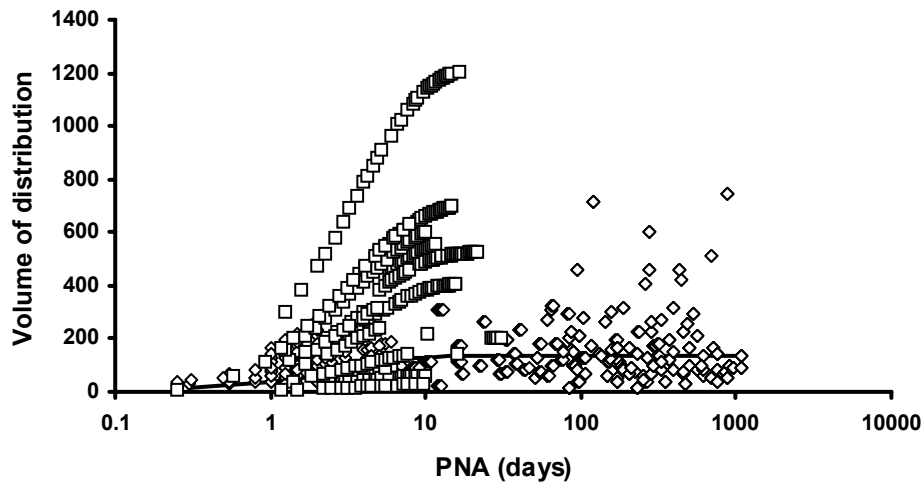
Figure 8 Volume of distribution change with postnatal age for postoperative infants (open diamonds). Individual predicted volumes, standardised to a 70-kg person, from the NONMEM post hoc step, are plotted against age. The solid line represents the non-linear relation between volume of distribution and age. Predictions from neonates on ECMO therapy are shown as open squares.

Co-administered drugs

The co-administration of furusemide, vecuronium, midazolam, and fentanyl had no effect on morphine formation clearance to M3G and M6G and the elimination clearance of M3G and M6G. Furusemide was administered to prevent the neonates from developing edema or to treat this. Edema, however, is related with distribution volume. In this study, no association was found between distribution volume and clearance. Beforehand we did not expect any effect of midazolam or fentanyl on morphine formation clearance to M3G and M6G or elimination clearance of these metabolites. This is because morphine is largely glucuronidated by uridine 5'-diphosphate glucuronosyltransferase UGT2B7 to M3G and M6G;[41] midazolam by the cytochrome P450 system, i.e. by CYP3A4, CYP3A5, and, to a much lesser extent, by CUP3A7;[41] and fentanyl by CYP3A4.[42]

Volume of distribution

The volume of distribution dramatically increased in the neonates receiving ECMO and was 2.8 times greater than that found in the postoperative neonates. The mechanisms for this phenomenon are unclear. It has been suggested that the mechanism for this change may be attributable to sequestration drugs to the ECMO circuit.[43] Dagan et al.[44] demonstrated that the uptake of drugs such as morphine is higher at ECMO initiation and decreases within several days after initiation of ECMO. Extracorporeal circuit changes during ECMO therapy will also have an impact. Our data suggest that this is an exponential, concentration-driven process reaching a state of equilibrium after about ten

days. Geiduschek et al.[3] refuted the claim that ECMO enhances distribution volume; they, however, studied the three first hours after initiation of ECMO only. Our findings, in addition, showed that in some ECMO neonates the distribution volumes did not increase exponentially but gradually like those of the postoperative neonates. This gradual increase in the postoperative neonates reflects age-related changes in distribution volumes.[45] Other factors other than sequestration may also be responsible for the exponential increases. Anderson et al.,[46] for example, reported an increase of 30% in body weight of newborn infants with respiratory failure after cannulation for ECMO; this increase also increases the volume of distribution.[47]

In summary

Formation clearance to M3G and M6G was reduced in critically ill neonates requiring ECMO, but improved rapidly and by two weeks was similar to that seen in postoperative neonates. Metabolite elimination clearance was related to creatinine clearance. ECMO flow had a small effect on metabolite clearance. Higher flows were associated with decreased formation clearances, possibly reflecting illness severity. Dopamine use reflected decreased renal clearance. Vecuronium, midazolam, and fentanyl did not affect metabolite clearance.

References

1. Dagan O, Klein J, Bohn D, et al.: Effects of extracorporeal membrane oxygenation on morphine pharmacokinetics in infants. Crit Care Med 1994; 22:1099-1101.

2. Dagan O, Klein J, Gruenwald C, et al.: Preliminary studies of the effects of extracorporeal membrane oxygenator on the disposition of common pediatric drugs. Ther Drug Monit 1993; 15:263-266.

3. Geiduschek JM, Lynn AM, Bratton SL, et al.: Morphine pharmacokinetics during continuous infusion of morphine sulfate for infants receiving extracorporeal membrane oxygenation. Crit Care Med 1997; 25:360-364.

4. Buck ML: Pharmacokinetic changes during extracorporeal membrane oxygenation: implications for drug therapy of neonates. Clin Pharmacokinet 2003; 42:403-417.

5. Hartley R, Quinn MW, Levene MI: Morphine glucuronidation in premature neonates. British Journal Clin Pharmacology 1993; 35:314-317.

6. Hartley R, Green M, Quinn MW, et al.: Development of morphine glucuronidation in premature neonates. Biol Neonat 1994; 66:1-9.

7. Kart T, Christrup LL, Rasmussen M: Recommended use of morphine in neonates, infants and children based on a literature review: Part 1--Pharmacokinetics. Paediatr Anaesth 1997; 7:5-11.

8. Kart T, Christrup LL, Rasmussen M: Recommended use of morphine in neonates, infants and children based on a literature review: Part 2--Clinical use. Paediatr Anaesth 1997; 7:93-101.

9. Faura CC, Collins SL, Moore RA, et al.: Systematic review of factors affecting the ratios of morphine and its major metabolites. Pain 1998; 74:43-53.

10. Lynn AM, Nespeca M, Bratton SL, et al.: Clearance of morphine in postoperative infants during intravenous infusion: the influence of age and surgery. Anesthesia & Analgesia 1998; 86:958-963.

11. Lynn AM, Nespeca M, Bratton SL, et al.: Intravenous morphine in postoperative infants: intermittent bolus dosing versus targeted continuous infusions. Pain 2000; 88:89-95.

12. McRorie TI, Lynn AM, Nespeca M, et al.: The maturation of morphine clearance and metabolism. AJDC 1992; 146: 972-976.

13. van Lingen RA, Simons SHP, Anderson BJ, et al.: The effects of analgesia in the vulnerable infant during the perinatal period. Clin Perinatol 2002; 29:511-534.

14. West GB, Brown JH, Enquist BJ: A general model for the origin of allometric scaling laws in biology. Science 1997; 276:122-126.

15. West GB, Brown JH, Enquist BJ: The fourth dimension of life: fractal geometry and allometric scaling of organisms. Science 1999; 284:1677-1679.

16. Holford NHG: A size standard for pharmacokinetics. Clin Pharmacokinet 1996; 30:329-332.

17. Anderson BJ, Meakin GH: Scaling for size: some implications for paediatric anaesthesia dosing. Paediatr Anaesth 2002; 12:205-219.

18. Bouwmeester J, Andersen B, Tibboel D, et al.: Developmental pharmacokinetics of morphine and metabolites in neonates, infants, and children. Br J Anaesth in press.

19. van Lingen R: Pain assessment and analgesia in the newborn: an integrated approach. Erasmus University Rotterdam, Rotterdam, 2000; pp 1-177.

20. Beal SL, Sheiner LB, Boeckmann: A 1999 Nonmem User's Guide. Division of Pharmacology, University of California, San Francisco.

21. Penson RT, Joel SP, Clark S, et al.: Limited phase I study of morphine-3-glucuronide. J Pharm Sci 2001; 90:1810-1816.

22. Hanna MH, Peat SJ, Knibb AA, et al.: Disposition of morphine-6-glucuronide and morphine in healthy volunteers. Br J Anaesth 1991; 66:103-107.

23. Verwey-van Wissen CP, Koopman-Kimenai PM, Vree TB: Direct determination of codeine, norcodeine, morphine and normorphine with their corresponding O-glucuronide conjugates by high-performance liquid chromatography with electrochemical detection. J Chromotogr 1991; 570:309-320.

24. Peters HP: Physiological correlates of size. In Beck E, Birks HJB, Conner EF: (Eds) The Ecological Implications of Body Size. Cambridge University Press, Cambridge, 1983, pp 48-53.

25. Karalis V, Macheras P: Drug disposition viewed in terms of the fractal volume of distribution. Pharm Res 2002; 19:696-703.

26. Cockcroft DW, Gault MH: Prediction of creatinine clearance from serum creatinine. Nephron 1976; 16:31-41.

27. Berkenstadt H, Segal E, Mayan H, et al.: The pharmacokinetics of morphine and lidocaine in critically ill patients. Int Care Med 1999; 25:110-112.

28. Pokela M, Olkkola KT, Seppälä T, et al.: Age-related morphine kinetics in infants. Dev Parmacol Ther 1993; 20:26-34.

29. Dagan O, Klein J, Bohn D, et al.: Morphine pharmacokinetics in children following cardiac surgery: effects of disease and inotropic support. J Cardiothorac Vasc Anesth 1993; 7:396-398.

30. Bouwmeester NJ, van den Anker JN, Hop WC, et al.: Age- and therapy-related effects on morphine requirements and plasma concentrations of morphine and its metabolites in postoperative infants. Br J Anaesth 2003; 90:642-652.

31. Hasselstrom J, Sawe J: Morphine pharmacokinetics and metabolism in humans. Enterohepatic cycling and relative contribution of metabolites to active opioid concentrations. Clin Pharmacokinet 1993; 24:344-354.

32. Somogyi AA, Nation RL, Olweny C, et al.: Plasma concentrations and renal clearance of morphine, morphine-3-glucuronide and morphine-6-glucuronide in cancer patients receiving morphine. Clin Pharmacokinet 1993; 24:413-420.

33. Van Crugten JT, Sallustio BC, Nation RL, et al.: Renal tubular transport of morphine, morphine-6-glucuronide, and morphine-3-glucuronide in the isolated perfused rat kidney. Drug Metab Dispos 1991; 19:1087-1092.

34. Hasselstrom J, Sawe J: Morphine pharmacokinetics and metabolism in humans. Enterohepatic cycling and relative contribution of metabolites to active opioid concentrations. Clin Pharmacokinet 1993; 24:344-354.

35. Milne RW, McLean CF, Mather LE, et al.: Influence of renal failure on the disposition of morphine, morphine-3-glucuronide and morphine-6-glucuronide in sheep during intravenous infusion with morphine. J Pharmacol Exp Ther 1997; 282:779-786.

36. Bjornsson TD: Use of serum creatinine concentrations to determine renal function. Clin Pharmacokinet 1979; 4:200-222.

37. Osborne R, Joel S, Grebenik K, et al.: The pharmacokinetics of morphine and morphine glucuronides in kidney failure. Clin Pharmacol Ther 1993; 54:158-167.

38. Barrington K, Brion LP: Dopamine versus no treatment to prevent renal dysfunction in indomethacin-treated preterm newborn infants. Cochrane Database Syst Rev 2002:CD003213.

39. Gorodischer R, Koren G: Cardiac Drugs. In Yaffe SJ, Aranda JV: (eds) Pediatric Pharmacology: Therapeutic Principles in Practice. W.B. Saunder Company, Philadelphia, 1992, pp 345-354.

40. Dagan O, Klein J, Bohn D, et al.: Morphine pharmacokinetics in children following cardiac surgery: effects of disease and inotropic support. J Cardiothorac Vasc Anesth 1993; 7:396-398.

41. de Wildt SN, Kearns GL, Leeder JS, et al.: Glucuronidation in humans. Pharmacogenetic and developmental aspects. Clin Pharmacokinet 1999; 36:439-452.

42. Phimmasone S, Kharasch ED: A pilot evaluation of alfentanil-induced miosis as a noninvasive probe for hepatic cytochrome P450 3A4 (CYP3A4) activity in humans. Clin Pharmacol Ther 2001; 70:505-517.

43. Mulla H, McCormack P, Lawson G, et al.: Pharmacokinetics of midazolam in neonates undergoing extracorporeal membrane oxygenation. Anesthesiology 2003; 99:275-282.

44. Dagan O, Klein J, Gruenwald C, et al.: Preliminary studies of the effects of extracorporeal membrane oxygenator on the disposition of common pediatric drugs. Ther Drug Monit 1993; 15:263-266.

45. Pokela ML, Olkkola KT, Seppala T, et al.: Age-related morphine kinetics in infants. Dev Pharmacol Ther 1993; 20:26-34.

46. Anderson HL, 3rd, Coran AG, Drongowski RA, et al.: Extracellular fluid and total body water changes in neonates undergoing extracorporeal membrane oxygenation. J Pediatr Surg 1992; 27:1003-1007.

47. Pokela M: Pain relief can reduce hypoxemia in distressed neonates during routine treatment procedures. Pediatrics 1994; 93:379-383.

Chapter 8

A CRITICAL ANALYSIS OF PAIN ASSESSMENT IN PREMATURE NEONATES: EVALUATION DURING A RANDOMIZED CONTROLLED TRIAL COMPARING MORPHINE AND PLACEBO IN VENTILATED NEONATES

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Submitted

Abstract

Introduction
Many neonatal pain instruments are developed in the last decades and guidelines for neonatal pain treatment have been published. However, standardized pain treatment including pain assessment, is not yet part of daily clinical care.

Objective
To determine which pain instrument, Premature Infant Pain Profile (PIPP), Neonatal Infant Pain Scale (NIPS), or COMFORT score, is the most appropriate and whether physiological indicators (Heart rate, Mean Arterial Pressure and Oxygen saturation) are sensitive enough to be useful for neonatal pain assessment.

Design, setting, and patients
During a randomized double-blind placebo-controlled trial in two level III NICUs pain scores were collected before, during and after endotracheal and nasal suctioning. Patients were randomized to receive either intravenous morphine (100 µg/kg and 10 µg/kg/hour) or placebo infusion during 7 days. Videotaped material was scored before, during and after endotracheal and nasal suctioning and before and after the administration of additional morphine. In this part of the study the behavioral and physiological indicators of the three instruments were analyzed to determine their usefulness.

Results
Non-metric principal component analyses showed respectively 63%, 78%, and 78% of explained variance by the first component for facial expression, body movements and behavioral state. On the second component differences between the three instruments showed the differences in item formulation.
Silent cry was observed in 45.9% of all 636 observations during endotracheal and nasal suctioning. The physiological indicators heart rate, blood pressure and oxygen saturation were significantly higher during suctioning compared to before or after suctioning. After additional morphine only MAP decreased significantly ($P < 0.001$).

Conclusions
The three instruments PIPP, NIPS and COMFORT scale each have their limitations. The indicators facial expression, body movements and behavioral state are relevant but should be translated into univocal response categories. Although physiological indicators

increased significantly during suctioning, this was not reflected by higher PIPP or COMFORT scores.

These findings call for an improved neonatal pain instrument, which is psychometrically sound and clinically valid.

Introduction

Many pain assessment instruments are developed for use in neonates during recent years. In addition to several reviews on this topic[1-4] and comparisons of pain assessment instruments,[5-7] a structural analysis in the context of a randomized controlled trial is not available in the literature.

We put forward that the continuous flow of newly developed pain instruments reflects dissatisfaction with the current state of pain measurement for neonates. More importantly, it is still uncertain if neonates perceive and express pain in the same way as adults or older children do. By extrapolating children's pain expression to (premature) neonates we might be able to partly capture the truth and objectify the amount of pain.

Despite consensus among pediatric health care professionals that pain assessment is required for optimal pain treatment[8,9] and a call for pain as the fifth vital sign by the American Pain Society, clinical practice is lagging behind using pain assessment as an integral part of daily care. Who is to blame? The developers of pain assessment instruments making these instruments unsuitable for daily clinical practice? Or the health care providers, who are reluctant to change their intuitive treatment into standardized treatment, including pain assessment? Or the premature neonates who are incapable of expressing pain in a for adults understandable way? Enough reasons for further exploring this area.

Among the most utilized instruments for neonates are the Neonatal Infant Pain Scale (NIPS)[6,10] and the Premature Infant Pain Profile (PIPP).[8,11] Both instruments were validated for acute pain in premature and term born neonates and are well accepted by international experts.[8,9,12] Because of the usefulness of the COMFORT behavior scale in postoperative neonates,[13-15] we decided to use these three instruments (NIPS, PIPP, and COMFORT) in a double blind randomized trial comparing continuous morphine with placebo in ventilated neonates. The Visual Analogue Scale (VAS) was included to obtain a global impression of pain by the observer.

In another analysis, we demonstrated that no significant differences in NIPS, PIPP and VAS pain scores were found between the two treatment conditions.[16] Because a considerable overlap is present in the three instruments, we were particularly interested to examine which description and number of response categories is best for neonatal pain assessment.

Despite the fact that the majority of neonatal pain instruments uses physiological items, sparse information is available about their sensitivity and specificity for pain.[5] In anesthesiology, it is generally accepted that physiological parameters, such as heart rate and blood pressure are indicators of pain. Because neonates are also unable to verbalize their pain, the same physiological parameters are therefore often used to assess neonatal pain. For postoperative pain assessment in neonates and infants, however, heart rate and mean arterial pressure were shown to have no additional value over behavioral pain assessment.[13,17]

This study had a two-fold aim: to determine which pain instrument is the most appropriate, and secondly, if the physiological indicators (i.e., heart rate, blood pressure and oxygen saturation) are sensitive enough as indicators of pain. For these aims we performed an in-depth analyses of the three validated pain assessment tools and the physiological data obtained during a randomized clinical trial in ventilated preterm and term neonates, comparing morphine with placebo.[16]

Methods

Patients/design

Pain assessment was performed during a randomized placebo controlled trial evaluating the effects of morphine in ventilated newborns. Ventilated neonates admitted to the Neonatal Intensive Care Unit (NICU) of the Erasmus MC-Sophia Rotterdam and the Isala Clinics in Zwolle, The Netherlands were included between December 2000 and October 2002. Other inclusion criteria were: postnatal age < 3 days, artificial ventilation for less than 8 hours, and the presence of an indwelling arterial catheter. Excluded were neonates with severe asphyxia (Apgar-score after 5 minutes of < 4 or cord blood pH < 7.0), severe IVH (grade III or IVH plus apparent periventricular hemorrhagic infarction), major congenital malformations and facial malformations (e.g. cleft lip and palate), neurological disorders, or receiving continuous or intermittent neuromuscular blockers. The procedure of this trial has been described extensively elsewhere.[16]

The COMFORT behavior scale and VAS were scored at bedside by caregiving nurses during 2-minute observation periods at standardized time-points: before, during and after endotracheal suctioning, before and half an hour after the start of analgesic therapy, and before and after the use of extra analgesic treatment. Simultaneously, videorecordings using two videocameras were used to film respectively face (from the side angular) and body (from above the incubator). All videotapes were scored by one of two observers assessing the PIPP, NIPS, COMFORT behavior and VAS in random order.

Data analysis
Oxygen saturation was assessed using a Hewlett Packard Monitor model 1092 A. During the 2-minute observation oxygen saturation (SAT), heart rate (HR) and mean arterial pressure were registered every 20 seconds (six times) on paper by pushing the button of the M1020A SaO_2module

Pain instruments
NIPS
The NIPS was originally validated in thirty-eight neonates of varying gestational age (GA) during needle stick.[10] The NIPS[10] was adapted from the CHEOPS[18] and contains the following five behavioral items 'facial expression', 'cry', 'arms', 'legs', and 'state of arousal', and one physiological item, 'breathing patterns'. Total scores range from 0 to 7. Other studies have used the NIPS comparing different analgesic treatments during painful procedures.[7,19-21]

PIPP
The Premature Infant Pain Profile was validated to assess acute pain in premature neonates.[11,22] It contains two contextual items, 'behavioral state' and 'gestational age', which are scored at baseline, and two physiological items, 'oxygen saturation' and 'heart rate' and three behavioral items focusing on facial expression, 'nasolabial furrow', 'brow bulge' and 'eye squeeze'. Total scores range from 0 to 18 in full term neonates and from 3 to 21 in premature neonates less than 28 weeks GA.

COMFORT scale
The COMFORT scale contains six behavioral items; 'alertness' 'calmness/agitation', 'facial tension', 'muscle tone', 'physical movement', and two physiological items; 'heart rate' and 'mean arterial pressure' (MAP ≈ diastolic blood pressure + 1/3 (systolic pressure-diastolic pressure). The COMFORT scale was originally developed to assess

distress on the Pediatric Intensive Care Unit (PICU)[23] and to determine optimal sedation.[24] Additionally, the COMFORT 'behavior' (excluding physiological items) was validated for postoperative pain in neonates and infants.[13] In the current study, all NICU nurses were trained to assess the COMFORT scale at bedside and participated in the study when their weighted Cohen's kappa exceeded 0.65.[25] Total scores for the COMFORT 'behavior' range from 6 to 30.

Visual Analogue Scale
The observational VAS pain[26,27] was scored at bedside after scoring of the COMFORT scale and from videotapes. The VAS score consists of a 10-cm horizontal line, that separates the boundaries 'no pain' (at the left side) and 'worst pain possible' (at the right side). Observers estimate the level of pain by making a mark on the line.
Table 1 gives the description of the response categories of the physiological items of NIPS, PIPP and COMFORT.

Procedure
All instruments were scored based on 2-minute observations. These observations included two minutes prior to, during, and thirty minutes after endotracheal and nasal suctioning. Suctioning was chosen because it is part of standard care on the NICU for which usually no standard pain treatment is given. NIPS, PIPP, COMFORT and VAS were scored from videotapes by two trained raters, COMFORT and VAS were also assessed at bedside.

Statistical analysis
The intraclass correlation[28] was used to estimate interrater reliability for continuous pain scores, and the linearly weighted Cohen's kappa[25] for categorical scores.
Categorical principal component analysis (PRINCALS analyses with SPSS 10.1(SPSS Inc, Chicago, Ill) was used to determine (dis)similarity between items of the same indicator.[29] PRINCALS, an acronym for Principal Component Analysis by Alternating Least Squares, reduces the number of variables to a smaller number of core variables (i.e. principal components) without substantial loss of information. Component loadings reflect the correlation of an item with the component. The goodness-of-fit for the solution is expressed by the explained variance for each component which is equal to the sum of the squared component loadings divided by the number of items. PRINCALS identifies nonlinear associations, if any, between variables of ordinal measurement level. The distances between component loadings on one or more components represent the dissimilarity between items.

Table 1 Content of physiological part of pain instruments (in chronological order) for (premature) neonates

	Breathing patterns	**Oxygen saturation**	**Heart rate**	**Blood pressure**
NIPS	1 = change in breathing (indrawing, irregular, faster than usual, gagging, breath holding)			
PIPP		0 = 0 to 2.4% decrease 1 = 2.5 to 4.9% ↓ compared to baseline 2 = 5 to 7.4% ↓ compared to baseline 3 = 7.5 or more ↓ compared to baseline	0 = 0 to 4 bpm increase 1 = 5 to 14 bmp increase 2 = 15 to 24 bpm increase 3 = 25 or more bpm increase	
COMFORT scale			1 = depressions to 15% below baseline 2 = baseline 3 = infrequent elevations ≥ 15% above baseline 4 = frequent elevations ≥ 15% baseline 5 = sustained elevation greater ≥ 15%	1 = depressions to 15% below baseline 2 = baseline 3 = infrequent elevations ≥ 15% above baseline 4 = frequent elevations ≥ 15% above baseline 5 = sustained elevation greater ≥ 15%

Bpm = beats per minute

For each two-minute observation period, the mean of six consecutively (each 20-seconds) determined HRs, MAPs and SATs were used as outcome variables. Because scoring around suctioning was performed twice a day during the study period of maximally seven days, repeated measures were summarized using the mean value.[30] These variables were converted into the response categories of PIPP (for heart rate and oxygen saturation) and COMFORT (for heart rate and mean arterial pressure) scores. Paired t-tests were used to compare mean HR, MAP and SAT levels before and during suctioning. For smaller sample sizes, i.e. before and after extra morphine administration, the Wilcoxon test for non-parametric data was applied.

Table 2   Background characteristics (n = 149)

		n (%)
Background		
Gender (boys / girls)		85/64 (57/43)
Condition (morphine/placebo)		72/77 (48/52)
Additional		
Gestational age*		29.4 (27.4 to 31.6)
Range		25.1 to 41.0
Birth weight (g)*		1195 (887 to 1645)
Postnatal age (hrs)*		8 (5 to 12)
Apgar	1 min.*	6 (4 to 8)
	5 min.*	8 (7 to 9)
CRIB [a]*		3 (1 to 6)
Number of painful procedures		
Day 1		14 (12 to 17)
Day 7		12 (7 to 16.5)
Study duration (in hrs)		49 (20 to 96)

* data are shown as median (25th and 75th percentile)
[a] Clinical Risk Index for Babies

Results

Background characteristics of 149 neonates are given in Table 2. From the original
sample, one neonate was excluded because a neuromuscular blocker was required,
making behavioral assessment impossible. GA ranged from 25.1 to 41.0 weeks. Median
postnatal age was 8 hours (IQR 5 to 12). Study duration was variable with a median of 49
hours (range 2 to 173 hours) primarily due to extubation before end of study duration.

Interrater reliability
Bedside
Interrater reliability between nurses was determined during the training period for the
COMFORT when each nurse scored ten times independently with a trained nurse. In this
manner ninety-six nurses obtained a median linearly weighted Cohen's kappa of 0.80
(range 0.65 to 0.94).

Videotapes

Both raters independently scored a random selection of videotaped material to determine the interrater reliability. The intraclass correlation for the observational VAS was 0.67 for n = 134 pairwise observations. The linearly weighted Cohen's kappa's for the COMFORT items ranged from 0.55 for facial tension to 0.73 for alertness in n = 115 pairwise observations.

The intraclass correlation coefficient for 153 paired NIPS scores was 0.92 and 0.89 for the PIPP in 26 pairwise ratings.

1. *Behavioral indicators*

Table 3 summarizes the results of PRINCALS analyses for facial expression, body movements and behavioral state. The first component explains respectively 63%, 78%, and 78% of the variance for facial expression, body movements and behavioral state. On the second component differences between the three instruments are demonstrated by the distances between component loadings as represented in Figure 1 to Figure 3 for facial expression, body movements and behavioral state respectively.

Table 3   PRINCALS, Structural analysis for indicators of pain

	Component loadings		**Explained variance in %**	
Indicator	I	II	I	II
Facial expression				
COMFORT facial tension	0.85	-0.41	63	24
NIPS facial	0.86	-0.49		
PIPP eye squeeze	0.74	0.53		
PIPP brow bulge[1]	0.73	0.52		
Body movements				
COMFORT physical movements	0.78	0.63	78	18
NIPS arms	0.93	-0.28		
NIPS legs	0.94	-0.24		
Behavioral state[2]				
COMFORT alertness	0.89	-0.35	78	17
COMFORT calmness	0.93	-0.16		
NIPS state of arousal	0.82	0.60		

1   *Nasolabial furrow was excluded because 88.4% of the observations during endotracheal suctioning were missing.*
2   *Behavioral state in the PIPP is assessed prior to the assessment and therefore excluded in this analysis.*

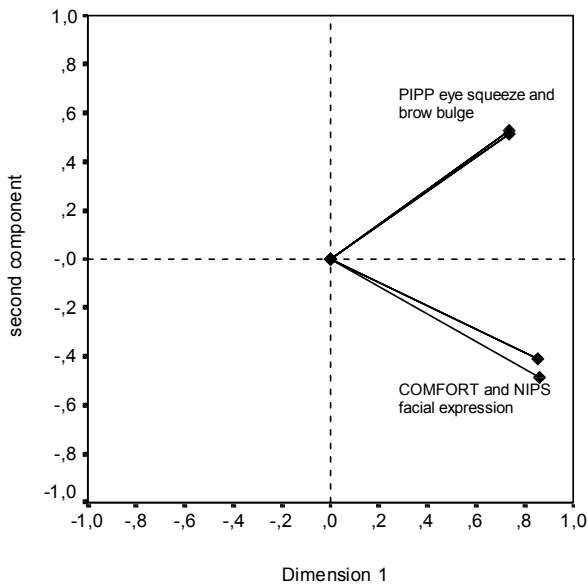Figure 1 component loadings for facial expression

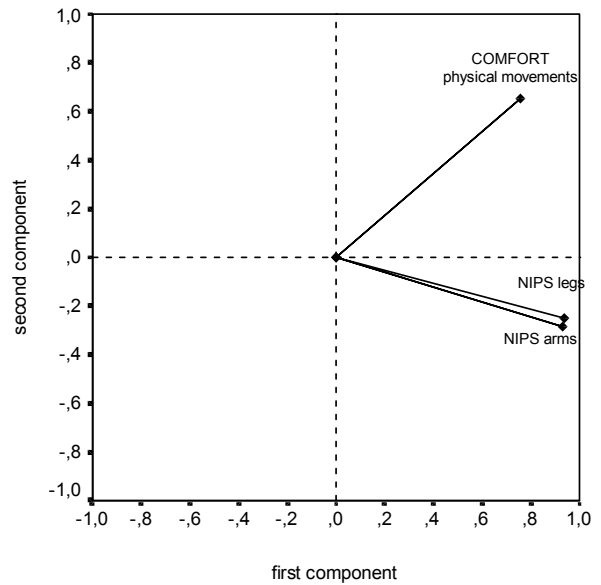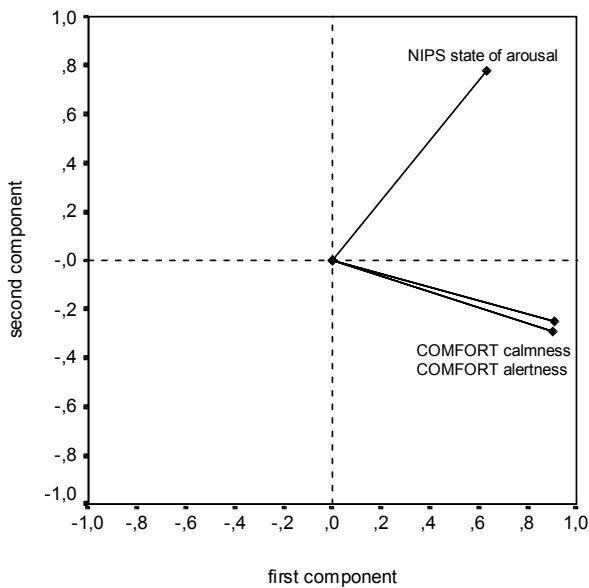Figure 2 Component loadings for body movements

Figure 3 Component loadings for behavioral state

Crying was only assessed with the NIPS and therefore not included in Table 3. During the observation of endotracheal suctioning, in 45.9% of all 636 observations no silent crying was observed, in 34.9% of observations whimpering (score 1) was observed, and the remaining 19.2% of observations showed crying with vigor (score 2). During all other observations (besides suctioning), whimpering was scored 5.5% of the observations, and crying in 1% of 1494 observations.

Muscle tone during endotracheal suctioning, as assessed by the COMFORT 'behavior' was scored 'normal' in 83.3% of all 730 scores.

2. *Physiological indicators*

Change during suctioning compared to before suctioning

The percentage of change in heart rate ranged from a decrease of 27% to an increase of 45%. The median increase was 1% (IQR -3 to 5%) across 668 observations. The percentage of change in MAP ranged from a decrease of 25% to an increase of 104% with a median MAP increase of 10% (IQR 2 to 19%) across 632 observations. SAT changed from a decrease of 22% to an increase of 10%. The median decrease of SAT was 2% (IQR 0 to 4%) across 622 observations.

Change at patient level

Table 4 gives the mean HR, MAP and SAT values before, during and after suctioning for our patient group. Mean HR during suctioning of 149 (SD 13) differed significantly compared to the mean HR of 146 before suctioning (paired t-tests: t = 4,5, *df* 130, t = 4.1, $P < 0.001$). Thirty minutes after suctioning the mean HR had returned to 146. MAP changed significantly, from 38 (SD 7) at baseline to 42 (SD 8), during suctioning (paired t-test:t = 11.2, *df* = 128, $P < 0.0001$). Thirty minutes after suctioning MAP returned to the level before suctioning. SAT decreased significantly from 95 (SD 2) to 93 (SD 3) during suctioning (paired t-tests: t = 11.6, *df* 126, $P < 0.0001$) and returned to before values after thirty minutes.

Bradycardia during endotracheal suctioning, defined as, a bpm of 80 or lower occurred in 37 out of 709 times (0.5%).

Desaturations (SAT 76 to 85%) occurred in 202 observations out of 2230 observations. The majority was measured during suctioning (76%). Desaturations of 75% or lower were measured 17 out of 2230 observations. Sixteen of which were during suctioning.

Table 4   Physiological characteristics before, during and after suctioning

	Endotracheal and nasal suctioning		
Outcome variables	**Before**	**During**	**After**
Heart rate			
Mean (SD)	146 (14)	149 (13)	146 (12)
n	132	131	133
Oxygen saturation (in percentage)			
Mean (SD)	95 (2)	93 (3)	95 (2)
n	129	128	128
Mean Arterial Pressure			
Mean (SD)	38 (7)	42 (8)	38 (7)
n	130	130	132

Physiological items of instruments

Figure 4 and Figure 5 give the percentage of observations for each response category of PIPP HR and COMFORT HR respectively, In the majority of cases there is either a decrease or a small increase in HR only, resulting in low scores.

Figure 6 shows the percentage of observations for the different response categories for MAP of the COMFORT scale during suctioning. The distribution of response categories for the PIPP SAT is given in Figure 7, indicating that in the majority of observations the score is 0.

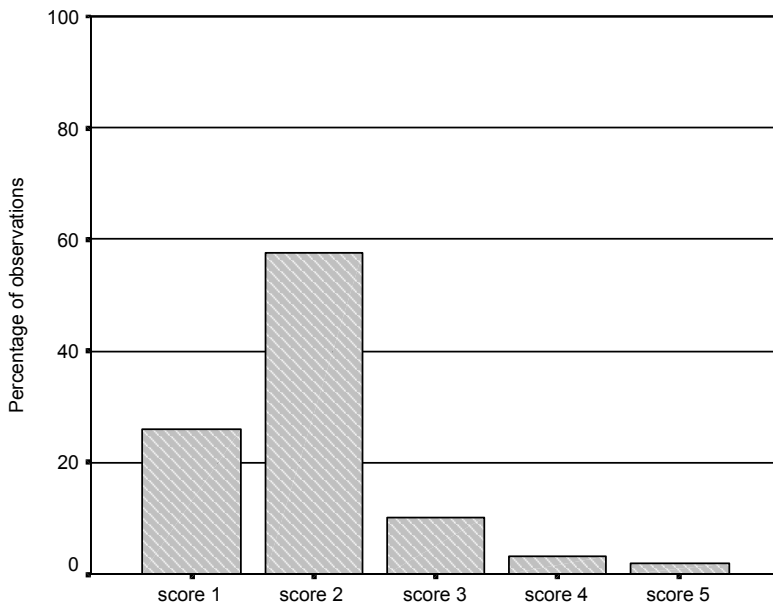Figure 4 PIPP heart rate during suctioning
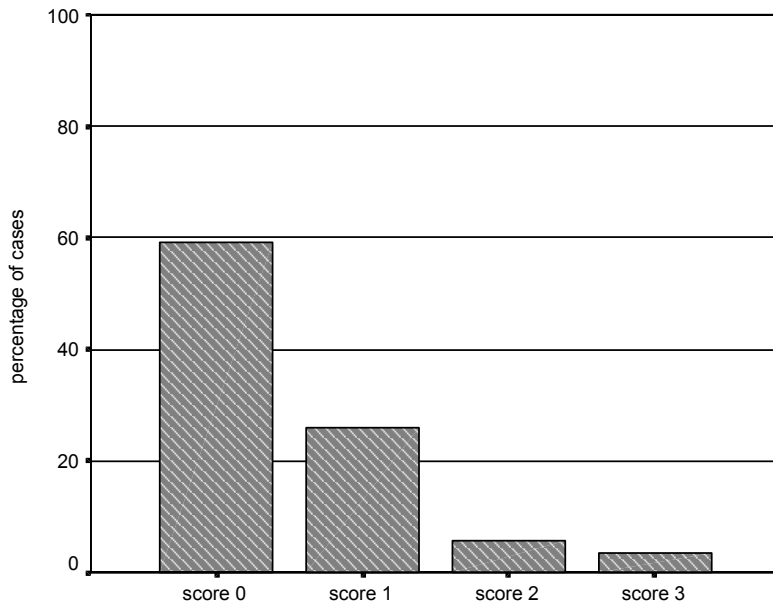
Figure 5 COMFORT heart rate during suctioning

Results before and after extra morphine

In total, fifty-one neonates received once, or more frequently additional open label morphine. Pain scores were assessed in 35 before and after morphine paired observations in twenty-nine neonates. These neonates (17 boys/12 girls) with a median GA of 29.1 (IQR 26.7 to 31.8) were considered to be in pain by the attending physician. Table 5 shows the comparisons of physiological parameters before and after additional morphine was administered. Only MAP decreased significantly (Wilcoxon test, $Z = -3.5$, $P < 0.001$) after treatment. The VAS bedside decreased significantly after additional morphine (Wilcoxon test, $Z = -4.1$, $P < 0.001$).
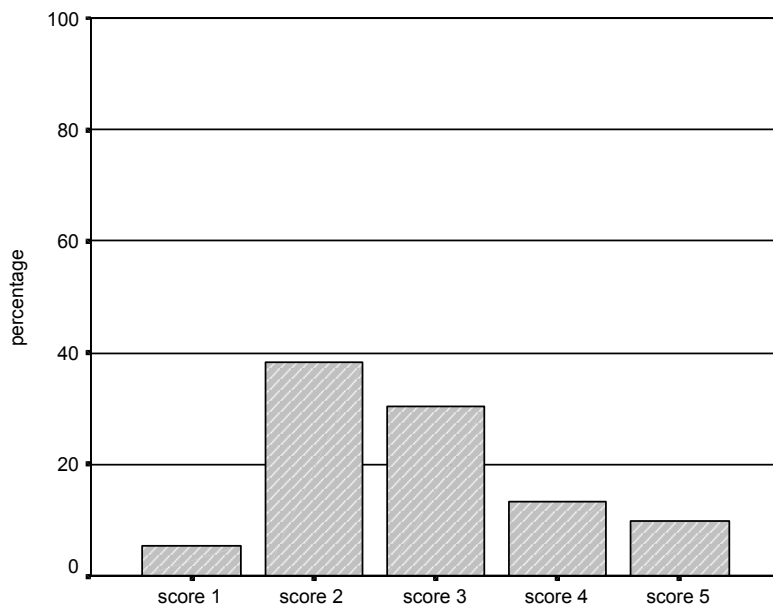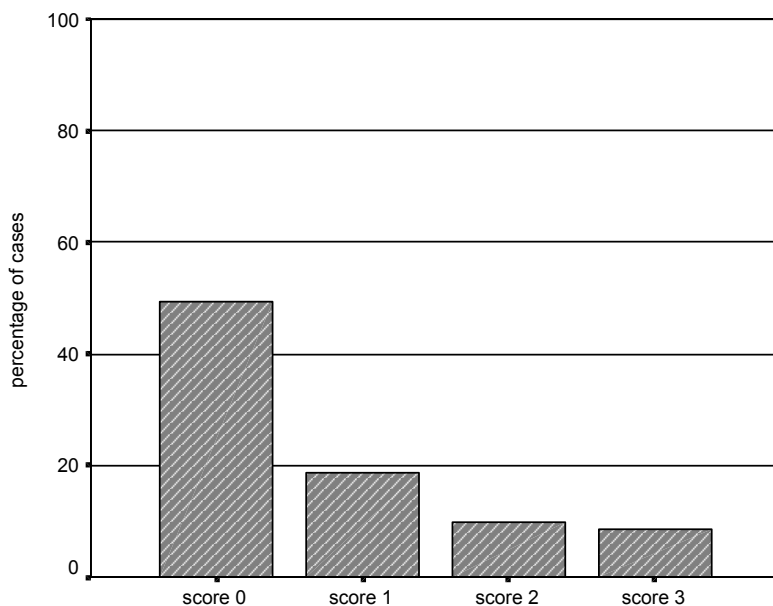
Figure 6 COMFORT 'MAP' during suctioning

Figure 7 PIPP oxygen saturation scores

Table 5   Physiological parameters and VAS bedside before and after extra morphine

	Before morphine	After morphine	*P**
HR			
Median	151	149	0.14
IQR	146 to 162	145 to 160	
n	31	31	
MAP			
Median	39	34	< 0.001
IQR	33 to 43	30 to 40	
n	29	29	
Oxygen saturation in %			
Median	95	95	
IQR	92 to 97	93 to 97	0.97
n	14	14	
Observational VAS bedside**			
Median	3.1	1.6	
IQR	1.9 to 5.0	0.6 to 2.4	< 0.001
n	34	34	

*Wilcoxon tests
**NIPS results are not given because videotaped observations were only available in 32% of the observations

Discussion

During data collection and analysis we found that the available instruments to assess pain in neonates each have their limitations. Some of the indicators used were less appropriate for the NICU environment, especially the physiological items in PIPP and COMFORT. The PRINCALS analyses revealed a common component for facial expression, body movements and behavioral state among the instruments. However, differences in component loadings on the second component can be attributed to the method of assessment. This might be explained by the different ways the instruments describe the indicators into visible behaviors.

Facial expression
The PIPP assesses three separate facial features while NIPS and COMFORT focus on the face as a whole. A drawback at least in our setting with the PIPP was that the presence of a nasolabial furrow was often not observable due to routinely used tape to fix the

endotracheal tube. The facial items of the PIPP were extrapolated from the Neonatal Facial Coding System (NFCS)[31,32] an instrument which scores ten facial features. Next to brow bulge, eye squeeze and nasolabial furrow, seven other features are vertical and horizontal mouth stretch, open lips, lip purse, tongue protrusion, taut tongue and chin quiver. Originally the NFCS was validated for scoring from videotapes, later also applied at bedside.[5,33] In infants 0 to 18 months old, Peters et al showed that a reduced five item NFCS improved the specificity for assessing postoperative pain.[34] When comparing preterm and term infants, differences in scoring are mentioned. For instance, tongue protrusion is considered a 'no pain' response in fullterm neonates, whereas in preterm neonates a high incidence of tongue protrusion was scored during the squeeze event of heel lance.[33] Although very useful for research purposes,[35,36] the NFCS seems not yet useful for daily practice, as cutoff scores for different gestational ages are lacking.

Body movements and muscle tone

The NIPS scores movement of arms and legs, while the COMFORT behavior scores duration and intensity of body movements (arms, legs, trunk and head). A drawback of the COMFORT behavior is the fact that both duration and intensity have been included in the item 'facial tension' and 'physical movements'.

Slight body movements are typical for the neurological immature neonate. This was reflected by the fact that in 63% of 1236 observations (excluding those during endotracheal suctioning) the 'body movements score' on the COMFORT item was 3, indicating frequent minor movements. This suggests that the quality of movement (relaxed versus tense), as is used in the NIPS, may be more appropriate than scoring the incidence of movements. It would be interesting to further explore this indicator, especially as it touches the field of General Movements (GM)[37] and Neonatal Individualized Developmental Care and Assessment of Prematures (NIDCAP®).[38-40] Abnormal GM in prematures, such as cramped synchronized movements, poor repertoire, and abnormal fidgety movements, have been shown to predict severe neurological impairment later in life.[37,41]

The usefulness of NIDCAP® in the context of pain might be found in its detailed examination of some of the body movements. More specifically, qualitative analyses suggest that finger splay and extension of extremities are promising indicators of pain.[42,43] The COMFORT behavior contains an item 'muscle tension' for which the observer has to lift an arm or leg to feel the degree of tension. During our study, we noticed that nurses sometimes estimated muscle tone by merely looking because they did not want to disturb the infant. As the introduction of NIDCAP® in many NICUs encourages, minimal

handling and clustering of care in premature neonates, this measuring muscle tone is less appropriate.

Behavioral state

For behavioral state, PIPP differs from the COMFORT and NIPS because it assesses behavioral state prior to the observation period and gives increasing points with decreasing alertness/wakefulness. The reason for this correction is that infants who are asleep show diminished pain responses.[44,45] The COMFORT scale and NIPS assess alertness and state of arousal during the observation period, implying that it is more difficult to be relaxed or sleep when pain is present. The question is whether behavioral state should be included as a indicator of pain or as mediating factor in pain assessment tools. Unfortunately, we were not able to investigate this question in the current study. As the behavioral state in preterm neonates may vary within minutes, it would be better to determine the variability in behavioral state opposed to assess an average impression of the level of behavioral state. Therefore it could be useful to score both sleep (duration and depth) and awakeness (quality and duration). In that manner an interval would be derived reflecting the variability of behavioral state.

Crying

The NIPS was the only instrument that includes crying. It is unclear from the original publication by Lawrence et al (1993), if they also tested the NIPS in ventilated neonates.[10] Because two points can be scored for crying opposed to one for the other items, much emphasis is put on this item. In our opinion, this is questionable as 45.9% of all observations during suctioning were without crying in our study. Other studies have reported comparable findings related to crying during different painful procedures in preterm neonates.[45,46] Probably, developmental differences in premature and term neonates may explain part of these differences. However, the Pearson's correlation coefficient between GA and mean crying score on the NIPS (mean of 2 to 44 assessments) in 139 neonates was low (0.12; 95% CI –0.05 to 0.28). Another factor may be the lack of energy resources in severely ill or very premature neonates, as less or shorter duration of cry was related to gestational age and severity of illness.[45,47,48] Furthermore, the observation of silent cry in ventilated infants is difficult to compare with audible crying and the reliability of scoring silent cry may be questioned.[49]

These problems with scoring crying may be circumvented by the fact that when an infant is crying, this also is reflected by increased facial tension, i.e. eye squeeze, deepening of nasolabial furrow and brow bulge.

Physiological indicators

Although heart rate was significantly different comparing the mean values during suctioning with before and 30 minutes after suctioning, clinically these differences are of minor significance, as they mostly can not be seen in the individual neonate. This is reflected in the actual scoring of pain. The majority of HR responses during suctioning on both COMFORT and PIPP were low or zero.

Blood pressure seems most promising as a significant increase during suctioning and a significant decrease after additional open label morphine was observed. However, MAP is constantly monitored in the NICU environment and treated when necessary, to keep it within acceptable intervals.

A practical drawback is the requirement of an indwelling arterial line to measure MAP. Therefore the value of blood pressure measured using non-invasive methods for pain assessment needs further evaluation.

Optimal oxygen saturation is a complex indicator in preterm infants. Desaturations in unstable neonates are not necessarily related to pain. According to hospital policy, during ventilation, optimal SATs may fluctuate between 87 and 93%. High saturations are not desirable for the risk of retinopathy of prematurity, while low saturations cause worse oxygen transport in the neonate. Therefore clinicians will frequently adapt the ventilator settings to achieve optimal oxygen saturations in the newborn.

In our study, oxygen saturation decreased significantly but not relevantly during suctioning. However, this was not well reflected in the PIPP oxygen saturation item (Figure 7). This may be attributed to the instantaneous adaptation of ventilator settings before and during endotracheal suctioning to avoid deoxygenation. As a consequence the relationship with pain is rather loose.

In general

Next to these considerations about the behavioral and physiological indicators, some other points should be made concerning the three instruments and pain assessment in this particular age group. Firstly, the PIPP is designed to assess pain directly after a painful procedure, while pain assessment in between procedures is important as well. For instance to assess more prolonged pain as in necrotizing enterocolitis, pain assessment should not be limited to responses to painful procedures.[50] Secondly, the way behavioral state is incorporated raises ethical questions as to whether a sleeping infant should or should not be awakened prior to a painful procedure. Thirdly, oxygen saturation and heart rate were not sensitive enough for pain in this study as shown by the distribution of the response categories during suctioning. One important reason may be the standard policy of

preoxygenation, increasing the inspired oxygen immediately prior to suctioning. Once again, this reflects how interventions affect physiological parameters to such an extent that they are less useful as pain indicators.

The NIPS is an 'all or nothing' kind of instrument which forces one to choose between two extremes while the real answer may be somewhere in the middle.

The items 'facial tension' and 'physical movements' of the COMFORT scale both score intensity and duration intertwined which proved confusing for the observers. Furthermore, attention should focus on the required observation period and the implementation of pain assessment. With regard to observation period, the COMFORT is scored after a 2-minute observation period as suggested by the developer Bruce Ambuel.[23] For the PIPP, the infant should officially be observed for 30 seconds immediately following the procedure. The developers of the NIPS give no instructions for scoring. For future purposes we feel that the optimal observation period should also be based on empirical research. As we have collected videotaped observations, it will be possible to compare for instance the equivalence or non-equivalence between a 1-minute and 2-minute observation. The duration of observation should be long enough to obtain a reliable impression but short enough to improve compliance by the caregivers on a busy NICU with critically ill newborn patients. Because implementation of pain assessment in clinical practice is difficult to achieve, publications should not only give psychometric evaluations of instruments but even more importantly for daily clinical practice also provide guidelines on how to use an instrument. Pivotal under these conditions is that nursing and medical staff should determine treatment policies rendering algorithms for pain assessment and treatment, as is current practice in postoperative newborns.[51]

Conclusions

Premature and term born neonates requiring NICU stay, are a complex group of patients to observe and to assess. Although many pain instruments are developed, the ideal instrument is not yet available. We feel that pain assessment instruments should at any rate incorporate facial expression and body movements, separating items in intensity and duration. The next step is to return to those who work in clinical practice and combine our research results with the needs from the working floor.

References

1. Abu-Saad HH, Bours GJ, Stevens B, Hamers JP. Assessment of pain in the neonate. Semin Perinatol. 1998;22(5):402-416.

2. Stevens BJ, Johnston CC, Gibbins S. Pain assessment in neonates. In: Anand KJS, Stevens BJ, McGrath PJ, eds. Pain in neonates, 2nd revised and enlarged edition. Vol 10. Amsterdam: Elsevier Science; 2000:101-134.

3. Franck LS, Greenberg CS, Stevens B. Pain assessment in infants and children. Pediatr Clin North Am. 2000;47(3):487-512.

4. Gibbins S, Stevens B, Asztalos E. Assessment and management of acute pain in high-risk neonates. Expert Opin Pharmacother. 2003;4(4):475-483.

5. Guinsburg R, Cassia Berenguel R, Cassia de Xavier R, Branco de Almeida MF, Kopelman BI. Are behavioral scales suitable for preterm and term neonatal pain assessment. In: Jensen TS, Turner JA, Wiesenfeld-Hallin Z, eds. Proceedings of the 8th World Congress on Pain. Vol 8. Seattle: IASP Press; 1997:893-901.

6. Blauer T, Gerstmann D. A simultaneous comparison of three neonatal pain scales during common NICU procedures. Clinical Journal of Pain. Mar 1998;14(1):39-47.

7. Pereira AL, Guinsburg R, de Almeida MF, Monteiro AC, dos Santos AM, Kopelman BI. Validity of behavioral and physiologic parameters for acute pain assessment of term newborn infants. Rev Paul Med. 1999;117(2):72-80.

8. Anand KJS. Consensus statement for the prevention and management of pain in the newborn. Arch Pediatr Adolesc Med. 2001;155(2):173-180.

9. American Academy of Pediatrics. Committee on Psychosocial Aspects of Child and Family Health. American Pain Society. Task Force on Pain in Infants C, and Adolescents. The assessment and management of acute pain in infants, children, and adolescents. Pediatrics. 2001;108(3):793-797.

10. Lawrence J, Alcock D, McGrath PJ, Kay J, Brock MacMurray S, Dulberg C. The development of a tool to assess neonatal pain. Neonatal Network. 1993;12(6):59-66.

11. Stevens BJ, Johnston CC, Petryshen P, Taddio A. Premature Infant Pain Profile: development and initial validation. Clinical Journal of Pain. 1996;12(1):13-22.

12. Mitchell P. Understanding a young child's pain. Lancet. 1999;354(9191):1708.

13. van Dijk M, de Boer JB, Koot HM, Tibboel D, Passchier J, Duivenvoorden HJ. The reliability and validity of the COMFORT scale as a postoperative pain instrument in 0 to 3-year-old infants. Pain. 2000;84(2-3):367-377.

14. Bouwmeester NJ, Anand KJS, van Dijk M, Hop WC, Boomsma F, Tibboel D. Hormonal and metabolic stress responses after major surgery in children aged 0-3 years: a double-blind, randomized

trial comparing the effects of continuous versus intermittent morphine. Br J Anaesth. 2001;87(3):390-399.

15. van Der Marel CD, van Lingen RA, Pluim MA, et al. Analgesic efficacy of rectal versus oral acetaminophen in children after major craniofacial surgery. Clin Pharmacol Ther. 2001;70(1):82-90.

16. 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.

17. Buchholz M, Karl HW, Pomietto M, Lynn AM. Pain scores in infants: a modified infant pain scale versus visual analogue. Journal of Pain and Symptom Management. Feb 1998;15(2):117-124.

18. McGrath PJ, Johnson G, Goodman JT, Schillinger J, Dunn J, Chapman JA. CHEOPS: A behavioral scale for rating postoperative pain in children. In: Fields HL, Dubner R, Cervero F, eds. Advances in Pain Research and Therapy. Vol 9. New York: Raven Press; 1985:395-402.

19. Saarenmaa E, Huttunen P, Leppaluoto J, Meretoja O, Fellman V. Advantages of fentanyl over morphine in analgesia for ventilated newborn infants after birth: A randomized trial [see comments]. J Pediatr. 1999;134(2):144-150.

20. Butler-O'Hara M, LeMoine C, Guillet R. Analgesia for neonatal circumcision: a randomized controlled trial of EMLA cream versus dorsal penile nerve block. Pediatrics. 1998;101(4):E5.

21. Shah VS, Taddio A, Bennett S, Speidel BD. Neonatal pain response to heel stick vs venepuncture for routine blood sampling. Archives of Disease in Childhood. 1997;77:F143-F144.

22. Ballantyne M, Stevens B, McAllister M, Dionne K, Jack A. Validation of the premature infant pain profile in the clinical setting. Clin J Pain. 1999;15(4):297-303.

23. Ambuel B, Hamlett KW, Marx CM, Blumer JL. Assessing distress in pediatric intensive care environments: the COMFORT scale. Journal of Pediatric Psychology. 1992;17(1):95-109.

24. Marx CM, Smith PG, Lowrie LH, et al. Optimal sedation of mechanically ventilated pediatric critical care patients. Critical Care Medicine. 1994;22(1):163-170.

25. Cohen J. Weighted kappa: Nominal scale agreement provision for scaled disagreement or partial credit. Psychological Bulletin. 213-220 1968;70(4).

26. Huskisson EC. Measurement of pain. Lancet. 1974;2(7889):1127-1131.

27. van Dijk M, Koot HM, Huijer Abu-Saad H, Tibboel D, Passchier J. The observational Visual Analogue Scale in pediatric pain assessment: Useful tool or good riddance? Clinical Journal of Pain. 2002;18(5):310-316.

28. Shrout PE, Fleiss J, L. Intraclass Correlations: Uses in Assessing Rater reliability. Psychological Bulletin. 1979;86(2):420-428.

29. Gifi A. Nonlinear multivariate analysis. Leiden: Department of Data Theory; 1981.

30. Matthews JNS, Altman DG, Campbell MJ, Royston P. Analysis of serial measurements in medical research. British Medical Journal 1990;300:230-235.

31. Grunau RVE, Craig KD. Facial activity as a measure of neonatal pain expression. In: Tyler DC, Krane EJ, eds. Advances in pain research and therapy. Vol 15. New York: Raven Press; 1990:147-155.

32. Grunau RVE, Johnston CC, Craig KD. Neonatal facial and cry responses to invasive and non-invasive procedures. Pain. 1990;42(3):295-305.

33. Grunau RE, Oberlander T, Holsti L, Whitfield MF. Bedside application of the Neonatal Facial Coding System in pain assessment of premature neonates. Pain. 1998;76(3):277-286.

34. Peters JW, Koot HM, Grunau RE, et al. Neonatal Facial Coding System for Assessing Postoperative Pain in Infants: Item Reduction is Valid and Feasible. Clin J Pain. 2003;19(6):353-363.

35. 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.

36. Scott CS, Riggs KW, Ling EW, et al. Morphine pharmacokinetics and pain assessment in premature newborns [see comments]. J Pediatr. 1999;135(4):423-429.

37. Prechtl HF, Einspieler C, Cioni G, Bos AF, Ferrari F, Sontheimer D. An early marker for neurological deficits after perinatal brain lesions. Lancet. 1997;349(9062):1361-1363.

38. Als H. Toward a synactive theory of development: promise for the assessment and support of infant individuality. Infant Mental Health J. 1982;3:229-243.

39. Als H. Reading the premature infant. Developmental interventions in the neonatal intensive care nursery. New York: Oxford University Press; 1999:18-85.

40. Als H, Lester B, Tronick EZ, Brazelton B. Manual for the assessment of preterm infants' behavior (APIB). In: Fitzgerald HE, Lester BM, Yogman MW, eds. Theory and research in behavioural Pediatrics. New York; 1982.

41. Ferrari F, Cioni G, Einspieler C, et al. Cramped synchronized general movements in preterm infants as an early marker for cerebral palsy. Arch Pediatr Adolesc Med. 2002;156(5):460-467.

42. Grunau RE, Holsti L, Whitfield MF, Ling E. Are twitches, startles, and body movements pain indicators in extremely low birth weight infants? Clin J Pain. 2000;16(1):37-45.

43. Morison SJ, Holsti L, Grunau RE, et al. Are there developmentally distinct motor indicators of pain in preterm infants? Early Hum Dev. 2003;72(2):131-146.

44. Grunau RVE, Craig KD. Pain expression in neonates:facial action and cry. Pain 1987;28:395-410.

45. Stevens BJ, Johnston CC, Horton L. Factors that influence the behavioral pain responses of premature infants. Pain. 1994;59(1):101-109.

46. Johnston CC, Sherrard A, Stevens B, Franck L, Stremler R, Jack A. Do cry features reflect pain intensity in preterm neonates? A preliminary study. Biol Neonate. 1999;76(2):120-124.

47. Johnston CC, Strada ME. Acute pain response in infants: a multidimensional description. Pain. 1986;24(3):373-382.

48. Johnston CC, Stevens BJ, Craig KD, Grunau RVE. Developmental changes in pain expression in premature, full-term, two- and four-month-old infants. Pain. 1993;52:201-208.

49. Craig KD, Korol CT, Pillai RR. Challenges of judging pain in vulnerable infants. Clin Perinatol. 2002;29(3):445-457.

50. Debillon T, Zupan V, Ravault N, Magny JF, Dehan M. Development and initial validation of the EDIN scale, a new tool for assessing prolonged pain in preterm infants. Arch Dis Child Fetal Neonatal Ed. 2001;85(1):F36-41.

51. Bouwmeester NJ, Hop WC, Van Dijk M, Anand KJ, Van Den Anker JN, Tibboel D. Postoperative pain in the neonate: age-related differences in morphine requirements and metabolism. Intensive Care Med. 2003;29(11):2009-2015.

PHARMACOGENETICS OF MORPHINE IN NEONATES AND INFANTS; EXAMINATION OF THE ROLES OF THE OPRM *asn*^{40}*asp* AND COMT *val*^{158}*met* SINGLE NUCLEOTIDE POLYMORPHISMS

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Submitted

Abstract

Background

Neonates and infants show large inter-individual differences in required amounts of morphine for adequate analgesia during postnatal and postoperative intensive care.

Objective

To determine the effects of two different single nucleotide polymorphisms (SNPs) – i.e. the human opioid receptor gene [OPRM] $asn^{40}asp$ and the catechol-O-methyltransferase [COMT] gene $val^{158}met$ – on postnatal and postoperative morphine requirements during intensive care treatment in neonates and infants.

Methods

A total of 385 patients from three different randomized controlled trials were included: I. Continuous morphine vs placebo in ventilated newborns (n = 150), II. Continuous morphine with and without acetaminophen after major surgery in infants aged less than 1 year (n = 54), III. Continuous vs intermittent morphine after major surgery in infants aged less than 3 years (n = 181). DNA was obtained from cheek-swabs and analyzed for both SNPs using PCR-RFLP. Pain was assessed using validated pain assessment scores (Comfort and VAS). Morphine dosing was tailored to the infants' individual needs using standard treatment protocols and available algorithms. Logistic regression analyses using additional morphine as outcome variable served to analyze the potential effect of either SNP on morphine requirements.

Results

The OPRM genotype was analyzed in 283 infants (wild type:214, heterozygous:63, homozygous:6). Additional morphine was required in 47.1%, 50.8% and 16.7% of the patients who were wild type, heterozygous or homozygous, respectively, but the genotype did not significantly influence morphine requirements. The COMT genotype was analyzed in 215 infants (wild type:54, heterozygous:105, homozygous:56). Additional morphine was required in 57.4%, 46.1% and 42.6% of patients who were wild type, heterozygous or homozygous, respectively. The COMT variant allele was significantly negatively correlated with the frequency of additional morphine use (OR 0.42; 95% CI: 0.18-0.98; $P = 0.045$) in the critically ill newborns and infants.

Conclusions

This is the first study evaluating genetic heterogeneity of important enzymes in relationship to the inter-individual differences in morphine requirements of pediatric patients. The COMT genotype was shown to influence morphine requirement in neonates and infants. The low frequency of additional morphine use in the homozygous $asp^{40}asp$ OPRM patients is a promising phenomenon that should be further evaluated in larger cohorts.

Introduction

The pharmacological treatment of pain in neonates and infants is complicated by a large inter-individual variability in analgesics required for pain relief. This variability makes it difficult to predict the effective analgesic doses of morphine in individual critically ill neonates during postnatal intensive care treatment and after major surgery. Previously we have shown, in a number of randomized controlled trials, that validated pain scores did not correlate with the amounts of morphine used in mechanically ventilated neonates,[1] that morphine plasma concentrations did not correlate with the severity of pain in post-surgical infants,[2,3] and that morphine requirements and plasma concentrations show large variability.

Pharmacogenetics, the study of how an individual's genetic inheritance affects the body's response to drugs, proposes to achieve individual dosing of drugs,[4,5] as was recently reviewed.[6,7] Genetic variability, among various other factors, might explain the unpredictable large inter-individual differences in morphine requirements in critically ill infants. Elucidation of potentially responsible polymorphisms might, therefore, improve the pharmacological treatment of pain in this vulnerable group of patients.

Pain perception is modulated by μ-opioid receptor activity in the central nervous system. Studies in knockout mice have shown that morphine and the endogenous opioids, such as β-endorphin and enkephalin, are μ-opioid receptor agonists, providing analgesia by binding to these receptors.[8] Beta-endorphin is a peptide, primarily produced in the anterior lobe of the pituitary gland and arcuate nucleus. It has morphine-like effects and is already present in preterm neonates.[9,10] Murine studies suggest that μ-opioid receptor gene (OPRM) alleles are strong candidates for contributing to individual differences in human nociception and opiate drug responses.[11] Recently a single nucleotide polymorphism

(SNP) in the OPRM gene, predicting an amino acid change at a putative N-glycosylation site, at codon 40, from asparagine (asn) to aspartic acid (asp),[12] was shown to bind β-endorphin three times more potently than did the receptor without this mutation.[13] Additionally, the same $asn^{40}asp$ SNP might also be responsible for a different μ-opioid receptor binding affinity for morphine or for its analgesically active metabolite morphine-6-glucuronide. Therefore this mutation might be associated with lower morphine requirements.

Another SNP, located in the gene encoding catechol-O-methyltransferase (COMT), codes the substitution of valine (*val*) by methionine (*met*) at codon 158 ($val^{158}met$). By reducing COMT activity this polymorphism has been suggested to influence human pain and inter-individual differences in responses to pain.[14] COMT is one of the enzymes metabolizing catecholamines, and as a consequence this SNP might influence dopaminergic and adrenergic/noradrenergic neurotransmission. The COMT mutation decreases COMT activity, which in its turn may reduce the neuronal content of enkephalin and as a compensatory mechanism increase μ-opioid receptor concentrations, as suggested in animal studies.[15] Consequently, the COMT mutation might be related to higher sensitivity to pain.

Relationships between either of these polymorphisms and variability in morphine requirements have not yet been studied in large pediatric populations. We, therefore, recruited a large number neonates and infants from three different randomized controlled trials on morphine performed by our group, for DNA collection and analysis,[1,2,16] in order to investigate possible relationships between the $asn^{40}asp$ and $val^{158}met$ SNPs and morphine requirements.

Methods

Patients from three different randomized controlled trials evaluating the analgesic effects of morphine in infants were enrolled in this study.

Study I
A blinded randomized placebo controlled trial evaluating the analgesic effects of routine continuous morphine infusion in ventilated newborns. All neonates admitted to the level III Neonatal Intensive Care Unit (NICU) of two centers in the Netherlands (Erasmus MC - Sophia, Rotterdam and the Isala Clinics, Zwolle) who required mechanical ventilation

were eligible for inclusion. Other inclusion criteria were: postnatal age less than 3 days, artificial ventilation for less than 8 hours, and an indwelling (peripheral or umbilical) arterial catheter. Excluded were neonates with severe asphyxia (Apgar-score after 5 minutes of < 4 or cord blood pH < 7.0), severe IVH (grade III or IVH plus apparent periventricular hemorrhagic infarction), major congenital malformations and facial malformations (eg cleft lip and palate), neurologic disorders, or receiving continuous or intermittent neuromuscular blockers. Enrolled patients received a loading dose (100 μg/kg) followed by a continuous infusion (10 μg/kg per hour) of either morphine or placebo. If patients from either group were judged to be in pain or distress, they were given additional morphine based on decisions of the attending physician (independent of the study). Additional doses of 50 μg/kg followed by 5-10 μg/kg per hour continuous open-label morphine were allowed. Analgesia was quantified from Comfort and VAS scores obtained twice a day during endotracheal suctioning.

Study II

A blinded randomized controlled trial evaluating the potential morphine sparing effect of acetaminophen following major abdominal and thoracic surgery in neonates and infants during their stay at the pediatric surgical intensive care unit (PSICU) of the Erasmus MC - Sophia, Rotterdam. Inclusion criteria were: neonates and infants aged 0-1 year, ≥ 36 weeks post-conceptual age, weight ≥ 1500 grams and abdominal or thoracic surgery. Patients receiving analgesics, sedatives or muscle relaxants < 12 hours prior to surgery, patients having abnormal hepatic or renal function, neurological damage, severe spasticity or hypotonia were excluded. Patients were randomly assigned to receive rectal acetaminophen (30 or 40 mg/kg loading dose [if below or above 4 kg bodyweight, respectively] directly after induction of anesthesia, followed by 20 mg/kg 6-hourly) or placebo as adjuvant to morphine infusions (loading dose 100 μg/kg at end of surgery; continuous morphine infusion [5 μg/kg/h if postnatal age < 45 weeks; 10 μg/kg/h if postnatal age ≥ 45 weeks]). Additional morphine was given when VAS scores were ≥ 4. Analgesia was quantified from VAS and Comfort scores obtained every 2 hours postoperatively for the first 24 hours, and every 3 hours during the second 24 hours after surgery.

Study III

A blinded randomized double-blind trial comparing the efficacy of continuous intravenous morphine infusions with intermittent intravenous morphine boluses after major abdominal or thoracic surgery in infants aged 0 to 3 years. Included were: neonates

(≥ 35 weeks gestation and bodyweight ≥ 1500 grams) and infants aged up to 3 years undergoing major thoracic or abdominal surgery. Exclusion criteria were: use of analgesics, neuromuscular blockers, hepatic or renal dysfunction, seriously compromised neurological status or altered muscle tone. At the end of surgery all patients were given an intravenous loading dose of morphine ≥ 100 µg/kg. Patients were randomly allocated to receive either morphine continuous infusions of 10 µg/kg/h or three-hourly morphine boluses of 30 µg/kg. Additional morphine was given if VAS scores were ≥ 4 (< 4 hours after surgery: 30 µg morphine/kg/15min; > 1hour after surgery 5 µg morphine/kg/10 min). Analgesia was quantified from Comfort and VAS scores obtained every 3 hours during the first 24 hours after surgery.

The local ethical committees of the participating centers approved this new study as an amendment, next to the original study protocols. The parents of eligible patients were asked to give additional written informed consent for DNA collection and analysis.

The influences of the $asn^{40}asp$ SNP of the OPRM gene and $val^{158}met$ SNP of the COMT gene on morphine requirements were determined. Primary outcomes of this study were the morphine requirements for adequate analgesia, calculated as the amounts of morphine (means per patient) needed for adequate analgesia (Study I: during maximally 7 days postnatal ventilation; study II: 48 hours after major surgery; study III: 24 hours after major surgery). Analgesia was measured using pain assessment tools validated for the different study populations and circumstances. In all three studies patients' pain was assessed using COMFORT-behavior and VAS scores. The mean values for COMFORT and VAS for each patient were calculated, as there were repeated measures. Pain was assessed by trained nurses (linearly weighted Cohen's Kappa's all > 0.75).

DNA isolation
DNA was isolated using buccal brushes (*MasterAmp*[tm], Epicentre). First, if possible, patients' mouths were cleaned with water. Tissue was collected by rolling the buccal brush on the inside of the patients' cheek, approximately 20 times on each side. Brushes were stored in the original packaging at 22-37°C for maximally 7 days before extracting the DNA.

DNA extraction
DNA was extracted using the following steps: First, 500 ml of the *MasterAmp* Buccal Swab DNA Extraction Solution was placed into 1.5 ml micro-centrifuge tubes and placed

on ice. Buccal brushes were placed into a tube containing DNA Extraction Solution and rotated 5 times. To ensure that most of the liquid remained in the tube, brushes were pressed against the side of the tube and rotated while removed from the tube. Afterwards we used the vortex mix for 10 seconds, incubated the tubes at 60°C for 30 minutes and vortex mixed for 15 seconds again. Then, tubes were transferred to 98°C (incubated for 8 minutes), vortex mixed for 15 seconds and returned to 98°C (incubated for an additional 8 minutes) and again vortex mixed for 15 seconds. Afterwards tubes were put on ice briefly to reduce the temperature. After centrifugation at 4°C for 5 minutes the supernatant containing the DNA was transferred to a clean tube and stored at -70°C until further analysis.

Genotyping: PCR-RFLP

OPRM

To detect the $asn^{40}asp$ SNP at the OPRM gene, a PCR amplification was performed in a 50 μl reaction volume, containing 2.5 μl of DNA template, 1x PCR Buffer II (Perkin Elmer), 1.5 mM MgCl$_2$, 0.2 mM each of the deoxynucleotide triphosphates (Roche), 1.25 U of Amplitaq Gold (Perkin Elmer) and 40 pmol each of forward primer 5'- GCTTGGAACCCGAAAAGTCT- 3'and reverse primer 5'- GTAGAGGGCCATGATCGTGAT- 3'. Amplification consisted of an initial denaturation step (7 min at 94°C), followed by 35 cycli (each consisting of 1 min at 94°C, 1 min at 55°C and 1 min at 72°C) and ending with an extension cycle (7 min at 72°C). PCR product was diluted 1:100 with distilled water, and 2 μl of the dilution was used as template in a nested PCR, final volume of 50 μl, containing 1x PCR Buffer II (Perkin Elmer), 1.75 mM MgCl$_2$, 0.2mM each of the deoxynucleotide triphosphates (Roche), 1.25 U of Amplitaq Gold (Perkin Elmer) and 40 pmol each of the forward primer 5'- GCTTGGAACCCGAAAAGTCT - 3' and reverse primer 5'- ACCGCATGGGTCGGA__AA__C__GT - 3'. Mismatches (underlined) in reverse primer were used to create a restriction site for *Psp*1406I. The PCR cycle conditions were the same as above except for an annealing temperature of 53°C. For restriction analysis, 10 μl from the nested PCR amplification was digested for 2h at 37°C in a final volume of 15 μl containing 1x restriction buffer and 10 U of *Psp*1406I (MBI Fermentas). The digested fragments were separated by electrophoresis on a 3% agarose gel with ethidium bromide staining. The fragments produced were 188 and 19 bp for the wild-type sequence, and 207, 188 and 19 bp for heterozygous sequences, whereas the 207 bp PCR fragment remained uncut for homozygous variant sequences.

COMT

For genotyping the *val*[158]*met* SNP of the COMT gene, a PCR amplification was performed in a 50 μl reaction volume, containing 2.5 μl of DNA template, 1x PCR Buffer II (Perkin Elmer), 1.25 mM $MgCl_2$, 0.2 mM each of the deoxynucleotide triphosphates (Roche), 1.25 U of Amplitaq Gold (Perkin Elmer) and 40 pmol each of forward primer 5'-CTCATCACCATCGAGATCAA - 3'and reverse primer 5'- CAGTGAACGTGGTGTGAACAC- 3'. Amplification consisted of an initial denaturation step (7 min at 94°C), followed by 45 cycli (each consisting of 1 min at 94°C, 1 min at 58°C and 1 min at 72°C) and ending with an extension cycle (7 min at 72°C). For restriction analysis, 10 μl from the nested PCR amplification was digested for 2h at 37°C in a final volume of 15 μl containing 1x restriction buffer and 10 U of NlaIII (New England Biolabs). The digested fragments were separated by electrophoresis on a 3% agarose gel with ethidium bromide staining. The fragments produced were 87, 54 and 44 bp for the wild-type sequence and 87, 69, 54, 44 and 18 bp for heterozygous sequences. Homozygous variant sequences were 69, 54, 44 and 18 bp.

Statistics

All results are shown as median values and their 25th and 75th percentiles when variables deviated from normal distribution. All data were analyzed using SPSS version 10.1.

The use of additional morphine (yes/no) served as outcome variable in the logistic regression analyses. The influence of the OPRM and COMT genotypes (using dummy variables: mutation yes/no, homozygous yes/no) on additional morphine use was analyzed in two separate analyses. Both analyses corrected for post-conceptual age, sex, COMFORT scores (means per patient), and allocation group using dummy variables: continuous morphine (yes/no), morphine + acetaminophen (yes/no), intermittent morphine (yes/no) and surgical vs non-surgical.

Results

The total number of infants included in the three studies was 385 (see Figure 1). DNA collection was successful in 344 patients, as 41 patients were lost to follow-up. Genotyping was successful in 283 patients for the OPRM *asn*[40]*asp* polymorphism and in 215 patients for *val*[158]*met* SNP of the COMT gene. Background characteristics of these infants are shown in table 1. DNA was analyzed for mutations in the OPRM gene

*asn*⁴⁰*asp* polymorphism in 118, 40 and 125 patients from study I, II, and III, respectively. The *val*¹⁵⁸*met* SNP of the COMT gene was analyzed in 88, 32 and 95 patients from study I, II, and III, respectively.
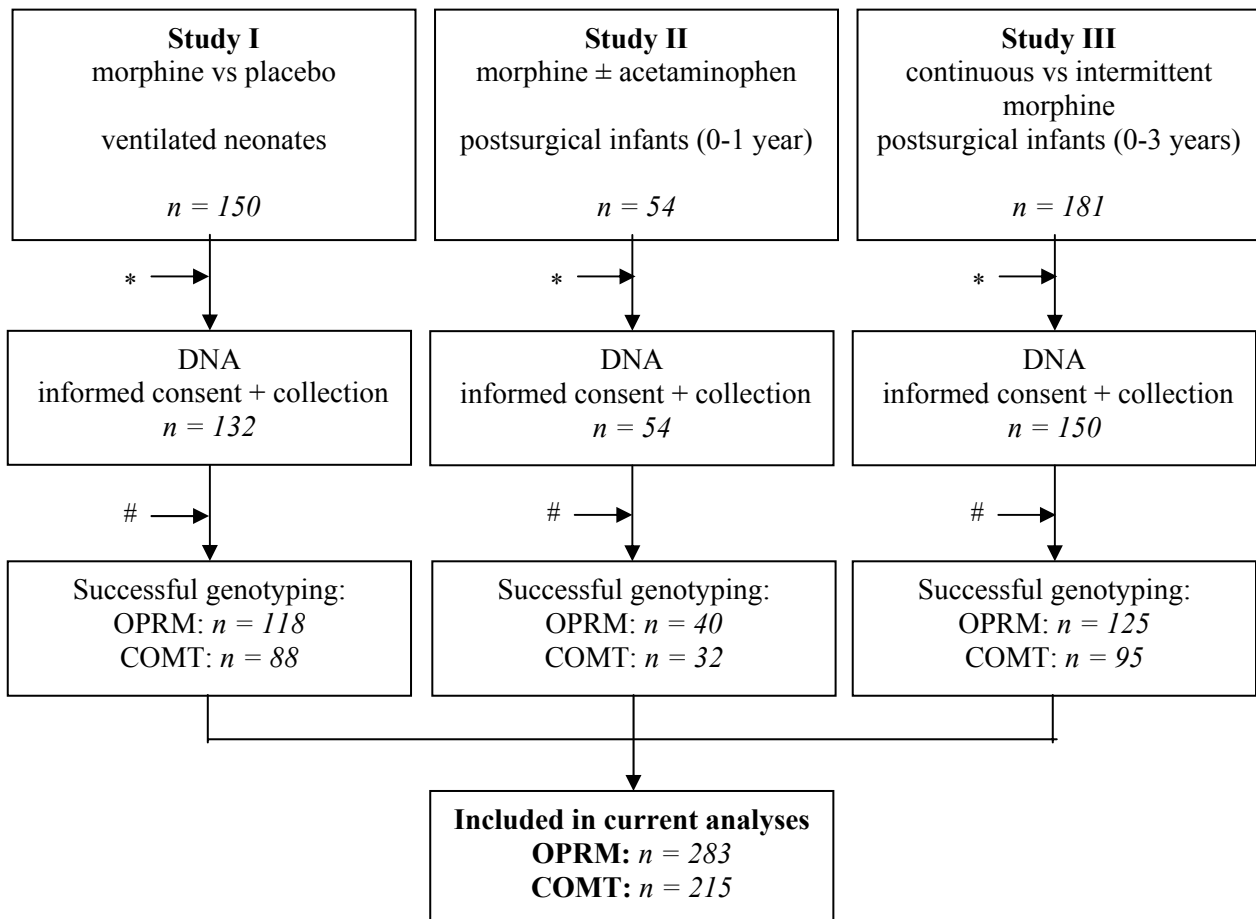

Figure 1   Flow diagram
* No DNA sampling in 49 patients for several reasons: death of patients, no parental informed consent, no address or telephone numbers because patients moved.
# Unsuccessful or inconsequent genotyping in 53 patients for the OPRM genotype and 121 patients for the COMT genotype.

Table 1   Background characteristics of the included patients with successful genotyping for both polymorphisms.

Characteristics:	OPRM gene ($asn^{40}asp$) $n = 283$	COMT gene ($val^{158}met$) $n = 215$
Age:		
Gestational age (weeks)	35 (30 to 40)	35 (30 to 40)
Post-conceptual age (weeks)	38 (31 to 50)	39 (31 to 48)
Bodyweight (g)	2809 (1446 to 5078)	2800 (1504 to 4500)
Sex (male/female)	160 / 123	124 / 91
Severity of illness:		
CRIB *	2.0 (1.0 to 5.0)	2.5 (1.0 to 5.0)
SSS †	9.0 (7.5 to 11.0)	9.0 (7.5 to 11.0)
Comfort$_{behavior}$ §	14.4 (12.3 to 16.3)	14.5 (12.3 to 16.4)
VAS §	1.5 (0.9 to 2.5)	1.5 (0.9 to 2.5)

*Results are shown as median (25th to 75th percentile) values, * Clinical risk index for babies determined in study I, † Surgical stress score determined in studies II and III. § Mean across repeated observations was calculated*

Genotype analysis

PCR analysis of the 283 neonates' DNA for the $asn^{40}asp$ SNP at the OPRM gene showed that 214 patients were wild type ($asn^{40}asn$), 63 patients were heterozygous ($asn^{40}asp$) and 6 patients were homozygous ($asp^{40}asp$) for this mutation. Predicting the number of homozygous subjects according to Hardy-Weinberg distribution (using the formulas $p = (2AA + 1 Aa)/2N$ and $p + q = 1$, where AA is the number of wild type subjects, Aa the number of heterozygous subjects, N the sample size, p the frequency of the wild type allele, q the frequency of the mutant allele, and q^2 the frequency of the homozygous genotype), the predicted number of homozygous, $asp^{40}asp$, subjects was 4.96. Genotyping for the $val^{158}met$ SNP of the COMT gene revealed that 54 patients were wild type ($val^{158}val$), 105 were heterozygous ($val^{158}met$), and 56 patients were homozygous ($met^{158}met$) for this mutation. The predicted number of homozygous, $met^{158}met$, patients was 54.7 according to Hardy-Weinberg distribution.

Morphine requirements

The median amount of used morphine during the study was 10.0 μg/kg/h (IQR: 6.8 to 11.5) and ranged from minimum 0.0 μg/kg/h to maximum 36.9 μg/kg/h per patient.

Figure 2 shows the morphine requirements of infants with increasing post-conceptual ages, labeled for the three different studies. In total 48% and 47% of patients needed additional 'open label' morphine during the study next to their study medication for the COMT and OPRM polymorphism analyses, respectively. Additional morphine was needed in the 62% of the 385 patients from the three original studies.
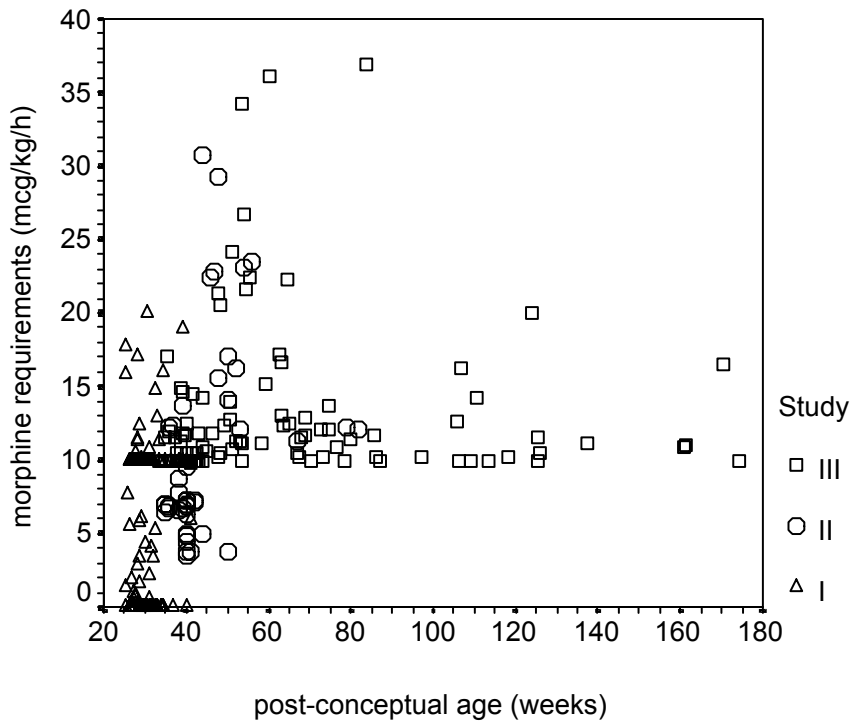

Figure 2 Morphine requirements (μg/kg/h) for individuals with increasing post-conceptual ages (weeks). Triangles show individuals for study I, circles for study II and squares for study III.

Table 2 Numbers of infants grouped for both genotypes in the three different studies

	Study I	Study II	Study III	Total
OPRM gene (asn[40]asp)				
Wild type	84	30	100	214
Heterozygous	30	9	24	63
Homozygous	4	1	1	6
Total	*118*	*40*	*125*	*283*
COMT gene (val[158]met)				
Wild type	21	7	26	54
Heterozygous	47	17	41	105
Homozygous	20	8	28	56
Total	*88*	*32*	*95*	*215*

Figure 3 shows the percentages of patients for the various genotypes of the OPRM. Additional morphine was required in 47.1% of the wild type patients, in 50.8% of the heterozygous patients, and in 16.7% of the OPRM $asp^{40}asp$ homozygous patients. It should be mentioned, however, that only six patients had homozygous genotypes. Results of the logistic regression analysis with the use of additional morphine as outcome variable, predicted by the OPRM genotype, is shown in table 3. No statistically significant effects of the different OPRM genotypes on the requirement of additional morphine were found.

Grouped for the $val^{158}met$ SNP of the COMT gene, additional morphine was required in 57.4% of the wild type ($val^{158}val$), patients, in 46.1% of the heterozygous patients and in 42.6% of the $met^{158}met$ homozygous patients. These results are shown in figure 4. Logistic regression analysis evaluating the effect of the COMT polymorphism on the use of additional morphine revealed that significantly more infants with a wild type, $val^{158}val$, genotype needed additional morphine (OR 0.42; 95% CI: 0.18-0.98; $P = 0.045$) compared to infants with the mutation (heterozygous and homozygous patients).

Table 3   Results of the OPRM gene polymorphism in logistic regression analyses with the requirement of additional morphine as outcome variable.

	Additional morphine use	
	OR (95% CI)	***p***
OPRM mutation (yes / no)	1.20 (0.60 to 2.43)	0.61
OPRM homozygous (yes / no)	0.69 (0.059 to 5.94)	0.66
COMFORT$_{behavior}$*, per 1-point difference	1.59 (1.36 to 1.87)	< 0.001
Post-conceptual age, per week	1.01 (1.00 to 1.03)	0.13
Sex (male/female)	1.05 (0.58 to 1.89)	0.88
Continuous morphine (yes / no)	0.51 (0.21 to 1.23)	0.14
Morphine + acetaminophen (yes / no)	0.37 (0.079 to 1.77)	0.22
Intermittent morphine (yes / no)	0.31 (0.090 to 1.03)	0.057
Surgery (yes / no)	13.2 (4.52 to 38.7)	<0.001
Nagelkerke R^2	0.37	
Hosmer-Lemeshow χ^2	4.3	0.83

Abbreviations: OR = odds ratio; CI = confidence interval
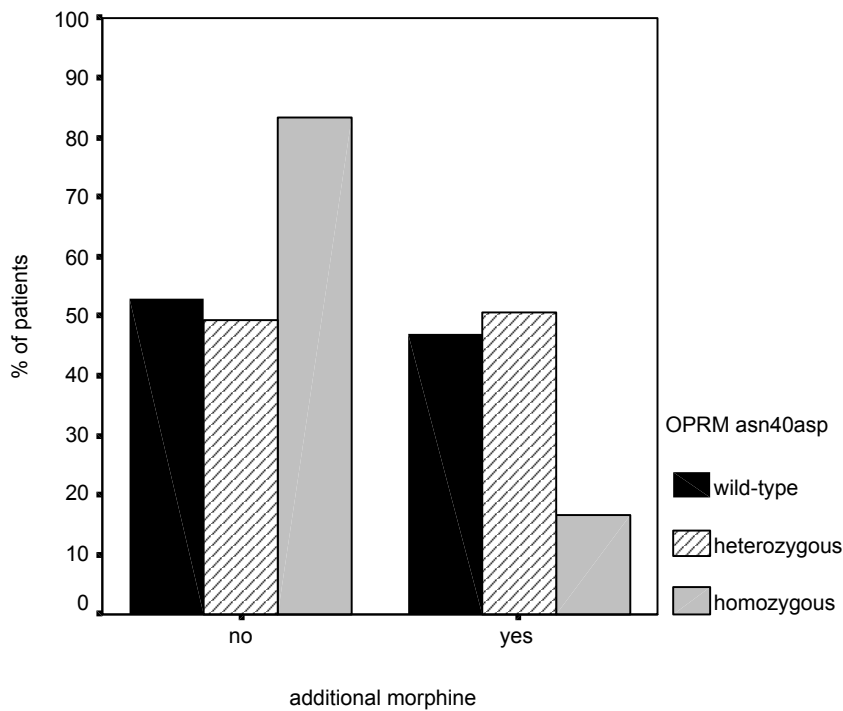** Means for COMFORT$_{behavior}$ per patient were calculated because of repeated measures*

Figure 3   Percentages of patients with and without additional morphine requirements for the different asn[40]asp genotypes of the OPRM gene. Numbers of patients without additional morphine were 110, 31 and 5, and with additional morphine were 98, 32 and 1, for the wild type, heterozygous and homozygous genotypes, respectively.
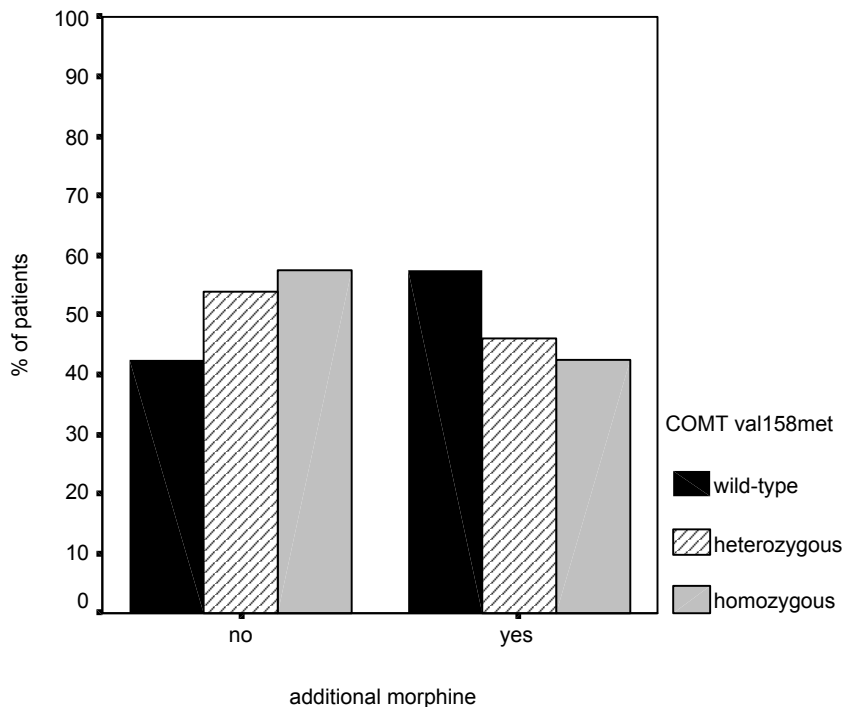

Figure 4   Percentages of patients with and without additional morphine requirements for the different val[158]met genotypes of the COMT gene. Numbers of patients without additional morphine were 23, 55 and 31, and with additional morphine were 31, 47 and 23, for the wild type, heterozygous and homozygous genotypes, respectively.

Table 4   Results of the COMT gene polymorphism in logistic regression analyses with the requirement of additional morphine as outcome variable.

	Additional morphine use	
	OR (95% CI)	*p*
COMT mutation (yes / no)	0.42 (0.18 to 0.98)	0.045
COMT homozygous (yes / no)	0.82 (0.35 to 1.92)	0.65
COMFORT$_{behavior}$*, per 1-point difference	1.64 (1.37 to 1.95)	< 0.001
Post-conceptual age, per week	1.01 (0.99 to 1.03)	0.26
Sex (male/female)	1.00 (0.50 to 2.00)	> 0.99
Continuous morphine (yes / no)	0.50 (0.18 to 1.40)	0.19
Morphine + acetaminophen (yes / no)	0.53 (0.091 to 3.03)	0.47
Intermittent morphine (yes / no)	0.12 (0.027 to 0.51)	0.004
Surgery (yes / no)	17.6 (5.10 to 61.1)	< 0.001
Nagelkerke R^2	0.40	
Hosmer-Lemeshow χ^2	2.69	0.95

Abbreviations: CI = confidence interval; OR = odds ratio
* Means for COMFORT$_{behavior}$ per patient were calculated because of repeated measures

Discussion

We investigated the role of DNA polymorphisms on inter-individual differences of morphine requirements in newborns and infants and found that the frequency of additional morphine use in patients with the COMT wild type genotype was significantly higher than that in patients with the COMT mutation (*P* = 0.045).

We evaluated the effect of two different single nucleotide polymorphisms that have previously been suggested to influence morphine analgesia and the responses to pain.[14,17] The available human pharmacogenetic data on morphine and analgesia is limited. Analysis of the first polymorphism in our study, a SNP in the COMT gene (*val*[158]*met*), showed that significantly more wild-type patients (*val*[158]*val*) needed additional morphine compared to the heterozygous (*val*[158]*met*) and homozygous patients (*met*[158]*met*). As the COMT enzyme metabolizes catecholamines, variability in its activity affects both dopaminergic (increased in homozygous patients) and (nor)adrenergic (decreased in homozygous patients) neurotransmission. Zubieta et al. showed, in young healthy adults, that the COMT homozygous *met*[158]*met* genotype was related to the highest pain sensitivity, activated with prolonged, but not acute, stressors, whereas the wild type *val*[158]*val* genotype showed the lowest pain sensitivity. They explained this variability in

pain sensitivity from a chronic activation of dopaminergic neurotransmission in the homozygous patients, which in its turn might reduce the neuronal content of enkephalin peptides.[18] Interestingly, the results of our study, showing that the wild-type infants required most morphine, suggest the opposite. Great variances in study design and study populations (healthy adults vs critically ill newborns and infants) may explain these contrasting results. In our study patients received morphine. Zubieta et al. hypothesized that, next to the relationship between COMT activity and μ-opioid receptor activity, COMT activity is also negatively correlated with μ-opioid receptor binding. Higher COMT activity, as is present in the wild type patients, might therefore also cause a lower morphine binding, and as a consequence result in higher morphine requirements. Furthermore, the pain induced in the infants by surgery and intensive care treatment can be considered an acute rather than a chronic stressor. Therefore the effect of chronic dopaminergic activation, as described above, might be absent. On the other hand, elevated noradrenergic neurotransmission, as is probably present in the homozygous, but not in the wild type infants, is suggested to potentiate morphine-induced anti-nociception in mice.[19] Direct activation of α_2-adrenergic-receptors also potentiates morphine-induced spinal analgesia,[20] and α_2-adrenergic- receptor agonists, such as clonidine, can be used for the treatment of pain.[21] This might also explain elevated additional morphine needs in wild type infant compared to heterozygous and homozygous COMT $val^{158}met$ patients. Although its role of in human pain experience, and related variability in dopaminergic and (nor)adrenergic neurotransmission, has not yet been elucidated, the COMT polymorphism might play an important role in morphine analgesia and in sensitivity to pain in humans. Variability in morphine requirements may depend on variability in the analgesic effects of morphine, but also on variability in pain sensitivity. In other words, an infant might need large amounts of morphine after surgery because the morphine has only minor effects or because the infant is extremely painful.

Polymorphisms acting on the drug target, resulting in, for instance, variability of μ-receptor binding, might directly influence morphine's analgesic effects. We showed, using multivariate logistic regression analysis, no significant difference in the use of additional morphine between the various OPRM $asn^{40}asp$ genotypes. Interestingly, however, only one out of six homozygous patients (16.7%) received additional morphine, compared to 47.1% and 50.8% of the wild type and heterozygous patients, respectively. This might indicate that the $asn^{40}asp$ SNP at the OPRM gene does influence neonatal pain, as is suggested by a more potent μ-opioid receptor binding of ß-endorphin.[13] The number of homozygous neonates in the current analyses was probably too small, however, to detect

significant decreases in morphine requirements and to determine the role of this homozygous genotype in neonatal morphine analgesia.

The importance of the μ-receptor in morphine analgesia was first shown from a down regulated analgesic response to morphine in rats who were prevented to produce μ-receptor protein using an anti-sense approach.[22,23] Studies in knockout mice have further confirmed the importance of the μ-receptor gene in morphine analgesia, as the anti-nociceptive effect of morphine is not apparent in animals without a μ-receptor gene.[24] Genetic variability of the human μ-receptor gene (OPRM) might therefore cause differences in responses to morphine. In our study no significant differences in morphine requirements between the wild-type and heterozygous patients were shown. A previous study showed, however, that a low morphine responder adult cancer patient was a heterozygous carrier of the $asn^{40}asp$ allele.[25] Probably other mutations in her DNA were responsible for the low response to morphine. As she was also shown to be heterozygous for the G1784A SNP of the OPRM gene, this polymorphism needs further evaluation.

Additionally, the $asn^{40}asp$ SNP might also be responsible for a different μ-opioid receptor binding affinity for morphine's metabolite morphine-6-glucuronide, which is analgesically active. Lötsch et al. have suggested reduced morphine-6-glucuronide (M6G) potency[17,26] and a protective effect against M6G-related opioid toxicity[27] by this polymorphism. As M6G plasma concentrations are generally very low in neonates, because of their immature metabolism, variability in M6G properties would probably have only minor effects in newborn infants.

Conclusion

This is the first study analyzing genetic heterogeneity of important enzymes in relationship to the inter-individual differences in morphine requirements in neonates and infants. The $val^{158}met$ polymorphism at the COMT gene was shown to significantly influence morphine requirements in neonates and infants. Additional morphine was most frequently used in the wild type patients. No difference was found between the wild type and heterozygous OPRM $asn^{40}asp$ genotype patients. Although the use of additional morphine in the OPRM homozygous $asp^{40}asp$ infants was lower compared to the heterozygous and wild type patients, the difference failed to reach statistical significance. As only few patients presented with this homozygous genotype, future research using

larger groups of patients should further evaluate the influence of the OPRM homozygous genotype on morphine needs. More research is needed to extend the knowledge about morphine, analgesia and neurotransmitters, in relationship to genetic variability in infants, but also in adults.

Acknowledgements

References

1. Simons SHP, van Dijk M, van Lingen RA, et al. Routine morphine infusion in preterm neonates who received ventilatory support: a randomized controlled trial. JAMA. 2003;290:2419-2427.

2. van Dijk M, Bouwmeester NJ, Duivenvoorden HJ, et al. Efficacy of continuous versus intermittent morphine administration after major surgery in 0-3-year-old infants; a double-blind randomized controlled trial. Pain. 2002;98(3):305-313.

3. Bouwmeester NJ, Hop WCJ, van Dijk M, Anand KJS, van den Anker JN, Tibboel D. Postoperative pain in the neonate: age-related differences in morphine requirements and metabolism. Intensive Care Med. 2003;29:2009-2015.

4. Roses AD. Pharmacogenetics and the practice of medicine. Nature. 2000;405(6788):857-865.

5. Roses AD. Pharmacogenetics and future drug development and delivery. Lancet. 2000;355(9212):1358-1361.

6. Evans WE, McLeod HL. Pharmacogenomics - Drug disposition, drug targets, and side effects. N Engl J Med. 2003;348:538-549.

7. Weinshilboum R. Inheritance and drug response. N Engl J Med. 2003;348:529-537.

8. Matthes HW, Maldonado R, Simonin F, et al. Loss of morphine-induced analgesia, reward effect and withdrawal symptoms in mice lacking the mu-opioid-receptor gene. Nature. 1996;383(6603):819-823.

9. Csontos K, Rust M, Hollt V, Mahr W, Kromer W, Teschemacher HJ. Elevated plasma beta-endorphin levels in pregnant women and their neonates. Life Sci. 1979;25(10):835-844.

10. Anand KJS, Hickey PR. Halothane-morphine compared with high-dose sufentanil for anesthesia and postoperative analgesia in neonatal cardiac surgery. N Engl J Med. 1992;326(1):1-9.

11. Uhl GR, Sora I, Wang Z. The mu opiate receptor as a candidate gene for pain: polymorphisms, variations in expression, nociception, and opiate responses. Proc Natl Acad Sci U S A. 1999;96(14):7752-7755.

12. Bergen AW, Kokoszka J, Peterson R, et al. Mu opioid receptor gene variants: lack of association with alcohol dependence. Mol Psychiatry. 1997;2(6):490-494.

13. Bond C, LaForge KS, Tian M, et al. Single-nucleotide polymorphism in the human mu opioid receptor gene alters beta-endorphin binding and activity: possible implications for opiate addiction. Proc Natl Acad Sci U S A. 1998;95(16):9608-9613.

14. Zubieta J, Heitzeg MM, Smith YR, et al. COMT val153met genotype affects μ-opioid neurotransmitter responses to a pain stressor. Science. 2003;299(5610):1240-1243.

15. Steiner H, Gerfen CR. Role of dynorphine and enkephalin in the regulation of striatal output pathways and behavior. Exp Brain Res. 1998;123:60-76.

16. Van der Marel CD. Paracetamol, widely used hardly understood. Rotterdam: thesis, Erasmus University Rotterdam; 2003.

17. Hollt V. A polymorphism (A118G) in the mu-opioid receptor gene affects the response to morphine-6-glucuronide in humans. Pharmacogenetics. 2002;12(1):1-2.

18. Bourgoin S, Rostaing-Rigattieri S, Nguyen JP, et al. Opposite changes in dopamine metabolites and met-enkephalin levels in the ventricular CSF of patients subjected to thalamic electrical stimulation. Clin Neuropharmacol. 1999;22(4):231-238.

19. Bohn LM, Xu F, Gainetdinov RR, Caron MG. Potentiated opioid analgesia in norepinephrine transporter knock-out mice. J Neuroscience. 2000;20(24):9040-9045.

20. Fairbanks CA, Wilcox GL. Spinal antinociceptive synergism between morphine and clonidine persists in mice made acutely or chronically tolerant to morphine. J Pharm Exp Ther. 1999;288(3):1107-1116.

21. Eisenach JC, DuPen S, Dubois M, Miguel R, Allin D. Epidural clonidine analgesia for intractable cancer pain. Pain. 1995;61:391-399.

22. Chen XH, Adams JU, Geller EB, DeRiel JK, Adler MW, Liu-Chen LY. An antisense oligodeoxynucleotide to mu-opioid receptors inhibits mu-opioid receptor agonist-induced analgesia in rats. Eur J Pharmacol. 1995;275(1):105-108.

23. Rossi G, Pan YX, Cheng J, Pasternak GW. Blockade of morphine analgesia by an antisense oligodeoxynucleotide against the mu receptor. Life Sci. 1994;54(21):L375-379.

24. Kieffer BL. Opioids: first lessons from knockout mice. Trends Pharmacol Sci. 1999;20(1):19-26.

25. Hirota T, Ieiri I, Takane H, et al. Sequence variability and candidate gene analysis in two cancer patients with complex clinical outcomes during morphine therapy. Drug Metab Dispos. 2003;31(5):677-680.

26. Lotsch J, Skarke C, Grosch S, Darimont J, Schmidt H, Geisslinger G. The polymorphism A118G of the human mu-opioid receptor gene decreases the pupil constrictory effect of morphine-6-glucuronide but not that of morphine. Pharmacogenetics. 2002;12(1):3-9.

27. Lotsch J, Zimmermann M, Darimont J, et al. Does the A118G polymorphism at the mu-opioid receptor gene protect against morphine-6-glucuronide toxicity? Anesthesiology. 2002;97(4):814-819.

Chapter 10

GENERAL DISCUSSION

Introduction

The International Association for the Study of Pain has defined pain as 'an unpleasant sensory and emotional experience associated with actual or potential tissue damage or described in terms of such damage'.[1] According to this definition, pain recognition requires self-report, which is still seen as the 'gold standard' to assess pain. As self-report is not feasible in pre-verbal and non-verbal individuals, it was feared that many of these individuals did not receive appropriate treatment for pain. The definition of pain was extended, therefore, adding physiological and behavioral responses as validated indicators of pain.[2] Pain has been acknowledged as an element of life that is also potentially present in pre-verbal infants.

In 1995 a multidisciplinary team in the Erasmus MC-Sophia Children's Hospital started to investigate pain and analgesia in newborns and older infants. Several clinical trials were conducted to investigate the effects of analgesics, such as morphine and acetaminophen, in infants.[3-5] Moreover, research efforts were directed towards assessment of pain in pre-verbal infants,[6] with special attention to 'facial' pain expression,[7] and in non-verbal, profoundly cognitively impaired children.[8] The studies described in this thesis were embedded in the pediatric pain research infrastructure of our hospital, and aimed at gaining more knowledge of pain and analgesia in newborn infants. Many unanswered questions[9] about the prevention of pain in neonates were addressed in the different studies presented in this thesis. For instance: How to measure pain and stress in newborns and how to differentiate pain from stress?; What analgesic agents to use in newborns?; How to explain inter-individual differences in pain responses? In this chapter the findings from our studies are discussed against the background of these main questions. Furthermore, suggestions for future research are given.

Pain experience during stay in the NICU

As a rationale for our studies, the first question we investigated was whether even today neonates will experience pain without adequate analgesia during intensive care treatment. Previous studies have shown that neonates admitted to a NICU daily undergo many painful procedures.[10-13] The necessity of neonatal pain management has been acknowledged over the last decade, resulting in consensus statements about pain management guidelines.[14,15] We hypothesized, therefore, that the frequency of painful

procedures would have been decreased and/or that the use of analgesics, at least for procedural pain, would have been optimized. Yet, in a prospective observational study among 151 newborns we showed that they were still subjected to a mean of 14 painful procedures per day in the NICU (**Chapter 2**). We are the first to report prospective data on a substantial number of failed procedures in the NICU (18% to 46% for the different studied procedures). Endotracheal intubation and the insertion of chest tubes were the only procedures routinely treated with analgesics. Overall, pharmacological treatment was limited, and 40 % of neonates did not receive any analgesic therapy during the first fourteen days of their stay at the NICU. We feel that it should be attempted to minimize the number of painful procedures and to give more attention to the use of non-pharmacological agents, such as sucrose, during NICU treatment.

Although nurses and physicians alike acknowledged the painfulness of almost all daily performed procedures, they did not routinely use analgesics to treat the presumed pain. Several explanations for this attitude may be suggested. The consensus statements do not take objective assessment of pain and optimal ways to achieve this into account.[14,15] As it is difficult to assess neonatal pain (**Chapter 3.2 and 8**), one might still disbelieve in the neonate's capacity to experience or remember pain. However, clinicians estimated 26 out of 31 procedures to be painful. Probably there is a lack of knowledge and evidence about the efficacy and safety of analgesic agents in neonates. Therefore, fear for adverse effects of analgesic agents may outweigh the short and long-term negative consequences of pain experience.

The premature neonate's capacity to feel pain

Several arguments support the idea that neonates, even the most premature, can feel pain. Pain is a complex experience that involves not only transduction of noxious stimuli (nociception), but also cognitive and emotional processing by the brain. Pain consists of a sensory-discriminative, an affective-emotional and a cognitive-interpretational component, suggesting that a certain stage of cortex development is required to experience painful stimuli.[16] Neuro-physiological afferent pain pathways reach the cortex between 20 and 26 weeks.[17,18] Although changes in pain behavior with increasing gestational age can be found (**Chapter 3.2**), even very prematurely born neonates show already behavioral and physiological reactions and hormonal stress responses to painful stimuli.[19-22] As spinal reflexes can not explain these reactions, the central nociceptive

pathways, including spino-thalamic and cortical fibers, must be already present in the very prematurely born neonates.

Supra-spinal pain processing in the developing brain has remained relatively unexplored. Evoked potentials signaling the arrival of sensory impulses at the cortex can be detected from 29 weeks of gestation.[23] Data about neurotransmitters and neuro-anatomical areas suggest that the pain system undergoes a major reorganization during the perinatal period of life.[24] Imaging techniques such as functional Magnetic Resonance Imaging or Positron Emission Tomography made visualization of cortical activity after painful stimuli possible. There is no 'pain-center' as such in the brain, but studies in adults undergoing painful experiences showed enhanced activity in the anterior cingulate, the thalamus, the lentiform nucleus and in the insular and prefrontal cortex as well as in the primary and secondary somatosensory cortices.[25] Comparable studies in neonates would provide more understanding of the developing pain system. At present the evidence about the premature neonate's capacity to experience pain is not yet conclusive.

Neonatal pain memory and long-term effects

When observing an "uncomfortable" neonate, it is hard to tell whether pain, hunger or fatigue causes the behavioral discomfort. The other way round, the neonate may not be able to discriminate between these emotions. It is unknown if a premature newborn even can remember pain. Memories for early painful events may not be accessible to explicit memory (conscious recall), but are probably incorporated in the implicit memory that operates at the level of conditioning without awareness, coded by structural or functional changes within the pain system and other neuronal assemblies.[26] In the spinal cord the excitation of the synaptic connection between $A\delta$- and C-fibers and dorsal neurons results in lower thresholds after repetitive stimulation, the so-called wind-up phenomenon. Furthermore, as receptive fields of adjacent dorsal neurons are overlapping, repetitive painful stimuli also cause spatial summation, leading to hyperalgesia and allodynia.[27] This plasticity is probably caused by activation of the glutamate N-methyl-D-aspartate (NMDA) receptors after repetitive activation of nociceptors.[28]

Although the pain itself might not be consciously remembered, pain might have effects on the short and long-term perspective, as suggested in clinical studies.[29-32] Murine studies have shown that abnormal or excessive activity in the developing central nervous system, due to pain, may alter normal synaptic development. This may lead to changes in the

somatosensory processing and neurobehavioral sequelae.[33-35] The induced degree and duration of inflammation in the used rat-models seem to far exceed, however, those caused by 'normal' procedural interventions in human neonates. Other studies have shown that lower doses of inflammation result in acute, but reversible spinal expansion.[36] One study showed weaker responses to painful stimuli at 32 weeks post-conceptual age in former extremely prematurely born neonates compared to those in neonates at 32 weeks gestational age.[37] The methodological drawback, however, is that all prematurely born neonates are exposed to pain during their intensive care treatment. As a consequence, negative effects of pain in neonates can only be compared with those in healthy term-born controls or between neonates receiving analgesia or placebo treatment. There are not yet follow-up data on the long-term effects of pain with and without analgesia of infants participating in a randomized placebo-controlled trial during NICU treatment immediately after birth.

As long as conclusive evidence about both the neonate's capacity to feel pain and possibly negative long-term effects of neonatal pain experience is lacking, ethical perspectives necessitate us to believe that neonates indeed experience pain. Therefore it should be attempted to alleviate neonatal pain and yet to minimize the adverse effects of analgesic treatment. In the late eighties of the last century clinicians' ideas about the treatment of post-surgical pain were changed by data from clinical trials showing the benefits of adequate analgesic therapy.[38,39] To establish adequate analgesic treatment during intensive care of the newborn, clinicians' opinions need to be altered again.

Need for a randomized controlled trial

Neonatal pain management might be improved by the outcome of randomized controlled trials on the effects of analgesic treatment.[40,41] The most widely used and studied opioid analgesics in newborns are morphine and fentanyl.[42] Although fentanyl might also effectively alleviate neonatal pain,[43] it is especially useful in specific clinical situations, such as in ventilated babies with persistent pulmonary hypertension (probably as a very strong sedative) or in those who have become tolerant to morphine. During ventilatory support, neonates are often sedated using midazolam. In a pilot study both morphine and midazolam were shown to cause significant analgesia compared to placebo, but morphine also protected against intraventricular hemorrhage in ventilated newborns.[44] These

findings made us opt for morphine as the analgesic of choice in our studies, administered in accordance with the internationally accepted dosage regimens.[45]

Our randomized placebo controlled trial had a threefold aim: to evaluate the effects of continuous morphine infusion in ventilated neonates during the first postnatal week:

1. on pain expression and stress responses,
2. on clinical outcome, and
3. on blood pressure.

Morphine, more fine?

The question whether continuous morphine infusion should be used as a standard of care in ventilated neonates, was the main question to be answered by our study. The effects of morphine on pain expression and stress responses were limited. No analgesic effect of continuous morphine could be determined using three validated pain assessment instruments, i.e. the Premature Infant Pain Profile (PIPP), the Neonatal Infant Pain Scale (NIPS) and the Visual Analog Scale (VAS), compared to placebo treated newborns (**Chapter 4**). Anand et al., however, previously showed significantly lower pain scores in the morphine treated neonates.[44] Concerning the neonatal stress responses, norepinephrine levels in the morphine treated infants were decreased, whereas no decrease of epinephrine levels was observed (**Chapter 5**). Quinn et al. previously showed a decrease in epinephrine, but not norepinephrine plasma concentrations after 24 hours of morphine treatment.[46] The only two previously performed studies investigating continuous morphine compared to placebo controls used higher morphine dosage regimens compared to our study. In our study, 27% of neonates in the morphine-group required additional open label morphine next to their study infusions. As overall pain scores were low, the morphine dosages (100 µg/kg loading dose +10 µg/kg/hour) used in our study do not seem to be too low. Although no clinically significant side-effects of morphine were found in any of the three studies, the included numbers of infants in the previous two studies, around 20 per group, might have been too small to detect significant adverse effects of the higher dosage regimen. Incidences of side-effects increase with increasing morphine and morphine-6-glucuronide plasma-levels. Therefore the amounts of morphine administered should be as low as possible. The absence of clinically significant analgesic effects of morphine in neonates leads us to conclude that continuous morphine should not routinely be given to ventilated neonates.

Effect of morphine on clinical outcome

The only positive effect of routine morphine infusion in our study was a significant decrease in the development of intra-ventricular hemorrhage (IVH). In our analysis the outcome measure IVH included all different grades. Morphine did not reduce poor neurologic outcomes, as suggested by a previous study of Anand et al.[44] In a second in-depth analysis we showed that blood-pressure-variability did not predict the development of IVH (**Chapter 6**). A recent study, however, has shown that superior cava vein blood flow during the first postnatal day is related with the development of periventricular and intra-ventricular hemorrhage.[47]

The prognosis of IVH at older ages, especially grades I and II, is not fully clear yet.[48] Therefore, long-term follow-up in our patients – using predetermined follow-up schedules and neurodevelopmental assessment instruments, as proposed by the Dutch Neonatal Network – is needed to evaluate if the positive effect on IVH will have a similar impact on neurodevelopmental outcome.[49] We did not find significant effects on any other clinical outcome measure, such as duration of ventilation or incidences of secondary infections. The findings from the large multi-center NEOPAIN trial,[50] which investigates the effects of routine continuous morphine infusions (again with higher dosage regimen compared to our study) in around 800 ventilated neonates, will tell whether morphine affects the incidences of periventricular leukomalacia and other major sequelae of neonatal intensive care, such as necrotizing enterocolitis.

Measuring pain in neonates

The absence of measurable analgesic effects during continuous morphine infusions compared to placebo (**Chapter 4**) combined with the shortcomings of neonatal pain instruments (**Chapter 3.2**), necessitated a critical analysis of these pain assessment tools. In **Chapter 8** the separate behavioral and physiological items included in three neonatal pain assessment instruments (PIPP, NIPS and COMFORT) were analyzed using the data of the 150 ventilated neonates who participated in the randomized controlled trial. The three instruments each have their specific drawbacks. The PIPP is useful only during painful procedures and the precise scoring of different facial features is problematic in intubated infants because tape will blur the observational area. The NIPS is an 'all or nothing' kind of instrument providing a choice between two extremes only. Furthermore, it incorporates the item 'crying', and we showed that half of the premature neonates do

not cry during painful procedures. The items 'muscle tension' and 'calmness' of the COMFORT scale were shown to be less useful in neonates, while 'facial tension' and 'physical movement' have both intensity and duration intertwined, which is also not helpful in scoring. The physiological pain indicators blood pressure, oxygen saturation and heart rate were shown to be not sensitive even during endotracheal suctioning. Most of the existing pain assessment instruments have psychometric and methodological flaws. Although a new instrument, next to the 16 existing ones, does seem superfluous, it would be a shortcoming not to develop one. The preferred alternative would be to revise the best fitting pain assessment tool. To this aim the available videotapes of our study will need to be re-analyzed, after which the shortcomings of the existing pain instruments might be filled in.

Morphine pharmacokinetics

Morphine is mainly metabolized by the glucuronidation pathway into morphine-3-glucuronide (M3G) and morphine-6-glucuronide (M6G). As is also shown in Figure 1, this reaction is catalyzed by the enzyme UDP-glucuronosyl-transferase 2B7 (UGT2B7) and occurs mainly in the liver. In **Chapter 7** the pharmacokinetics of morphine were studied and the effects of co-medication were evaluated using population parameter estimates with non-linear mixed effects modeling (NONMEM). Data from neonates receiving extra-corporeal membrane oxygenation (ECMO) were compared to non-ECMO treated controls who participated in a randomized controlled trial comparing continuous versus intermittent morphine after major surgery in infants.[4] Although morphine glucuronidation was reduced during the start of ECMO, it improved rapidly and reached similar values as in postoperative neonates within 2 weeks.

During our randomized placebo controlled trial evaluating morphine in ventilated newborns at the NICU, blood was also sampled for pharmacokinetic analyses. The plasma concentrations of morphine and the metabolites, M3G and M6G are now being determined. As the neonates admitted to NICUs are heterogeneous groups of patients, in whom for ethical reasons only limited amounts of blood can be sampled, these samples will also be analyzed in the near future using population parameter estimates with NONMEM. These analyses could adjust for population parameter variability between and within subjects. Polymorphisms in the UGT2B7 gene[51] that might be responsible for between subject variability, determined in the available DNA of our patients, should also

be incorporated in these analyses. As previous studies were not able to correlate morphine plasma levels with analgesia, NONMEM analyses should also be used to further investigate pharmacodynamics of morphine.

Pharmacogenetics

The Human Genome Project has raised expectations for medicines that can be customized to match the genetic make-up of patients, thereby dramatically improving efficacy and safety.[52,53] The study of the role of inheritance in the individual variation in drug response is called pharmacogenetics. The prospects of this area of research look very promising,[54,55] and pharmacogenetics of acetaminophen were already studied by our research group.[5] As morphine use in neonates and infants shows a very narrow range between effect and side-effect,[56] which is complicated by a large inter-individual variability, prediction of individual effects would be very useful. In **Chapter 9** the influence of two single nucleotide polymorphisms (SNPs), *val*[158]*met* at the COMT gene and *asn*[40]*asp* at the human μ-opioid receptor gene (OPRM), on the morphine requirements in neonates and infants was evaluated. The DNA of patients from three different randomized controlled trials evaluating morphine in critically neonates and infants was collected and analysis succeeded in 215 patients for the COMT gene and 283 patients for the OPRM gene. The *val*[158]*met* polymorphism at the COMT gene was shown to influence morphine requirements in neonates and infants. Additional morphine was most frequently used in the wild type patients. This indicates that the COMT mutation increases the efficacy of morphine, or that patients having this mutation are less sensitive to pain. No difference was found between the wild type and heterozygous OPRM *asn*[40]*asp* genotype patients. A promising lower frequency of additional morphine use in the OPRM homozygous *asp*[40]*asp* infants was found compared to the heterozygous and wild type patients. There were, however, only 6 patients with this homozygous genotype and the effect failed to reach statistical significance. Future research using larger groups of patients should further evaluate the influence of the OPRM homozygous genotype on morphine needs.

The two investigated polymorphisms probably only determine part of the genetic variability involved in morphine requirements. Next to genetic differences in the genes encoding the receptors, the so-called drug targets, genetic differences might also be found in the metabolizing enzyme (UGT2B7) and in drug transporters (Figure 1).
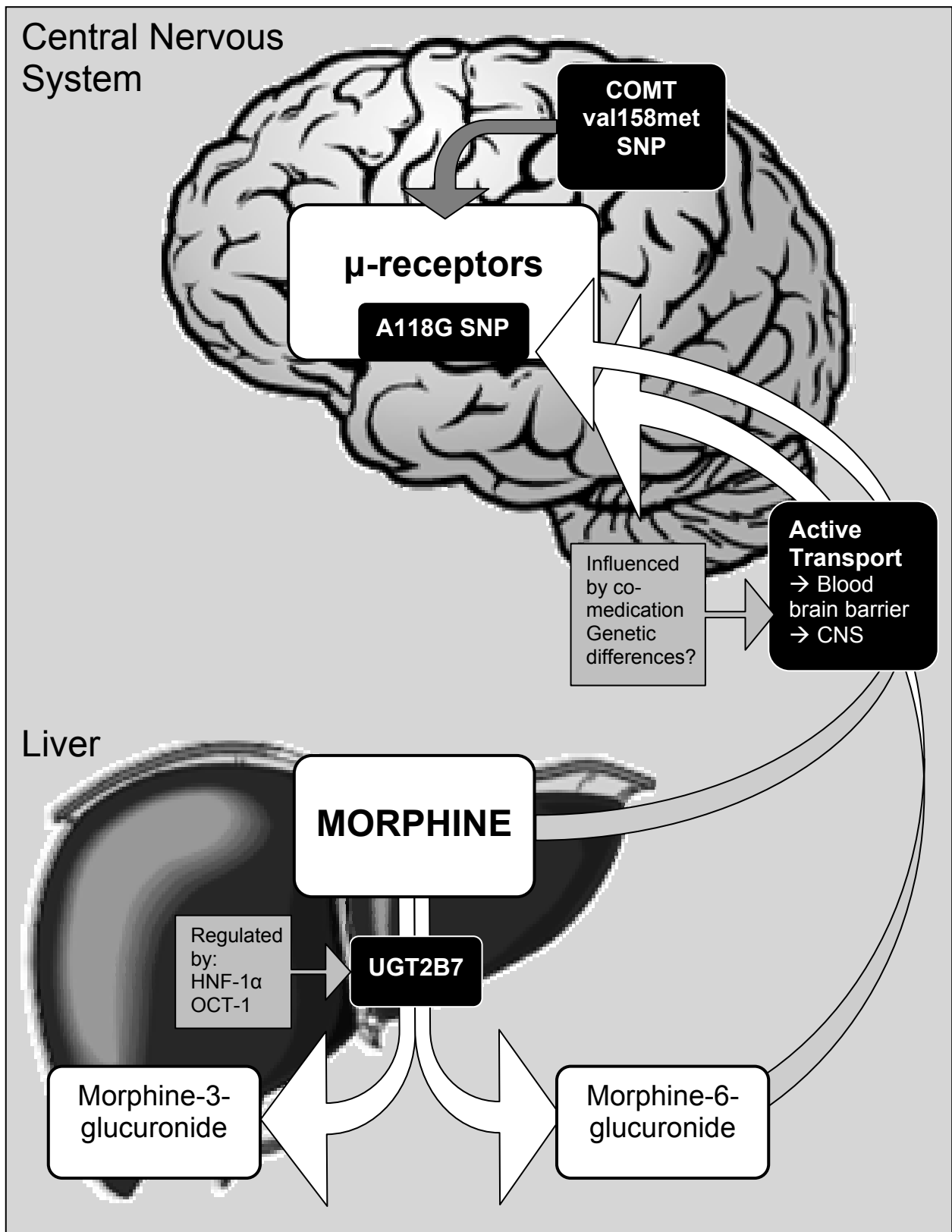
Figure 1   Morphine metabolism and site of action

Furthermore, probably all involved genes are regulated by others, causing a cascade of regulatory reactions that might all influence morphine requirements. More than 1.4 million SNPs were identified in the initial sequencing of the human genome,[57] with over 60,000 of them in the coding region of genes. Bearing in mind that genes might interact with each other and that each SNP causes wild type, heterozygous and homozygous genotypes, the variability seems endless. Nevertheless, polymorphisms causing poor and extensive drug metabolizers have already been found, such as in the CYP2D6 gene for codeine,[58] which polymorphism was recently shown to be also clinically relevant for postoperative tramadol analgesia.[59] Further research is needed to improve our knowledge about morphine, analgesia and neurotransmitters, in relationship to genetic variability in infants. Databases including drug-drug interactions and relevant polymorphisms are available on the internet. As genotyping methods are also developing very rapidly, testing for the expression of thousands of genes using micro-arrays[60] in clinical practice will soon be possible. This might enable to compile 'drug-passports' for individual patients including the relevant polymorphisms and related drug dosages for adequate treatment.

Future perspectives

Evaluation of the current knowledge about neonatal morphine use and analgesia in general shows that still much has to be done. Despite relevant research in the past and present, questions remain unanswered. Conclusive evidence about the neonate's capacity to experience pain should be provided using modern imaging techniques, and long-term effects of pain and analgesic use should be further investigated. We started to investigate the genetics of analgesic effects of morphine and pain sensitivity in newborns. Further research should bring pharmacokinetics, pharmacodynamics and pharmacogenetics of morphine, and of other analgesics as well, closer together by applying analytic methods such as NONMEM.

An enormous gap between findings from research and incorporation of this knowledge into daily clinical practice of critically ill newborns needs to be bridged. This calls for the development of a pain assessment instrument that incorporates the highest standards of psychometric analysis with a well-defined cut-off point. The implementation of such an instrument incorporated in an algorithm for the administration of analgesic agents in newborns can be considered a good start.

Overall, morphine use in newborns may be fine, but the major challenge for future research will be to search for finer and finest. Much can be expected from the recent availability of intravenous acetaminophen and proparacetamol,[61] the use of which in neonates is under investigation. Recent data about the development of a new class of 'physiological' analgesics activating endogenous opioids, are promising.[62] In this way analgesia might be improved and the use of opioids could even become superfluous in the future.

References

1. Merskey H, Bogduk N. Classification of Chronic Pain: Description of Chronic Pain Syndromes and Definitions of Pain Terms. Seattle: IASP Press; 1994:209-214.

2. Anand KJS, Craig KD. New perspectives on the definition of pain. Pain. 1996;67(1):3-6; discussion 209-211.

3. Lingen van RA. Pain Assessment and Analgesia in the Newborn: An Integrated Approach. Rotterdam: thesis Erasmus University Rotterdam; 2000.

4. Bouwmeester NJ. Paediatric Pain Management: from personal-biased to evidence-based. Rotterdam: thesis Erasmus University Rotterdam; 2002.

5. Van der Marel CD. Paracetamol, widely used hardly understood. Rotterdam: thesis Erasmus University Rotterdam; 2003.

6. Dijk van M. Pain unheard? Postoperative pain assessment in neonates and infants. Rotterdam: thesis Erasmus University Rotterdam; 2001.

7. Peters JWB. Facing Pain in Infancy and Childhood. Rotterdam: thesis Erasmus University Rotterdam; 2001.

8. Terstegen C, Koot HM, de Boer JB, Tibboel D. Measuring pain in children with cognitive impairment: pain response to surgical procedures. Pain. 2003;103(1-2):187-198.

9. 6th International Symposium on Paediatric Pain. The Big Questions, Sidney. 15-19 June. http://dcconferences.com.au/ispp2003/ISPP_thebigquestions.html.

10. Barker DP, Rutter N. Exposure to invasive procedures in neonatal intensive care unit admissions. Arch Dis Child Fetal Neonatal Ed. 1995;72(1):F47-48.

11. Johnston CC, Collinge JM, Henderson SJ, Anand KJS. A cross-sectional survey of pain and pharmacological analgesia in Canadian neonatal intensive care units. Clin J Pain. 1997;13(4):308-312.

12. Porter FL, Anand KJS. Epidemiology of pain in neonates. Res Clin Forums. 1998;20:9-18.

13. Benis MM, Suresh GK. Frequency of invasive procedures in very low birth weight (VLBW) infants in the neonatal intensive care unit (NICU), abstract. Pediatric Research. 2001;49(2):392A, 2253.

14. Anand KJS. Consensus statement for the prevention and management of pain in the newborn. Arch Pediatr Adolesc Med. 2001;155(2):173-180.

15. Prevention and management of pain and stress in the neonate. American Academy of Pediatrics. Committee on Fetus and Newborn. Committee on Drugs. Section on Anesthesiology. Section on Surgery. Canadian Paediatric Society. Fetus and Newborn Committee. Pediatrics. 2000;105(2):454-461.

16. Melzack R, Casey KL. Sensory, motivational, and central control determinants of pain. A new conceptual model. In: Kenshalo DR, ed. The Skin Senses. Charles, C. Thomas. IL: Springfield; 1968:423-443.

17. Mrzljak L, Uylings HB, Kostovic I, Van Eden CG. Prenatal development of neurons in the human prefrontal cortex: I. A qualitative Golgi study. J Comp Neurol. 1988;271(3):355-386.

18. Kostovic I, Rakic P. Developmental history of the transient subplate zone in the visual and somatosensory cortex of the macaque monkey and human brain. J Comp Neurol. 1990;297:441-470.

19. Johnston CC, Stevens BJ, Yang F, Horton L. Differential response to pain by very premature neonates. Pain. 1995;61(3):471-479.

20. Giannakoulopoulos X, Sepulveda W, Kourtis P, Glover V, Fisk NM. Fetal plasma cortisol and beta-endorphin response to intrauterine needling. Lancet. 1994;344(8915):77-81.

21. Giannakoulopoulos X, Teixeira J, Fisk N, Glover V. Human fetal and maternal noradrenaline responses to invasive procedures. Pediatr Res. 1999;45(4 Pt 1):494-499.

22. Fisk NM, Gitau R, Teixeira JM, Giannakoulopoulos X, Cameron AD, Glover VA. Effect of direct fetal opioid analgesia on fetal hormonal and hemodynamic stress response to intrauterine needling. Anesthesiology. 2001;95(4):828-835.

23. Klimach VJ, Cooke RW. Maturation of the neonatal somatosensory evoked response in preterm infants. Dev Med Child Neurol. 1988;30(2):208-214.

24. Coskun V, Anand KJS. Development of supraspinal pain processing. Anand KJS, Stevens BJ, McGrath PJ. Pain in Neonates 2nd Revised and Enlarged Edition, Elsevier Amsterdam. 2000;Vol 10:23-54.

25. Chen ACN. New perspectives in EEG / MEG brain mapping and PET/fMRI neuroimaging of human pain. Int J Psychophysiology. 2001;42:417-159.

26. Grunau RE. Long-term consequences of pain in human neonates, Chapter 4. Anand KJS, Stevens BJ, McGrath PJ. Pain in Neonates 2nd Revised and Enlarged Edition, Elsevier Amsterdam. 2000;Vol 10:55-76.

27. Goldschneider KR, Anand KJS. Long-term consequences of pain in neonates, Chapter 4. In: Schechter NL, Berde C, Yaster M, eds. Pain in Infants, Children, and Adolescents. 2nd ed. Philadelphia: Lippencott Williams & Wilkens; 2003:58-70.

28. Fitzgerald M, Howard RF. The neurobiologic basis of pediatric pain, Chapter 2. In: Schechter NL, Berde C, Yaster M, eds. Pain in Infants, Children, and Adolescents. 2nd ed. Philadelphia: Lippencott Williams & Wilkens; 2003:19-42.

29. Grunau RV, Whitfield MF, Petrie JH, Fryer EL. Early pain experience, child and family factors, as precursors of somatization: a prospective study of extremely premature and fullterm children. Pain. 1994;56(3):353-359.

30. Taddio A, Katz J, Ilersich AL, Koren G. Effect of neonatal circumcision on pain response during subsequent routine vaccination. Lancet. 1997;349:599-603.

31. Porter FL, Grunau RE, Anand KJS. Long-term effects of pain in infants. J Dev Behav Pediatr. 1999;20(4):253-261.

32. Anand KJS. Effects of perinatal pain and stress. Prog Brain Res. 2000;122:117-129.

33. Reynolds ML, Fitzgerald M. Long-term sensory hyperinnervation following neonatal skin wounds. Journal of Comparative Neurology. 1995;358(4):487-498.

34. Anand KJS, Coskun V, Thrivikraman KV, Nemeroff CB, Plotsky PM. Long-term behavioral effects of repetitive pain in neonatal rat pups. Physiol Behav. 1999;66(4):627-637.

35. Ruda MA, Ling QD, Hohmann AG, Peng YB, Tachibana T. Altered nociceptive neuronal circuits after neonatal peripheral inflammation. Science. 2000;289(5479):628-631.

36. Walker SM, Meredith-Middleton J, Cooke-Yarborough C, Fitzgerald M. Neonatal inflammation and primary afferent terminal plasticity in the rat dorsal horn. Pain. 2003;105:185-195.

37. Oberlander TF, Grunau RE, Whitfield MF, Fitzgerald C, Pitfield S, Saul JP. Biobehavioral pain responses in former extremely low birth weight infants at four months' corrected age. Pediatrics. 2000;105(1):e6.

38. Anand KJS, Sippell WG, Aynsley-Green A. Randomised trial of fentanyl anaesthesia in preterm babies undergoing surgery: effects on the stress response. Lancet. 1987;1(8524):62-66.

39. Anand KJS, Hickey PR. Pain and its effects in the human neonate and fetus. N Engl J Med. 1987;317(21):1321-1329.

40. Kennedy KA, Tyson JE. Narcotic analgesia for ventilated newborns: are placebo-controlled trials ethical and necessary? J Pediatr. 1999;134(2):127-129.

41. Franck LS, Miaskowski C. The use of intravenous opioids to provide analgesia in critically ill, premature neonates: a research critique. J Pain Symptom Manage. 1998;15(1):41-69.

42. Anand KJS. Systemic analgesic therapy. Anand KJS, Stevens BJ, McGrath PJ. Pain in Neonates 2nd Revised and Enlarged Edition, Elsevier Amsterdam. 2000;Vol 10:159-188.

43. Saarenmaa E, Huttunen P, Leppaluoto J, Meretoja O, Fellman V. Advantages of fentanyl over morphine in analgesia for ventilated newborn infants after birth: A randomized trial. J Pediatr. 1999;134(2):144-150.

44. Anand KJS, 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. 1999;153(4):331-338.

45. Berde CB, Sethna N. Analgesics for the treatment of pain in children. N Engl J Med. 2002;347(14):1094-1103.

46. Quinn MW, Wild J, Dean HG, et al. Randomised double-blind controlled trial of effect of morphine on catecholamine concentrations in ventilated pre-term babies. Lancet. 1993;342(8867):324-327.

47. Osborn D, Evans N, Kluckow M. Hemodynamic and antecedent risk factors of early and late periventricular/intraventricular hemorrhage in premature infants. Pediatrics. July 2003;112(1 Pt 1):33-39.

48. Volpe JJ. Intracranial Hemorrhage: Germinal Matrix-Intraventricular Hemorrhage of the Premature Infant. Neurology of the Newborn. 4th ed. Philadelphia: Saunders; 2000:428-491.

49. Van Baar Aldo AL, Kollee LAA. Ontwikkeling van kinderen met perinatale risicofactoren: theoretische achtergrond, literatuurgegevens en implementatie in de praktijk. Tijdschr Kindergeneeskd. 2000:210-216 (in Dutch).

50. NEOPAIN Multicenter Group. Effects of Morphine Therapy on Neurologic Outcomes in Ventilated Preterm Neonates: Primary Outcomes from the NEOPAIN (Neurologic Outcomes & Pre-emptive Analgesia In Neonates) Multicenter Trial. Pediatric Research. 2002;51(4 Suppl):361A.

51. Coffman BL, King CD, Rios GR, Tephly TR. The glucuronidation of opioids, other xenobiotics, and androgens by human UGT2B7Y(268) and UGT2B7H(268). Drug Metab Dispos. 1998;26(1):73-77.

52. Evans WE, McLeod HL. Pharmacogenomics - Drug disposition, drug targets, and side effects. N Engl J Med. 2003;348:538-549.

53. Weinshilboum R. Inheritance and drug response. N Engl J Med. 2003;348:529-537.

54. Roses AD. Pharmacogenetics and the practice of medicine. Nature. 2000;405(6788):857-865.

55. Roses AD. Pharmacogenetics and future drug development and delivery. Lancet. 2000;355(9212):1358-1361.

56. Kart T, Christrup LL, Rasmussen M. Recommended use of morphine in neonates, infants and children based on a literature review: Part 2--Clinical use. Paediatr Anaesth. 1997;7(2):93-101.

57. Sachidanandam R, Weissman D, Schmidt SC. A map of human genome sequence variation containing 1.42 million single nucleotide polymorphisms. Nature. 2001;409:928-933.

58. Sindrup SH, Brosen K. The pharmacogenetics of codeine hypoalgesia. Pharmacogenetics. 1995;5(6):335-346.

59. Stamer UM, Lehnen K, Höthker F, et al. Impact of CYP2D6 genotype on postoperative tramadol analgesia. Pain. 2003;105:231-238.

60. Gerhold DL, Jensen RV, Gullans SR. Better therapeutics through microarrays. Nature Genet. 2002;32:547-552.

61. Allegaert K, van der Marel C, Debeer A, et al. Pharmacokinetics of single dose intravenous propacetamol in neonates: effect of gestational age. Arch Dis Child. In press.

62. Le Guen S, Mas Nieto M, Canestrelli C, et al. Pain management by a new series of dual inhibitors of enkephalin degrading enzymes: long lasting anti-nociceptive properties and potentiation by CCK2 antagonist or methadone. Pain. 2003;104((1-2)):139-148.

Chapter 11

SUMMARY/SAMENVATTING

Chapter 11.1

SUMMARY

Summary

The past 20 years have seen a burst of pediatric pain research that underlined the necessity of analgesic treatment in different groups of infants. However, against the background of the existing undertreatment of pain, the preterm born, critically ill neonate admitted to a Neonatal Intensive Care Unit (NICU) appears to be a changeling.
Considered the most appropriate analgesic, morphine is also the most widely used opioid analgesic for the treatment of postoperative and procedural pain in neonates and infants. It is, however, not often routinely used as a standard of care in NICUs, seeing that the effects of continuous morphine in neonates admitted to a NICU have never been properly investigated.

Considering the increasing attention given to neonatal pain and analgesia during the last decades, we hypothesized that procedural pain during neonatal intensive care treatment would now have been reduced and be adequately treated with pharmacological agents. **Chapter 2** was used as a rationale for our studies. In a prospective study we counted all painful procedures, including attempts, in 151 neonates during their first two weeks in our NICU, and meanwhile noted the used analgesic therapies as well. Furthermore, nurses and physicians were asked to judge the painfulness of each procedure on a 10-pointscale. We found that these neonates on average underwent 14 painful procedures per day. Overall, the number of procedures was highest during the first day on the NICU, and neonates receiving respiratory support underwent the highest amount of procedures. Although most procedures were scored as painful by the clinicians (painfulness scores above 4.0 on a 10-point scale for 26 out of 31 procedures), pre-emptive pharmacological analgesic treatment was limited to less than 35% of neonates per studied day. Overall, 40% of the neonates did not receive any analgesic therapy during all studied days. The discrepancy between the high number of painful procedures, predominantly in those on ventilatory support, and the limited administration of analgesics, confirmed the necessity of further research.

In **Chapter 3** we evaluated the present state of knowledge about analgesics and pain assessment in neonates. The pharmacodynamics and kinetics of the most frequently used opioids in neonates and infants are reviewed in **Chapter 3.1**. Opioids are compared and the ontogeny of opioid pharmacodynamics and kinetics, taking place after birth and during the first years of life, is extensively discussed. In general, the metabolism of opioids is immature at birth, especially in prematurely born neonates, and reaches adult levels in early childhood. As a consequence, clearances and the analgesic effects of opioids vary during the first years of life. This age-dependent variability combined with a

large unpredictable inter-individual variability in kinetics and dynamics of opioids, necessitates titration of opioids according to the individual infant's needs.

Around sixteen different multidimensional pain assessment instruments have by now been developed to make pain assessment in neonates less subjective. **Chapter 3.2** critically reviews their content, consisting of behavioral and physiological indicators. Behavioral indicators are, for instance, facial expression and body movements, while blood pressure and heart rate are often used as physiological indicators of pain. Methodological, psychometrical shortcomings of the available tools are discussed in the context of tools that are needed for research purposes and those for daily clinical practice. The usefulness of physiological indicators for neonatal pain assessment has been tested insufficiently. Furthermore, procedural issues concerning pain assessment, such as the duration of observation, are often lacking or incomplete. Currently, none of the neonatal pain assessment instruments seems ideal, and much room is left for improvement.

We hypothesized that the restrained use of morphine in newborns is partly explained by ignorance of the effects of routine morphine infusion in neonates. To fill the knowledge gap, we performed a blinded randomized placebo-controlled trial evaluating the effects of continuous morphine infusion in ventilated newborns. The aims of this study were to determine the effects of morphine on the levels of pain experience, stress response, and neurologic and clinical outcome of newborns. Furthermore we aimed to determine if continuous morphine causes adverse effects in newborn infants, such as hypotension. During the inclusion period 150 ventilated newborns, admitted to the NICUs of the Erasmus MC - Sophia in Rotterdam and the Isala Clinics in Zwolle, were randomly allocated to receive either a morphine loading dose (100 μg/kg) followed by a continuous morphine dose (10 μg/kg/h) or placebo during maximally 7 days.

Chapter 4 reports how continuous morphine infusion in ventilated neonates effected their pain responses, neurologic outcomes, and other clinical outcome measures (such as duration of ventilation). Pain was measured with three validated pain assessment tools: the Premature Infant Pain Profile (PIPP), the Neonatal Infant Pain Scale (NIPS) and the Visual Analog Scale (VAS). Neurologic outcome was defined as IVH all grades and as poor neurologic outcome including severe IVH, PVL and death before 28th postnatal day. An analgesic effect of morphine could not be detected as pain scores of infants receiving morphine did not differ with those of placebo-treated infants: PIPP, 10.1 (8.2-11.6) vs 10.0 (8.2-12.0)($P = 0.94$); NIPS, 4.8 (3.7-6.0) vs 4.8 (3.2-6.0)($P = 0.58$); and VAS, 2.8

(2.0-3.9) vs 2.6 (1.8-4.3)($P = 0.14$), respectively. Logistic regression analysis showed that the incidence of IVH (all grades) was significantly lower in the morphine group compared to the placebo group (23% vs. 40%, $P = 0.04$). Otherwise, no significant effect of morphine on the incidence of poor neurologic outcome (10% in the morphine group vs. 16% in the placebo group; $P = 0.66$), or on the other clinical outcome measures was found. These results, we feel, do not support the routine use of morphine infusions as a standard of care in preterm ventilated newborns. However, follow-up is needed to evaluate the long-term effects of morphine on the neurobehavioral outcomes of prematurity.

Next to the effects of morphine on neonates' pain responses, we evaluated the effects on stress responses, as measured by epinephrine and norepinephrine plasma concentrations. In **Chapter 5** we report that median plasma concentrations of epinephrine during infusion of study medication were 0.12 nmol/l (IQR: 0.28) and 0.18 nmol/l (IQR: 0.35) for the morphine- and placebo-treated infants, respectively. Median norepinephrine concentrations were 2.8 nmol/l (IQR: 3.7) and 3.8 nmol/l (IQR: 4.0) for the morphine- and placebo-treated infants, respectively. Multivariate analysis showed that norepinephrine ($P = 0.029$), but not epinephrine ($P = 0.18$), concentrations were significantly lower in the morphine group compared to the placebo group. As continuous morphine infusion significantly reduced norepinephrine plasma concentrations in ventilated newborns as compared to placebo treatment, the results of this study support the idea that routine morphine administration decreases stress responses in ventilated neonates.

Next to positive effects, continuous morphine administration in newborns might also be associated with adverse reactions. Fear for side-effects is probably also an important reason for the restrained use of analgesics in the critically ill neonate. As a separate in-depth analysis of data from the randomized placebo controlled trial we evaluated the effects of morphine on blood pressure in **Chapter 6**. We tested the hypothesis that continuous morphine infusion would (a) cause hypotension and (b) decrease blood pressure variability. Mean arterial blood pressures during the first 48 hours of study medication infusion were comparable between the morphine group (median 36 mmHg; IQR 6) and the placebo group (median 38 mmHg; IQR 6)($P = 0.11$). However, significantly more morphine-treated patients (70%) showed hypotension during study medication infusion compared to the placebo-treated patients (47%)($P = 0.004$). As the use of volume expansion and vasopressor drugs was comparable (morphine group: 44%; placebo group: 48%; $P = 0.87$) the clinical significance of hypotension as a side-effect of

morphine was limited. Multiple regression analysis showed that blood pressure variability was not influenced by routine morphine analgesia ($P = 0.81$) or additional morphine ($P = 0.80$). Patients with and without intraventricular hemorrhage showed comparable blood pressure variability ($P = 0.51$), mean arterial blood pressure ($P = 0.14$) and incidence of hypotension ($P = 0.28$). Therefore no relationship between blood pressure and intraventricular hemorrhage could be determined. The clinical effects of low-dose morphine treatment on blood pressure in neonates were minimal.

In **Chapter 7** the pharmacokinetics of morphine in neonates during veno-arterial extra-corporeal membrane oxygenation (ECMO) were studied and compared with the pharmacokinetics of morphine in 0 to 3-year-old post-surgical infants. Fourteen neonates receiving ECMO were included and plasma concentrations of morphine, morphine-3-glucuronide (M3G), and morphine-6-glucuronide were determined. Data from these neonates were combined with the data from post-surgical infants using non-linear-mixed effects modeling (NONMEM). Formation clearances from morphine to M3G and M6G on day 1, at the start of ECMO, were lower (3.5 and 0.5 l/h/70kg) compared to the formation clearances in the postoperative infants (10.6 and 0.6 l/h/70kg). The clearances matured more rapidly in the ECMO-treated patients (25.5 and 13.6 days) compared to the post-surgical patients (56.6 days). Elimination clearances of M3G and M6G also increased with postnatal age with a maturation half-life of 174 days. Concomitant medication during ECMO, such as midazolam and fentanyl, did not affect the clearances of morphine metabolites.

As our review of neonatal pain assessment tools (Chapter 3.2) showed many of them to have drawbacks, we evaluated the usefulness of the Premature Infant Pain Profile (PIPP), Neonatal Infant Pain Scale (NIPS) and COMFORT score in neonates, using the prospectively collected data during the randomized controlled trial (**Chapter 8**). We looked at both the behavioral pain indicators of these three pain assessment tools and the physiological indicators blood pressure, heart rate and oxygen saturation. Heart rate during suctioning differed significantly from that just before and that 30 minutes after suctioning, but clinically these differences are minor, as they mostly can not be seen in the individual neonate. A significant increase of blood pressure during suctioning and a significant decrease after additional morphine were found. Oxygen saturation decreased significantly but not relevantly during suctioning. Of the behavioral indicators, the items facial expression, body movements and behavioral state were shown to be good indicators of neonatal pain. We conclude that the ideal neonatal pain assessment instrument has not yet emerged.

In **Chapter 9** we aimed to determine if polymorphisms would explain the large inter-individual differences in the amounts of morphine required for adequate analgesia during postnatal and postoperative intensive care in neonates and infant. To this aim we focussed on two single nucleotide polymorphisms, i.e. $asn^{40}asp$ of the OPRM gene and $val^{158}met$ of the COMT gene. Patients from three different randomized controlled trials on the effects of morphine using validated pain assessment scores in newborns and infants were included. The COMT mutation significantly decreased the frequency of additional morphine use (OR 0.42; 95% CI: 0.18-0.98; $P = 0.045$) in critically ill newborns and infants. Additional morphine was required in 57.4% wild type, 46.1% heterozygous and 42.6% homozygous patients for the COMT gene polymorphism. Additional morphine grouped for the OPRM genotype was required in 47.1% of the wild type, 50.8 of the heterozygous and 16.7% of the homozygous patients. This genotype did not significantly influence morphine requirements. Only six patients were found to be homozygous for the OPRM genotype. The low frequency of additional morphine use in these patients seems to be promising and warrants further evaluation in larger cohorts of patients.

The results of our studies are discussed in **Chapter 10**. Future perspectives are indicated.

Chapter 11.2

SAMENVATTING

Samenvatting

De noodzaak van pijnstilling bij bepaalde groepen kinderen wordt onderschreven door de resultaten van het steeds intensievere onderzoek naar pijn bij kinderen gedurende de afgelopen 20 jaar. Daar pijn bij te vroeg geboren pasgeborenen opgenomen op een Intensive Care Neonatologie (ICN) nog steeds wordt onderbehandeld, lijkt de premature patiënt wat dit betreft een ondergeschoven kindje te zijn.

Omdat morfine gezien wordt als het beste pijnstillende middel, wordt het ook bij pasgeborenen en kinderen bij voorkeur gebruikt ter bestrijding van postoperatieve en procedurele pijn. Omdat het effect van continue morfine toediening nog nooit goed is onderzocht wordt het nog maar zelden routinematig, als standaardbehandeling, bij pasgeborenen op de ICN gebruikt.

Gezien het feit dat er de laatste decennia steeds meer aandacht is besteed aan pijnbestrijding bij pasgeborenen, was onze hypothese dat pasgeborenen tijdens hun ICN-opname tegenwoordig minder pijn zouden ervaren van alle handelingen die ze moeten ondergaan, en dat deze pijn op een adequate manier behandeld zou worden. **Hoofdstuk 2** werd gebruikt als een basis voor onze studies. In een prospectief onderzoek telden we alle pijnlijke handelingen, inclusief de handelingen die mislukten, die 151 pasgeborenen ondergingen tijdens de eerste twee weken van hun opname op de ICN. Tegelijkertijd werd alle gebruikte pijnstilling vastgelegd. Verder werd aan verpleegkundigen en artsen door middel van een enquête gevraagd om de pijnlijkheid van de verschillende procedures te schatten op een schaal van 0 tot 10.

Het bleek dat de pasgeborenen gemiddeld 14 pijnlijke procedures per dag ondergingen. De frequentie van de uitgevoerde handelingen was het hoogst gedurende de eerste dag van opname op de ICN. Het aantal handelingen was het hoogst bij de pasgeborenen die ademhalingsondersteuning nodig hadden. Hoewel de artsen en verpleegkundigen 26 van de 31 verschillende handelingen als pijnlijk hadden aangegeven (pijnscore hoger dan 4.0 op de 10-punts schaal), kreeg minder dan 35% van de pasgeborenen per dag adequate pijnstilling. Gedurende hun gehele observatieperiode kreeg 40% van de patiënten überhaupt geen pijnstillers toegediend. Er bleek dus een discrepantie: een hoge frequentie van (pijnlijke) procedures, met name bij degenen die beademd werden, en een laag gebruik van pijnstillende middelen. Dit bevestigde de noodzaak van ons verdere onderzoek.

In **hoofdstuk 3** werd de huidige kennis over pijnstillende middelen en pijnmeting bij pasgeborenen geëvalueerd. In **hoofdstuk 3.1** wordt een overzicht gegeven van de farmacodynamiek en -kinetiek van de meest gebruikte opioïden bij pasgeborenen en jonge kinderen. De verschillende opioïden worden met elkaar vergeleken en de ontwikkeling van de farmacodynamiek en -kinetiek van opioïden na de geboorte en gedurende de eerste levensjaren wordt uitgebreid besproken. In het algemeen is het metabolisme van opioïden nog onderontwikkeld bij de geboorte, met name bij te vroeg geborenen, en bereikt het volwassen waarden in de jonge kinderjaren. Derhalve veranderen de klaring en het pijnstillende effect tijdens de eerste levensjaren. Omdat naast deze leeftijdsgebonden variabiliteit er ook nog een grote onvoorspelbare variabiliteit bestaat bij kinderen van dezelfde leeftijd, dient het gebruik van opioïden aangepast te worden aan de behoefte van het individuele kind.

Er zijn zo'n 16 verschillende multi-dimensionele pijnmeetinstrumenten ontwikkeld met het doel de meting van pijn bij pasgeborenen minder subjectief te laten zijn. **Hoofdstuk 3.2** geeft een kritisch overzicht van deze pijnmeetinstrumenten, die zijn samengesteld uit gedrags- en fysiologische indicatoren. Gedragsindicatoren zijn bijvoorbeeld gezichtsuitdrukking en lichaamsbewegingen, terwijl de hoogte van de bloeddruk en de hartslag vaak gebruikt worden als fysiologische indicatoren van pijn. De methodologische en psychometrische tekortkomingen van deze pijnmeetinstrumenten – voor gebruik bij onderzoek of voor dagelijks gebruik in de kliniek – worden besproken. Het blijkt dat de bruikbaarheid van de fysiologische indicatoren voor pijnmeting bij pasgeborenen onvoldoende is getest. Verder worden aanwijzingen voor het gebruik, bijvoorbeeld hoe lang er geobserveerd dient te worden, onvoldoende of niet gegeven. Geen van de huidige instrumenten lijkt ideaal voor het meten van pijn bij pasgeborenen, en is er nog veel verbetering mogelijk.

Onze hypothese was dat het terughoudend gebruik van morfine bij pasgeborenen gedeeltelijk te wijten is aan onduidelijkheid over de effecten van morfinetoediening bij deze pasgeborenen. Om het tekort aan kennis aan te vullen, verrichtten we bij beademde pasgeborenen een geblindeerde placebo-gecontroleerde studie naar de uitwerking van continue morfinetoediening, met name op de mate van pijn, de stress-respons, de neurologische uitkomst en de mate van ziek zijn. Bovendien wilden we vaststellen of continue morfinetoediening schadelijke bijwerkingen, zoals hypotensie, veroorzaakt bij pasgeborenen. De studiepopulatie bestond uit 150 beademde pasgeborenen die opgenomen waren op de ICN van het Erasmus MC - Sophia te Rotterdam en de Isala

Klinieken te Zwolle, 'at random' verdeeld in twee groepen. De ene groep kreeg een startdosering morfine (100 µg/kg) gevolgd door continue morfine (10 µg/kg/uur), de andere groep kreeg placebo gedurende maximaal 7 dagen.

In **hoofdstuk 4** worden de effecten van continue morfinetoediening op de pijnrespons, de neurologische uitkomst en andere klinische uitkomstmaten (zoals beademingsduur) van beademde pasgeborenen beschreven. De mate van pijn werd gemeten met drie verschillende gevalideerde pijnmeetinstrumenten: de *Premature Infant Pain Profile* (PIPP), de *Neonatal Infant Pain Scale* (NIPS) en de Visueel Analoge Schaal (VAS). De neurologische uitkomst werd gedefinieerd als intraventriculaire bloedingen (IVH), waarbij alle graden werden meegenomen, en als slechte neurologische uitkomst, waartoe ernstige intraventriculaire bloedingen, periventriculaire leukomalacie (PVL) en overlijden voor de 28ste postnatale dag werden gerekend.

Een pijnstillend effect van morfine kon niet worden vastgesteld: de pijnscores van de kinderen die morfine kregen bleken statistisch niet te verschillen van de scores van degenen die placebo kregen; respectievelijk PIPP: 10.1 (8.2 – 11.6) versus 10.0 (8.2 – 12.0)($P = 0.94$), NIPS: 4.8 (3.7 – 6.0) versus 4.8 (3.2 – 6.0)($P = 0.58$) en VAS: 2.8 (2.0 – 3.9) versus 2.6 (1.8 – 4.3) ($P = 0.14$). Uit logistische regressie-analyse bleek dat de incidentie van IVH (alle graden) significant lager was in de morfine-groep in vergelijking met de placebo-groep (23% versus 40%; $P = 0.04$). Overigens was er noch een significant verschil in de incidentie van de slechte neurologische uitkomst tussen de twee groepen (10% in de morfine groep versus 16% in de placebo groep; $P = 0.66$), noch in de incidentie van de andere klinische uitkomstmaten. Naar onze mening pleiten deze resultaten dus niet voor het routinematige gebruik van continue morfine als standaardbehandeling bij beademde pasgeborenen. Vervolgonderzoek is echter noodzakelijk om de lange termijn gevolgen van morfine op de neurologische ontwikkeling ten gevolge van prematuriteit te evalueren.

Naast de uitwerking van morfine op de pijnrespons van de pasgeborene, evalueerden wij ook de mate van de stressrespons, door middel van het meten van de concentraties van adrenaline en noradrenaline in het bloedplasma. In **hoofdstuk 5** rapporteren we dat de mediane plasmaconcentratie van adrenaline tijdens de toediening van studiemedicatie 0.12 nmol/l (IQR: 0.28) bedroeg in de morfine-groep en 0.18 nmol/l (IQR: 0.35) in de placebo-groep. De mediane noradrenaline-concentratie bedroeg respectievelijk 2.8 nmol/l (IQR: 3.7) en 3.8 nmol/l (IQR 4.0) in de met morfine en met placebo behandelde kinderen. Multipele regressie-analyse liet zien dat wel de noradrenaline-concentraties ($P =$

0.029), maar niet de adrenaline-concentraties ($P = 0.18$) significant lager waren in de morfine-groep in vergelijking met de placebo-groep. Omdat de toediening van morfine de concentraties van noradrenaline in bloedplasma significant verlaagde, onderschrijven de resultaten van deze studie de veronderstelling dat routinematige toediening van morfine bij beademde pasgeborenen tot een verlaagde stressrespons leidt.

Naast positieve effecten zou het gebruik van continue morfine bij pasgeborenen ook bepaalde schadelijke bijwerkingen met zich mee kunnen brengen. Angst voor bijwerkingen is waarschijnlijk ook een belangrijke reden voor het terughoudend gebruik van pijnstillende middelen bij de ernstig zieke pasgeborene. De gegevens uit de gerandomiseerde placebo-gecontroleerde trial werden in een uitvoerige secundaire analyse gebruikt om de uitwerking van morfine op de bloeddruk vast te stellen in **hoofdstuk 6**. We onderzochten de hypothese dat continue toediening van morfine (a) hypotensie en (b) een verlaagde variabiliteit van de bloeddruk zou veroorzaken. De gemiddelde arteriële bloeddruk (MAP) gedurende de eerste 48 uur toediening van studie-medicatie in de morfine-groep (mediaan 36 mmHg; IQR 6) bleek vergelijkbaar met die in de placebo groep (mediaan 38 mmHg; IQR 6)($P = 0.11$). Significant meer morfine-behandelde patiënten (70%) bleken echter perioden met hypotensie te hebben gehad in vergelijking met de placebo-behandelde patiënten (47%)($P = 0.004$). Daar het gebruik van volume-expansie en bloeddrukverhogende middelen vergelijkbaar was (morfine-groep: 44%; placebo-groep: 48%; $P = 0.87$), lijkt de klinische relevantie van hypotensie als bijwerking van morfine minimaal te zijn. Multipele regressie-analyse toonde aan dat de bloeddruk-variabiliteit niet werd beïnvloed door routinematige morfine-toediening ($P = 0.81$) of door het gebruik van extra morfine ($P = 0.80$). Patiënten met en zonder intraventriculaire bloedingen hadden vergelijkbare bloeddruk-variabiliteit ($P = 0.51$), gemiddelde arteriële bloeddruk ($P = 0.14$), en incidentie van hypotensie ($P = 0.28$). Er kon derhalve geen relatie tussen bloeddruk-variabiliteit en intraventriculaire bloedingen worden vastgesteld. De klinische effecten van lage morfine-doseringen op de bloeddruk van pasgeborenen waren minimaal.

In **hoofdstuk 7** werd de farmacokinetiek van morfine bestudeerd bij pasgeborenen tijdens *veno-arterial extra-corporeal membrane oxygenation* (ECMO) en vergeleken met de farmacokinetiek van morfine bij postoperatieve kinderen in de leeftijd van 0 tot 3 jaar. Van veertien pasgeborenen die ECMO-behandeling kregen werden de plasma-concentraties van morfine, morfine-3-glucuronide (M3G) en morfine-6-glucuronide (M6G) bepaald. De gegevens van deze pasgeborenen werden gecombineerd met die van

postoperatieve kinderen door gebruik te maken van *non-linear-mixed-effects modeling* (NONMEM). De formatieklaringen van morfine naar M3G en M6G op de eerste dag, bij aanvang van de ECMO-behandeling, waren lager (3.5 en 0.5 l/h/70kg) in vergelijking met de formatieklaringen bij de postoperatieve patiënten (10.6 en 0.6 l/h/70kg). De maturatie van de klaringen bij de ECMO behandelde patiënten (25.5 en 13.6 dagen) was veel sneller in vergelijking met de postchirurgische patiënten (56.6 dagen). De eliminatieklaringen van M3G en M6G namen ook toe met de postnatale leeftijd, met een uitrijpings-halfwaardetijd van 174 dagen. Aanvullende medicatie, zoals midazolam en fentanyl, die tegelijkertijd met morfine gebruikt werd tijdens de ECMO-behandeling had geen invloed op de klaring van de morfine-metabolieten.

In ons overzicht van de neonatale pijnmeetinstrumenten (**hoofdstuk 3.2**) lieten wij zien dat veel van deze instrumenten nog tekortkomingen hebben. Daarom gebruikten we de prospectief verzamelde gegevens van de gerandomiseerde trial om de bruikbaarheid van de *Premature Infant Pain Profile* (PIPP), de *Neonatal Infant Pain Scale* (NIPS) en de COMFORT schaal te evalueren (**hoofdstuk 8**). We keken naar de gedragsindicatoren en naar de fysiologische indicatoren – zoals bloeddruk, hartslag en zuurstofsaturatie – van deze drie pijnmeetinstrumenten. Tijdens het uitzuigen van de beademingstube week de hartslag significant af van de hartslag gemeten net voor en een half uur na het uitzuigen, maar klinisch zijn deze verschillen minimaal, omdat ze meestal niet bij de individuele pasgeborene kunnen worden waargenomen. De bloeddruk was significant hoger tijdens het uitzuigen en na het gebruik van extra morfine. De zuurstofsaturatie liet een significante, maar niet relevante, daling zien tijdens het uitzuigen. Van de gedragsindicatoren bleken de items gezichtsuitdrukking, lichaamsbeweging en gedragstoestand goede indicatoren voor pijn bij de pasgeborene te zijn. Wij concluderen dat het ideale instrument om pijn bij de pasgeborene te meten nog niet beschikbaar is.

In **hoofdstuk 9** was het doel vast te stellen of bepaalde afwijkingen in het DNA, zogenaamde polymorfismen, de grote inter-individuele variabiliteit in hoeveelheden morfine kunnen verklaren, die nodig zijn voor adequate pijnstilling tijdens postnatale en postoperatieve intensive care bij pasgeborenen en jonge kinderen. Voor dit doel concentreerden wij ons op twee *single-nucleotide*-polymorfismen, te weten *asn*^{40}*asp* van het OPRM-gen en *val*^{158}*met* van het COMT-gen. Patiënten uit drie verschillende gerandomiseerde trials, naar het effect van morfine bij pasgeborenen en jonge kinderen aan de hand van gevalideerde pijnscores, werden bestudeerd. De COMT-mutatie bleek gerelateerd te zijn aan een significant lagere frequentie van extra morfinegebruik (OR

0.42;95%CI: 0.18-0.98; P = 0.045) bij ernstig zieke pasgeborenen en kinderen. Extra morfine was noodzakelijk bij 57.4% van de wild-type, 46.1% van de heterozygote en 42.6% van de homozygote COMT-patiënten. Extra morfinegebruik gegroepeerd voor het OPRM genotype was 47.1% voor de wild-type, 50.8 % voor de heterozygote en 16.7% voor de homozygote patiënten. Dit genotype was niet significant gerelateerd aan het extra morfinegebruik. Slechts 6 patiënten waren homozygoot voor het OPRM genotype. De lage frequentie van extra morfinegebruik bij deze patiënten ziet er veelbelovend uit en dient in grotere groepen patiënten geëvalueerd te worden.

De resultaten van onze studies worden in **hoofdstuk 10** bediscussieerd. Tevens worden daarin toekomstperspectieven geschetst.

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Curriculum Vitae

Sinno Simons werd op 24 december 1975 geboren te Roermond. In 1995 behaalde hij het VWO diploma aan het Bisschoppelijk College Schöndeln in dezelfde stad. In de twee daarop volgende jaren studeerde hij geneeskunde in België aan het Limburgs Universitair Centrum te Diepenbeek, waar hij in 1997 zijn tweede kandidatuursjaar met onderscheiding behaalde. Vanaf 1997 vervolge hij zijn studie geneeskunde aan de Erasmus Universiteit Rotterdam. Zijn afstudeeronderzoek deed hij op de afdeling Kinderheelkunde in het Erasmus MC-Sophia onder leiding van professor Tibboel en hij behaalde zijn doctoraalexamen in september 2000. In de daaropvolgende periode deed hij promotieonderzoek onder leiding van professor Tibboel en professor van den Anker dat gesubsidieerd werd door de Nederlandse Organisatie voor Wetenschappelijk Onderzoek (NWO) en beschreven is in dit proefschrift. Dit onderzoek vond plaats op de afdeling kinderheelkunde (intensive care chirurgie) en neonatologie van het Erasmus MC-Sophia en op de afdeling neonatologie van de Isala Klinieken in Zwolle.

In april 2003 startte hij met het volgen van zijn co-schappen, die hij in oktober 2004 af zal ronden.