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PROJECT FINAL REPORT

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Project acronym: NEOOPIOID

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4.1 Final publishable summary report

Executive summary

A summary description
The main objectives of this project were to study the current clinical practices regarding these drugs in the EU and develop more personalized sedative/analgetic (S/A) drug therapy of neonates considering individual pharmacogenetic and kinetic properties. The ultimate aim was to develop more personalized S/A drug therapy considering individual pharmacogenetic and kinetic properties.

Main Results
Assessment of pain and the use of S/A drugs for infants cared at neonatal intensive care units (NICU) were studied using special web-based questionaires. About 6700 patients from 18 EU countries were included. Most centres use either morphine or fentanyl. However, there is a trend to use more fentanyl. Paracetamol is also very often used either alone or in combination with the other drugs. More than half of the NICUs did not practice any kind of pain assessment of ventilated neonates. Most ventilated infants were treated with continuous and bolus administration of S/A drugs. Only 12 % of the non-ventilated babies received S/A treatment,

For the clinical trial a lot of effort has been spent to develop a protocol and optimize the scoring of pain in preterm infants. These scores are studied in relation to the PK/PG properties of the analgetic drugs. This part of the project has been delayed due to considerable changes in neonatal intensive care during the last years. Newly updated European consensus guidelines emphasize a minimum handling of the preterm infant, ventilatory treament with CPAP and with very limited use of opiods and fentanyl. This explains to some extent the slow recruitment of infants.

The methods to analyse opioids and their metabolites have been improved with special regard to the small blood samples from neonates. Of particular interest is the active morphine-6-glucuronide metabolite, which was detected in some infants.

The relation between polymorphisms in candidate genes (COMT and KCNJ6 genes) have been studied in relation to the pain relieving effect.

Child friendly formulations of fentanyl have been developed and used for the clinical trial.

A number of ad hoc studies on the preterm brain have been performed. Of particularly interest are the studies of the spontaneous resting activity of both preterm and term infants. These studies are of particular importance to understand the effect of the analgesic drugs.

Expected final results and potential impact:
The results of the survey may lead to the development of uniform recommendations how to assess and treat neonatal pain with S/A drugs.
The results of the PK/PG studies are of importance to develop a more personalized treatment with S/A drugs.
More child friendly drugs may be developed.

The most important impact is the increase of knowledge how to assess and alleviate pain in newborn infants. This knowledge will hopefully be disseminated among pediatricians and nurses in the entire EU and also worldwide. We will then be able to treat infants in the same individualized way as adult patients and fulfil the Hippocratic oath to alleviate without damage. Furthermore reducing procedural pain as well as chronic distress is essential for normal brain development.

Project website: www.neoopioid.eu

A summary description of project context and objectives

It is now well established that newborn infants not only show physiological and behaviour responses to pain but also reach pain to conscious level. Newborn infants seem to be even more sensitive to pain, since some of the modulating neurosystems are not developed (Fitzgerald et al, 2000). Newborns admitted to neonatal intensive care units (NICU) may experience prolonged pain and repeated painful procedures as part of their medical treatment. Several hundreds of invasive procedures may be performed in extremely preterm infants during neonatal intensive care, particularly during the first weeks after birth. The most frequent procedures include heel sticks for blood sampling, endotracheal suctioning during mechanical ventilation, and intravenous line insertion (Baker DP et al, 1995, Simons SH et al, 2003).

The current management of neonatal pain is not optimal in spite of an increased awareness among clinicians to reduce the burden of pain in ill newborn infants. Several aspects of pain management need to be improved, which includes treatment and prevention of continuous pain and stress, as well as intermittent procedural pain.Data from a French prospective multicentre study, The French EPIPAIN Study showed that 30,018 painful procedures and 30,951 mainly stressful procedures were recorded during the first 14 days after NICU-admission in 430 newborn infants. Analgesic treatment was given only during 27.4% of painful procedures (Carbajal R et al, 2008).

The experimental evidence for adverse neurodevelopmental outcome follow painful stimuli is strong. Human studies also show evidence of adverse long-term effects of repeated or prolonged pain in the newborn period, which includes cognitive outcomes, adverse behavioural and neuroendocrine responses and alterations in somatosensory perception (both hypo- and hypersensitivity). Pain may also lead to increased blood pressure and fluctuations in cerebral blood flow, which in the preterm infants can be deleterious with development of intracranial haemorrhages. It was recently shown that painful experiences are processed at a cortical level also in preterm infants, and that there are clear gender effects with increased responses in male infants (Bartocci et al, 2006, Slater et al, 2006).

During the last decade major efforts have been made to improve standards of care for pain management in sick newborn infants. Two large multicentres randomised clinical trials (RCTs) evaluated morphine versus placebo during mechanical ventilation, with slightly different doses of morphine. One of the studies was conducted in the Netherlands and included 150 infants born 2000 to 2002 (Simons et al, 2003). The other study included 898 very preterm neonates (gestational age <33 weeks) in 16 centres in Europe and USA during the time-period 1999 to 2001 (Anand et al, 2004). Pain and stress responses (plasma catecholamines) were reduced in infants receiving morphine. However, one of the studies showed a small, but significant, increase in intraventricular haemorrhages (IVH) in infants at 27-29 gestational weeks and in those who receive additional boluses of open-label morphine. A Finnish RCT compared effects from morphine and fentanyl during mechanical ventilation in 163 infants born 1994-1996. Both drugs were equally effective in reducing pain and stress responses, although fentanyl was associated with fewer gastrointestinal side effects (Saarenmaa et al, 1999).

Although treatment with morphine and fentanyl analgesia is associated with reduced pain and stress responses, the current knowledge about long-term safety from newborn treatment with these drugs, as compared to placebo, is limited. The problem is significant since around 100 000 infants are born very preterm in Europe, with increasing survival rate, a large number of infants are exposed to opioid or suboptimal pain relief.

The alleviation of pain is a basic and human right regardless of age – also newborn and preterm infants feel pain and require analgesics. We present a comprehensive study designed to investigate the clinical efficacy and safety effects of opioid therapy in newborns. The NeoOpioid project will advance the science of neonatal pain and stress, the clinical practice of analgesia, its pharmacokinetic determinants, while documenting current therapies and guidelines and developing a new European standard of care for pain relief in its most vulnerable population.

- To provide new standards of care regarding the use of opioids in neonatal intensive care.
- To evaluate acute effects of pain, and treatment with opioids, in relation to pharmacokinetics.
- To develop a pharmacokinetic (PK) model for morphine and fentanyl dosing in newborn infants that takes into account developmental stage.
- To compare safety and effects on pain and stress responses in relation to pharmacokinetic predisposition in a new cohort of newborn infants who will receive morphine or fentanyl, which will be administered as judged by pain scoring.
- To develop new drug formulations for safe administration of opioids to newborn infants.
- To implement and disseminate new knowledge on effects of neonatal pain and treatment with opioids in the newborn period.
- To submit and get approvals for two Paediatric Use Marketing Authorisation (PUMA) with application to the EMEA (European Medicines Agency).

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4.2 The main S&T results and foregrounds

The progress and results of each work package are hereby presented.

1. Management
2. Dose optimization of morphine and fentanyl in the newborn infants
3. Safety and clinical effects of morphine and fentanyl in ventilated newborn infants in relation to drug levels and pharmacokinetic profiles
4. New child friendly formulation and subsequent approval by the EMEA and European Commission via PUMA's submission
5. European survey of sedation and analgesia practices for ventilated newborn infants
6. Data collection with Dissemination, Implementation, Exploitation & Knowledge Transfer and Education.

Work Package 1 - Management

NeoOpiod was coordinated by Karolinska Institutet, where management is executed by the management office, which is a part of Department of Women's and Children's Health. The management office has provided the NeoOpioid consortium with a structure supporting management to all members.

- Organization of steering committee meetings, telephone conferences, and other project meetings and workshops in collaboration with other Work Packages
- Arrange meetings with External advisory board of international experts have been updated.
- Monitoring of progress by external advisor.
- GCP training including rules enforcement with compliance with regulatory and ethical guidelines and regulations within consortia agreement, including gender issues.
- Provide support throughout the progress of the work in each work package
- Keeping of Essential Documents
- Financial oversight to ensure proper financial management within the consortium and appropriate communication of related matters with the European Commission.
- Educational workshops providing evidence for safe and effective use of analgesic drugs in newborn infants (e.g. "pain management, diagnosis, monitoring and treatment")

The Steering committee has consisted of Principal Investigator from each beneficiary and is chaired by the Coordinator.

NeoOpioid Steering committee
Hugo Lagercrantz (coordinator), Lena Bergqvist (PI, WP 1 and WP 6), and Anders Rane (PI, WP 2), Karolinska Institutet, Sweden
Vineta Fellman (PI WP 3b), Lund University, Sweden
Ricardo Carbajal (PI, WP 5), Assistance Publique – Hopitaux de Paris, France
Bart van Overmeire (PI WP 3a), Université Libre de Bryxelles, Belgium

Mats Eriksson, Örebro University, Sweden
Ron van Schaik, Erasmus Universitaire Medisch Centrum, Netherlands
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Sture Andersson, Helsinki University Hospital, Finland
Gopi Menon, University of Edinburgh, UK
Michael Schroth, Friedrich-Alexander Universitaet Erlangen-Nuernberg, Germany
Elaine M Boyle, University of Leicester, UK

Work Package 2 - Dose optimization of morphine and fentanyl in newborn infants

Elementary information on enzymes and other pathways that eliminate drugs in our target study group is essential for establishment of a treatment strategy.

Hypotheses:

1. Morphine is mainly transformed by glucuronidation in the (preterm) infant and sulphate conjugation is also taking place but subsides with maturation. Fentanil is mainly oxidised by cytochrome P450 (CYP)3A4. These reactions are related to gestational and postnatal age. (Enzymatic activity of UGT2B7 can be characterized by the formation clearances, (morphine to M3G (CLf,M3G), and morphine to M6G (CLf,M6G)) and the enzymatic activity of CYP3A4 can be characterized by the formation clearance (fentanyl to norfentanyl (CLf, norfentanyl)).
2. The enzyme activity is related to various gene (allele) variants encoding for UGT2B7 and CYP3A4, respectively.
3. The elimination rate of metabolites of morphine and fentanyl newborn infants is dependent on the glomerular filtration rate, and therefore on the stage of development.
4. Clinical response to morphine and fentanyl in newborn infants is related to variation in the COMT, the μ-opioid receptor, beta-arrestin2, stat6, and other pain mechanism related genes.

Based on investigations of the above hypotheses, we propose to develop a PK/PD model for morphine and fentanyl dosing in newborn infants that takes into account both developmental stage and genetic variability. The long-term goal of the investigations is to optimise dosing of morphine and fentanyl in critically ill newborn infants, leading to improved pain control and decreased adverse drug reactions. More immediately, we will develop a PK/PD model for morphine and fentanyl dosing based on relevant developmental and genetic characteristics. Achievements of these goals are awaiting availability of patients, but this has been impeded by circumstances discussed elsewhere.

Main analytical achievements
Studies of drug disposition in neonates require highly sensitive and specific analytical methods that have been developed specifically for these projects. Below follow details of the work with specifications of methodological performance and pharmacological findings.

Analytical method for morphine
The validation of morphine (M) and the glucuronide M3G, M6G and sulphate metabolites M3S M6S has been finished. The procedure regarding the sample preparation has been revised to fit the instrumentation more preferably. Results for morphine metabolites are now available, and it is also satisfactory that the methods allow measurement of the active M6G (morphine-6-glucuronide) metabolite.

Method development
The first method used reversed phase chromatography. During the validation we discovered significant ion suppression for the polar morphine-3,6-sulfate and morphine-3-glucuronide analytes. There was a 40-80 % reduction of the signal for these analytes. A high sensitivity and robust method is needed to determine the plasma concentrations in neonates. Plasma samples frequently need to be reanalysed which often gives a problem due to the small sample volumes from the neonates. Therefore, we needed to the develop a new method that decreased the severe matrix effect.
The method was modified regarding chromatography, instrumentation and the sample preparation. We also developed a new analytical method to determine the phospholipid content to assess the matrix effect as well as a reference material to determine the effectiveness of the sample preparation for removal of the (phospho)lipids.
The table compares the two methods regarding retention of the polar analytes, sample preparation and the matrix effect.

	Former method	**New method**
Chromatography	Reversed Phase	Stragiht Phase
Instrumentation	Quatro Premiere UPLC-MS/MS	Xevo Tqd UPLC-MS/MS
Sample preparation	Proteine precipitation	-Proteine precipitation -Phospholipid removal plates
Analytes	-Morphine -Morphine-3,6-glucuronide -Morphine-3,6-sulfate	-Morphine -Morphine-3,6 glucuronide -Morphine-3,6-sulfate -Normorphine
Matrix effect	-Post column infusion -Standard addition experiment	-Post column infusion -Standard addition experiment -Analytical determination of lipids -Reference folch lipid extract

We concluded that the reversed phase chromatography with Xevo Tqd UPLC-MS/MS will be used together with the Phree phospholipid removal plate from Phenomenex for sample preparation.. This method is more robust and sensitive than the earlier developed method, especially for the critically low morphine-6-sulfate.

A linear calibration response was obtained between 5-5000 ng/ml in plasma with a correlation coefficient of >0.99 (n=6) for all analytes. Lowest limit of quantification (LLOQ) was set to 5 ng/ml with a total imprecision between 7-19 % (n=20) for all analytes. A summary of imprecision and accuracy data are shown in Table 1 calculated according to CLSI EP15-A2.

Table 1

Analyte	n	Conc. Added (ng/ml)	Accuracy (%)	Intra assay CV (%)	Total imprecision CV (%)
M3G	25	15	110	3.6	5.0
	25	200	97	1.5	2.1
	25	3000	99	1.2	1.2
M6G	25	15	111	3.3	4.9
	25	200	98	1.6	1.6
	25	3000	96	1.6	1.6
Morphine	25	15	109	4.9	4.9
	25	200	100	2.7	2.7
	25	3000	100	2.3	2.3
M3S	25	15	100	2.7	5.1
	25	200	84	1.3	6.8
	25	3000	99	2.0	3.6
M6S	25	15	104	2.3	3.6
	25	200	89	1.6	5.4
	25	3000	103	2.2	3.9

Calculations of the pharmacokinetics have begun and some plots have been made. Additionally a total of 66 patient samples from 22 patients from the Lund site have been analysed.

A total of 25 patients have been included. Twenty one patients of a total of 62 samples have been analysed and evaluated. Six patient samples were not analyzed due to not enough plasma volume after centrifugation. Four patients (twelve samples) were not analyzed due to misplace of samples (during relocation of the laboratory) Seventeen samples out of sixty two contained M3S. Interestingly, a low concentration of M6S was measurable in one plasma sample. This has not been demonstrated before. Thirty six samples were within the measuring range regarding M3G, thirty two samples for M6G and thirty five samples for morphine. In most cases M3S levels correlate positively with M6G, i.e. those with measurable M3S have high M6G levels. The patient results are summarized in Table 2.

Table 2

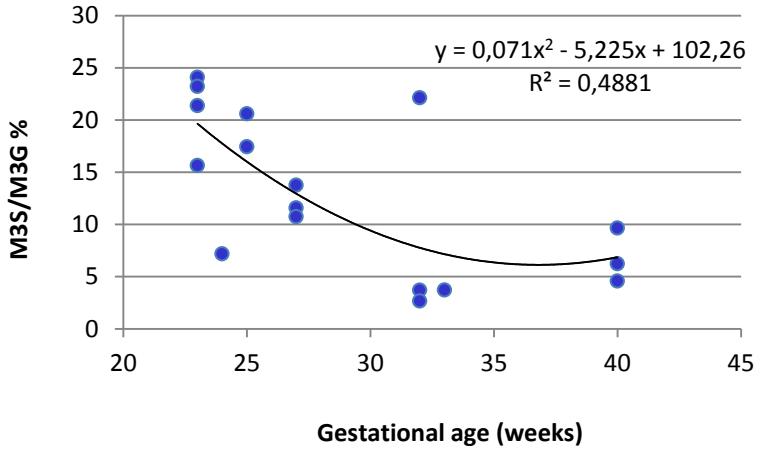
Study	M3S			M6S			M3G			M6G			M		
	n	ng/ml	median	n	ng/ml	n	ng/ml	median	n	ng/ml	median	n	ng/ml	median
patients plasma (n=62)	17	5-21	9	1	7	36	6-300	82	32	5-90	27	35	5-130	34

Calculations of the M3S/M3G ratio for all samples containing quantifiable concentrations of M3S are shown in Table 3. When plotting the M3S/M3G ratio versus gestational age for the newborn group, we found that the ratio decreased with age indicating a decreasing sulfation capacity, alternatively an increasing capacity to glucuronidate morphine (Figure 1).

Table 3

Study	M3S/M3G %			
	N	%	Average	median
Newborn patients plasma (n=62)	17	2.66-24.1	12.8	12.7

Fig 1



Sulfation of morphine is obviously catalysed early in ontogeny. We compared the gene expression of SULT2A1 in fetal and adult livers, to study the intra-individual tissue distribution, and investigated if expression is associated with a SULT2A1 copy number variation polymorphism. We used quantitative real time polymerase chain reaction. In contrast to other drug metabolizing enzyme systems the expression of SULT2A1 did *not* differ between fetal and adult liver samples and it was not affected by maternal smoking or gestational age. SULT2A1 was consistently expressed in livers and adrenals, being 7 times more abundant in adrenals, but was absent in the lungs. The SULT2A1 copy number variation was proportional to gene expression in liver and adrenals. Our results show that the ontogeny of SULT2A1 is different from other phase II metabolizing enzymes investigated so far and that SULT2A1 is important in the first trimester; particularly in the adrenals (Genetic Variation, Expression and Ontogeny of Sulfotransferase SULT2A1 in Humans, Ekström and Rane, submitted 2014).

Remifentanil kinetics in preterm infants

The synthetic opioid remifentanil was studied in preterm infants. The metabolism was about as rapid as in older infants. This is reassuring for infants with liver dysfunction or liver failure, a common situation in asphyxiated newborn infants.

Morphine metabolite profile in patients with treatment of intubation induced pain (University Hospital of Lund)

Pain treatment at intubation was studied in babies from a study in Lund. Out of 66 samples 49 contained measurable concentrations of M and M3G and 42 samples contained measurable concentrations of the analgesic metabolite M6G. Morphine-3-sulphate was analysed in 27 samples but detectable only in 21 samples. No samples contained any detectable M6S metabolite.

These findings are essentially consistent with the previous pilot study at Karolinska (see above).

Pharmacogenomic studies

Report on the interface between developmental and pharmacogenetic background in newborn infants

We have studied the relation between polymorphisms in a number of candidate genes in relation to the pain relieving effect of morphine and remifentanil over time (together with prof Ron v Schaik´s group. The study was performed in the above mentioned patients from Lund University Hospital and results are described below.

Genotype analyses were developed and validated for genes involved in pain sensitivity and in morphine and fentanyl metabolism, and included the PK genes MRP3 (-211C>T), UGT2B7 (-842G>A, -78G>A, 802C>T), PPARa and CYP3A4 (*1B, *1G, *20, *22), the PD genes ß-arrestin2 (C8622T), COMT (1947G>A), OPRM1 (118A>G), STAT6 (6080C>T), and the pain sensitivity genes GCH1 (4279C>G, -9610G>A), KCNJ6(-1250G>A, 1032A>G), SCN9A (R1160W), WDFY4 (A>C). Genes added to this, based on new insights and publications: OCT1 (*2, *3, *4, *5), IL1R1*2, ARBB2 8622C>T, POR*28 and METTL21A (rs2952768). In total, we now have 26 candidate SNPs in 17 genes involved in the opioid PK/PD pathway.

The effect of COMT, OPRM1 and ßarrestin-2, and KCNJ6 was investigated in a group of newborn infants enrolled in a randomized controlled trial. The combined OPRM1/COMT genotype was significantly associated with need for additional morphine boluses to achieve pain relief (OR 6.8; p=0.025) in neonates **(Matic et al 2014, in manuscript)**. Further we showed that the UGT2B7 -842G>A SNP was significantly associated with M3G/morphine ratio in the 34 preterm newborns **(Matic et al 2014, manuscript in preparation).** In addition, we found that the KCNJ6 and COMT polymorphism were correlated with opioid response in preterm infants (n=34) **(Elens 2014, to be submitted).**

Report on relevant SNPs to be investigated in genes involved in morphine and fentanyl PK and PD.

Two candidate genes were selected; the *KCNJ6* gene that encodes the G protein-activated inwardly rectifying potassium channel 2 (GIRK2) and the *COMT* (catechol O-methyl transferase) enzyme gene important for the metabolism of catecholamines in the interneuronal space.

A significant relation was found between the COMT (catechol O-methyl transferase) and the KCNJ6 genetic polymorphisms and analgesia mediated by the two opioids together, and of morphine alone.

Report on development and validation of pharmacogenetic analyses

For CYP3A4, a new intronic polymorphisms was recently described (R van Schaik et al; August 2011, intron 6 SNP), which was shown to affect CYP3A4 activity on midazolam, erythromycin,

tacrolimus, cyclosporine [1-10]). It may also be of importance for fentanyl metabolism. Assays for related genes affecting CYP3A4 activity, POR*28 and PPARalpha, were also developed and validated [11-13] but not yet related to the therapeutic outcome.

The start of the core study has been delayed because the study plan had to be amended a couple of times for various reasons. We aim to find a pharmacokinetic model for morphine and fentanyl dosing in newborn infants at different stages of development. This will probably be the first study of the pharmacokinetics of these drugs in extremely preterm infants (from the 23rd gestational week).

Work Package 3 - Safety and clinical effects of morphine and fentanyl

Objectives

1) To assess safety of opioid therapy in newborn infants requiring mechanical ventilation (short term morbidity variables).
2) To describe the pharmacodynamics of opioid therapy by assessment of physiologic stress responses, hemodynamics in relation to validated pain scores (PIPP, EDIN), and plasma levels of morphine or fentanyl with metabolites.
3) To investigate whether specific pharmacokinetic profiles (particularly the following candidate genes: the μ1-opioid receptor, UGT2B7, catechol-*O*-methyltransferase (COMT), β-arrestin2 and stat6 genes) explains the pharmacokinetic and/or pharmacodynamic phenotypes of opioid analgesia in newborn infants, or their responsiveness to pain
4)

The NeoOpioid original application submitted to European Commission (EC) 2007 included a complete organisation to follow up infants and children due to a paediatric pain perspective. Negotiation phase with EC resulted in exclusion of two third of i.e. the follow up work. As part of the applied call approved Paediatric Investigational Plan (PIP) from the European Medicines Agency's Paediatric Committee (EMA/PDCO) was requested. Never less these follow up data were requested from experts at PDCO as an obligation. The process has delayed the progress of the NeoOpioid project. This issue is partly solved with mathematical calculations.

EMA/PDCO first requested a randomized controlled trial, comparing the two drugs morphine and fentanyl. Solved via negotiation and discussions resulted in separated PIP applications that took some extra time.

The start of the clinical trials was delayed due to disagreement between the European Commission and European Medicine's Agency´s (EMAs) Paediatric Committee (PDCO) regarding the study protocol. Instead of one clinical trial including patients both on Morphine and Fentanyl the PDCO requested a randomised clinical trial comparing morphine and fentanyl, which the consortium considered not possible to conduct for various reasons, mainly because they do not have exactly the same indications. The alternative from PDCO/EMA was that separate two separate Paediatric Investigational Plans, one for morphine and one for fentanyl were submitted and that separate clinical trials for morphine and fentanyl were conducted. This however, caused a significantly increased work load as EC had granted money for only one Paediatric Investigational plan and one clinical trial, with the use of morphine and fentanyl in the same trial (but not a randomized trial).

Paediatric Investigational Plans

Finally two Paediatric Investigational Plans (PIPs) were approved by the EMA/PDCO.

A PIP for a child friendly fentanyl formulation - NeoFentanyl for procedural pain, was developed in collaboration with work package 4 and work package 1. The PIP was approved 08 October 2010 by the EMA/PDCO.
A Paediatric Investigational Plan for a child friendly formulation for the use of Morphine for continuous pain was approved by the EMA in December 2011.

The proposed morphine and fentanyl doses in the clinical trials are on purpose smaller than those currently used in clinical practise and are in line with the recent suggestions to use small doses of opioids. The dose will be titrated based on individualised needs.

Safety and clinical effects of Morphine

Method development study

A pilot study has been performed at Karolinska University Hospital, Stockholm in collaboration with WP 2. Morphine plasma samples were collected from 25 newborn infants. In addition to the findings described in the WP2 section, one of the aims of this pilot study was to evaluate the procedure for collecting, handling, storage and shipment of the pharmackokinetic samples, as this is an important part of the clinical trials of both fentanyl and morphine. Another aim was to determine the minimum amount of blood needed per sample.

NeoMorph Clinical study

Since morphine has been substituted by fentanyl or remifentanil at many NICU:s in the EU we have first focused on starting the fentanyl study. We have however finalised a study protocol for the Morphine clinical trial in accordance with the PIP approved by EMA/PDCO, and the study will be performed in Belgium and France.

NeoMorph Clinical Trial Protocol Summary

Title: Morphine treatment in newborn infants: A Pharmacokinetic, Pharmacodynamic, and Pharmacogenetic Study (PK/PD model)

Trial Objectives: To assess the morphine total body clearance (dose related to concentration AUC) and to derive an algorithm for morphine administration in preterm and term infants with moderate to severe prolonged pain. To determine other pharmacokinetic and pharmacodynamic parameters of morphine in preterm and term newborn infants (e.g. volume of distribution, serum half life) and safety outcomesTo determine the influence of the pharmacogenetic profile on pharmacokinetics and on analgesic effects. To test efficacy of morphine using validated pain assessment methods for titration to the desired clinical effect – i.e. pain relief.

Study drug: Morphine hydrochloride, solution for infusion. Depending on local routines, morphine administration will then be started and titrated according to the 2 possible dosing schemes: either the intermittent bolus or the continuous scheme.

Trial Design: Open, Prospective interventional trial

Inclusion Criteria

Male and female patients meeting all criteria listed below can be included in the study:

1. Infants born at a gestational age of at least 23 weeks up to a post-conceptional age of 46 weeks.
2. At least 12 hours of age
3. Clinical indication for analgesia in case of moderate to severe prolonged pain such as:
   a. post-operative pain
   b. necrotizing enterocolitis
   c. inflammatory pain
   d. severe birth injuries
   e. burns or major invasive procedures
4. Admitted to the Neonatal intensive care unit (NICU) or Paediatric Intensive Care units (PICU)
5. Informed written parental consent

Exclusion Criteria :

Patients will not be included in the study if any of the following criteria applies:

1. Concurrent or previous other opioid administration:
2. Major chromosomal anomaly, e.g. trisomy
3. Neonatal encephalopathy (e.g. seizures, lethargy/coma)
4. Hypothermia treatment for a neonatal asphyxia
5. Clinical or biochemical evidence of hepatic failure (e.g. cholestasis, hepatic coagulopathy, hypoalbuminaemia) or renal failure (serum creatinine > 132 micromol/L corresponding to >1.5 mg/dL
6. Use of muscle relaxants during the last 4-8 hours
7. Participation in other clinical intervention trial (including blood sampling procedures other than clinical routine, and administration of other investigational medicinal product) within 24 hours before study start and throughout the study
8.

Efficacy Parameters: Pain scores EDIN and Comfort B

Safety Parameters: Occurrence of hypotension chest wall rigidity convulsion, hypoventilation, apnea, gastrointestinal motility dysfunction

Number of Patients: 200

Morphine dosing schedule:

Depending on local routines of participating units, one of two options for morphine dosing is possible; intermittent bolus or continuous infusion.

Dosing schedule when intermittent boluses are used

	Intermittent bolus dose (µg/kg over 60 minutes) as required by pain score

Postconceptional age	First bolus	Second bolus (if pain is still present after the first bolus)	Third bolus (if pain is still present after the second bolus)
23-27 weeks	5	7.5	15
28-31 weeks	10	15	30
32-36 weeks	15	25	50
37-42 weeks	20	30	60
43-46 weeks	25	35	75

The patient will receive a first bolus. **Within 15 minutes** after the completion of the infusion of the bolus, pain will be assessed:

1. If **no pain**, no additional bolus will be given and additional pain scores will be performed every 3 hours. If one of the subsequent scores indicates that the infant has pain, a re-administration of another bolus with the same dose as the initial one will be infused.

2. If **pain is present** after the first bolus, a second bolus (with approximately 50% increase of the dose of the first bolus), will be administered, followed again by a pain score. If still pain after this second bolus a third bolus will be administered (with approximately 100% increase of the dose of the 2nd bolus), followed again by a pain score. Subsequently, scoring will be repeated every 3 hours and if one of these scores indicates pain, another bolus with the same dose of the preceding bolus will be administered.

3. If the baby has sufficient analgesia after the initial bolus, no systematic increase of doses will be applied.

If the patient has still a high score indicating pain after the administration of the maximal proposed dose of morphine, the clinician should decide to add additional analgesic drugs and directly address the underlying cause of the increased pain (see rescue treatment).

Dosing schedule when continuous infusion are used

Continuous administration is less preferable according to our previous studies. However, if there are special indications the following dosing schedule has been proposed:

Patients will start with a continuous infusion of 5 µg/kg/h. A pain score will be performed every 3 hours. Based on the results of the pain scores, the continuous infusion dose might be adjusted, up to a maximum dose of 10 µg/kg/h (23- 27 weeks), 15 µg/kg/h (28-31weeks), 20 µg/kg/h (32-36 weeks), or 30 µg/kg/h (37-46 weeks).

Continuous infusion rates should be adjusted according to following steps:

a stepwise increase of 2.5 µg/kg/h for the 23-27 weeks' infants, increments of 5 µg/kg/h for the 28-31 and 32-36 weeks' infants, and increments of 10 µg/kg/h for the 37-42 and 43-46 weeks' infants.

Blood sampling

Blood samples of **0.8 ml blood** will be taken from indwelling arterial/venous catheters.

The sampling will be performed at three possible time windows:

The first sample is optional and will be taken between t= 0.25h – 1.10 h (15 – 66 minutes) after start of study drug administration

The second sample between t= 3 – 5 h after start of study drug administration

The third sample between t= 15 – 24 h after start of study drug administration.

For this study the intervention observation will end 24 hours after the last sampling moment. Follow-up of the patient will be according the predefined follow-up protocol.

A **baseline blood sample** will only be taken from the first 5-10 infants that weigh more than 600 g. If zero, no more baseline samples will be taken.

In infants **600-1000 g** no more than **three** samples should be taken in 24 h.

In infants <600 g no more than two samples should be taken in 24 h.

Safety and Clinical Effects of Fentanyl

A Paediatric Investigational Plan (PIP) for the child friendly fentanyl formulation - NeoFentanyl was developed. The PIP was approved 08 October 2010 by the European Medicines Agency's Paediatric Committee (PDCO).

A clinical study protocol was developed based on the requirements requested by PDCO in the approved PIP.

Within the consortium, very few centers used fentanyl since 2011 and it was also difficult to recruit study centers outside the consortium that had the possibility to participate in the clinical trial. Therefore the investigational drug NeoFentanyl 5 mikrog/ml is studied only in Sweden and Finland.

The study protocol was finalised in Sweden and approved by the Ethics Committee in Sweden 2011 06 28 and the Medical Products Agency 2011 09 08 (EudraCT 2011000310-19). Due to administrative issues and unclear responsibilities within the organisation concerning insurance for the study patients, the final approval in Finland from authorities was delayed with one year (Research Ethics approval 2012 03 19).

NeoFent Clinical Trial Protocol Summary

Title: NeoFent-I study - Fentanyl treatment in newborn infants: A Pharmacokinetic, Pharmaco-dynamic, and Pharmacogenetic Study (PK/PD model)

Trial Objectives: The study will assess the efficacy of fentanyl using a PK/PD model where log concentration of the drug is related to effect curve (pain score change in response to standardised procedural pain). Further, cortical, physiological, biochemical responses to fentanyl will be assessed, elucidating the feasibility before the main study project (NeoOpioid)

Test Product: Fentanyl, Solution for injection and infusion

Trial Design: open, interventional study

Dose/Duration: BOLUS: 0.5 µg/kg (0.1 ml/kg of a solution with fentanyl 5 µg/ml) over 1 min starting 3-5 minutes before the procedure. INFUSION: loading 2 µg/kg (0.4 ml/kg of a solution with fentanyl 5 µg/ml over 10 min) before the start of the procedure. MAINTENANCE: according to clinical decision: 0.5 µg/kg/h.
24 hours follow-up of dose given

Primary Endpoint: PK/PD based algorithm for fentanyl dosing based on fentanyl clearance.

Secondary endpoint: Ten percent reduction in cerebral hemodynamic response to pain and pain score

Number of Subjects: 65 for interim analyses of NeoFent PK/PD model, and **total number 180** in this study

Inclusion criteria:

1. Clinical indication (or pain scoring, see 7.2.1: PIPP $\geq$ 7, or BIIP $\geq$ 3, or EDIN $\geq$ 6) for analgesia before any of following procedure:
    a. Insertion of peripheral IV-catheter
    b. Insertion of arterial cannula
    c. Other skin breaking procedure
    d. Insertion of chest tube

Other painful/stressful procedure might be considered if indicated by pain scoring used, eg. endotracheal suction.

2. Possibility to obtain blood sample after the procedure (indwelling line or the procedure is insertion of line as clinically indicated)
3. Infants of all gestational ages (preferably preterm born at a gestational age of 24 0/7 to 32 6/7 weeks and term infants at 38-41 gestational weeks (gw) for the first 65 in the PK/PD model)
4. Postnatal age 0-28 days, ie up to an age corresponding to 44 post conceptional weeks.
5. Informed written parental consent.

Exclusion criteria:

1. Concurrent or previous other opioid administration (72 h interval required)
2. Abdominal surgery (since intra-abdominal pressure influences fentanyl metabolism)
3. Major chromosomal anomaly, e.g. trisomy
4. Neonatal encephalopathy (e.g. seizures, lethargy/coma)
5. Use of muscular relaxant
6. Hypothermia treatment after hypoxic-ischemic insult

7. Clinical or biochemical evidence of hepatic failure (e.g. cholestasis, hepatic coagulopathy, hypoalbuminaemia) or renal failure (serum creatinine > 132 micro mol/L corresponding to >1.5 mg/dL[42])
8. Participation in other clinical intervention trial (including blood sampling procedures other than clinical routine, and administration of other investigational medicinal product) within 72 hours before study start and throughout the study

- The fentanyl dose :
  - BOLUS dose: 0.5 µg/kg (0.1 ml/kg of a solution with fentanyl 5 µg/ml) over 1 min 10 minutes before the estimated peak pain of the procedure. The dose is on purpose smaller than those used for premedication of intubation intubation[16] and in the fentanyl morphine trial intubation[9] in line with the present suggestions to use small doses of opioids based on individualised needs.[4] This dose effect will be assessed in the model after recruiting 65 infants, and accordingly changed, if needed, for the following cases. If too small, an additional similar dose can be given (see below point 6).
  - LOADING dose: 2 µg/kg (0.4 ml/kg of a solution with fentanyl 5 µg/ml over 10 min) before the start of the procedure (insertion of the chest tube, additional local infiltration anaesthesia according to routine). The loading dose of 2 µg/kg is the same as recently was used in a premedication RCT for intubation.[16]
  - MAINTENANCE INFUSION, if clinically decided: 0.5 µg/kg/h (0.1 ml/kg/h of a solution with fentanyl 5 µg/ml).

1. A blood sample will be obtained immediately after the procedure (10 min after administration, however NOT later than 30 min) with concomitant pain scoring, see figure 7, 8, and 10.
2. If pain scoring indicates that the patient is in need of additional pain relief, an additional dose of 0.5 µg/kg fentanyl can be given 10 minutes (or later) after the first dose, see figure 9 and 11. In this case if the weight is <1000 g, the blood sampling time will be adjusted
   - an additional blood sample and pain scoring will be performed at baseline before the second dose, 10 minutes and 2 h after the administration. Five-six blood samples can thus be obtained in infants>1000g during the first 24 h, see figure 11.
   - In infants 600-1000 g only three samples should be obtained in 24 h, see figure 9.
   - In infants <600 g only 10 min and 2 h samples are obtained during 24 h.
3. If the infant needs additional procedures (any of 7.4 1.a-d) the protocol (points 3-5) is repeated but in addition a blood sample is obtained before the procedure at the same time as pain scoring. The number of investigational procedures for each infant is decided depending on volume blood sampled that must not exceed 5% of total blood volume (amount per kg body weight will be indicated in the CRF).

Pain assessment will be performed regularly and frequently during the study period. **Continuous pain** will assessed (1-)4-8-hourly with the EDIN score, and **procedural pain** will be assessed with the PIPP and BIIP in conjunction to **PK blood sampling** at 0, 10 min 2 h, 4h 8 h and 24 hours, respectively. The procedural pain to be studied is preferably a skin breaking procedure, however endotracheal (ET) suctioning procedure is accepted, since this has been used in several other studies.

If the baby is not on mechanical ventilation or in need of ET suctioning, other specified procedures will be evaluated (see above).

Good Clinical Practice
The study is monitored to ensure that it is conducted in accordance with Good Clinical Practice (GCP), the study protocol and European and local regulations. The initiation visit by the monitor was performed in Lund on 25 January 2012, in Stockholm Karolinska Hospital, Solna and Huddinge on 26 November 2012, and on 24 October 2012 in Helsinki, Finland. Patient recruitment is still ongoing.

Pain Scoring
The goal was to use pain scales in a standardized way and thus we produced verified translations of the scales proposed to use: BIIP, PIPP, and EDIN. EDIN was previously used by us in a RCT, for which purpose it was translated from French to Swedish and back. This translated scale turned out to be useful in the clinical setting in Lund, Sweden (Norman et al J Pediatr 2011;159:893). Thus we could foresee that it could be used in the NeoFentanyl trial. In addition, a Swedish pain scale (ALPS-Neo) was designed in parallel.
Teaching and training of personnel in all sites were performed to secure that the pain scoring will be used in a structured similar way. In all sites, the Lund group and the local personnel (n=6-10) performed simultaneous scorings from video recordings. The interrater reliability was assessed and considered good. In addition a Swedish score was used and validated (see publ Lundqvist et al).

The experience obtained from the scales, including a proposal for an algorithm how to use pain scales and provide pain relief, was written in Swedish for a Finnish medical journal to be available for both countries.

Pharmacokinetic and Pharmacogenetic Sampling
The samples are collected, handled, stored and analysed according to the methods developed in the NeoOpioid pilot study conducted at Karolinska. All samples are sent to the Karolinska Clinical Pharmacology. The Fentanyl Pharmacokinetics are analysed at Karolinska Institutet, Sweden, and from the samples, cells are extracted and sent to Erasmus Universitaire in the Netherlands for pharmacogenetic analyses.

Database
The Lund group developed together with the Karolinska Clinical Research Center, Karolinska University Hospital an electronic Case Report Form (eCRF), PhEdit for electronic data capture.

Patient recruitment
Patients are recruited in Lund, Stockholm and Helsinki. This part of the project is still in progress

Preliminary Report on clinical pain responses
Our initial findings in a RCT comparing two strategies with different opioids – morphine and remifentanil (Norman E…. Fellman V. Rapid sequence induction is superior to morphine for intubation of preterm infants – a randomized controlled trial. *J Pediatr* 2011;59:893-9) showed that significant changes in both heart rate and blood pressure occurred in response to the pain of the intubation procedure. However, fortunately the premedication and handling ameliorated the pain response to such an extent that no changes were found in regional cerebral oxygenation. By use of increase in scores of ALPS-Neo, EDIN, and PIPP scales as indicator of pain, we identified pain in

one third of the patients after the intubation. We used these increases as an indication for administration of additional opioids (morphine) that resulted in decreases of the scores. Thus, we considered a pain scale for assessment of procedural pain in combination with another pain scale measuring continuous stress/pain to be needed for the clinical judgment whether the infant has pain or not. For the NeoFentanyl study, we have chosen PIPP-r and BIIP to assess procedural pain and EDIN for continuous pain. In addition, since ALPS-Neo turned out to be easy to use we included it as well in the Swedish centers.

The assessment of pain by use of the scales might be difficult and needs training. Therefore, we produced a set of videotapes of preterm infants in normal NICU-setting when they were pain-free or in mild pain/severe pain. This set was used in all centers participating in the trial for teaching. Doctors and nurses of the two NICUs in Karolinska Hospital, Stockholm, as well as in Helsinki University Central Hospital (HUCS) scored together with the Lund research group all videos and interrater variability was assessed. Thus we could standardize pain assessment for the NeoFentanyl study.

ALPS-Neo has been thoroughly investigated in a large group of newborn infants and proven to be easily implemented with a high interrater reliability in the NICU both for preterm and sick term newborn infants (Lundqvist P…Norman E. Manuscript submitted to *Acta Paediatr*).

Report on pain and stress responses in relation to pharmacokinetics

We have previously investigated whether a pain response can be detected in EEG in response to painful skin breaking procedures and found that EEG does not reliably show a robust pain reaction (Norman E et al *Pediatr Res* 2008;64:429-34). However, in the pilot RCT intubation study, we found that a prolonged cerebral depression was present during 24 hours after intubation when morphine had been used. Thus EEG can be used for assessing opioid effects. Therefore, we will investigate EEG in the NeoFentanyl study. We have added aEEG monitoring to the protocol to be able to assess whether the investigational drug has a similar cerebral effect as found when using morphine (Norman et al J Pediatr 2011;159:893 and Norman E…Fellman V, Hellström-Westas L. Premedication for intubation with morphine causes prolonged depression of electrocortical background activity in preterm infants. *Pediatr Res* 2013;73:87-94).

A traditional biomarker of stress/pain is the cortisol level, which can be measured from blood, saliva or urine. In collaboration with the HUCS laboratory, we used a sensitive cortisol LC-MS/MS method to determine cortisol concentrations in plasma before, and after intubation. We found a high variation in the baseline levels and after the intubation both increases as well as decreases in the concentrations (Norman et al J Pediatr 2011;159:893). This study revealed clearly that there is an ongoing stress in the sick preterm infants in need of intubation, ie in need of opioid administration. Thus, we consider cortisol measurements not feasible during the NICU treatment when the infants are undergoing repeated procedures. Therefore, we have not included this measure in the NeoFentanyl study to restrict amount of blood volume obtained for the study. We only use plasma for fentanyl PK and the cells for PG.

In a review, we have summarized our experience on how to administer opioids and assess effects. (Norman E, Fellman V. Premedication for semi-urgent intubations in newborn infants – new evidence support short-acting analgosedatives. *Cambridge Research Center, Treatment Strategies Paediatrics* 2012; 2: 68-72.) We have obtained the conclusions from the pilot intubation study and the ongoing NeoOpioid Fentanyl study.

Considerable change in neonatal intensive care practice
During the project period a considerable change in neonatal intensive care has occurred. The new updated European consensus guidelines (initially published 2010) were recently published (Sweet et al Neonatology 2013;103:353) emphasizing minimal handling of the preterm newly **born** infant. Thus the majority of the pretem infants are nowadays receiving ventilatory support with CPAP and if in need of surfactant that is instilled often without premedication through a catheter or endotracheal tube which are withdrawn as soon as possible. This has led to a very limited use of opioids in NICU care nowadays, explaining why the recruitment rate is slow. These principles of minimal handling had already been taken into use in the partner NICUS of this consortium since about 2010. Thus, during this reporting period very few newborn infants needed opioids.

Work Package 4 – New Child friendly formulation

Main objectives of WP 4

1. To get agreement of the European Medicines Agency's Paediatric Committee (PDCO) on the fentanyl and morphine Paediatric Investigation Plan (PIP).
2. To prepare and distribute investigational medicinal products to clinical sites :
   - Manufacturing of injectable solutions of Fentanyl (5 µg/ml) and Morphine (50µg/ml) in compliance with Good Manufacturing Practices (GMP) applicable to samples for clinical trials
   - Quality Control and release for shipment
   - Shipment of samples in compliance with applicable regulations for narcotics
   - Preparation of the IMPDs (Investigational Medicinal Product Dossiers) needed to obtain regulatory clearance to start clinical trials
3. Development of "child friendly formulations" of Fentanyl and Morphine for commercial purposes in order to get the information necessary to support submissions of Paediatric Use Marketing Authorisations :
   - Pharmaceutical development and validation

Manufacturing validation

- Stability studies
- Preparation of pharmaceutical reports for regulatory submissions
- Ensure the submission of PUMA's application for new child friendly formulation of Fentanyl and Morphine.

Morphine
A Paediatric Investigational Plan (PIP) was submitted to the PDCO on 19 October 2009, the review started on 19 November 2009. A request for modification of our proposed PIP was received on in January 2010. Given the significant changes requested for our morphine PIP, including significant protocol amendments, not in line with the grant agreement, our response document was only

submitted to the PDCO on 30 August 2011. Our proposed modifications to the PIP were approved by the PDCO on 9 december 2011.

Fentanyl

A Paediatric Investigational Plan (PIP) for the child friendly fentanyl formulation - NeoFentanyl was approved 08 October 2010 by the European Medicines Agency's Paediatric Committee (PDCO). The clinical study for fentanyl was initiated, and clinical samples of fentanyl were shipped to the clinical sites in Finland and in Sweden.

We had to manufacture samples for Finland and Sweden, therefore the labelling was different for each country, and we had to define in advance the number of samples for each country.

The original Shelf life applied to the clinical trial samples of Fentanyl 5 mcg/ml solution for injection was 12 months based on literature data. A protocol for our stability studies was finalized in November 2011, and the first two batches of fentanyl solution for injection 2 and 10ml glass ampoule manufactured in December 2012, were put in stability on 12 December 2011.

The clinical trial samples were then controlled, released (according to the pre-defined released specifications) and shipped to the clinical sites in Sweden (the CTA being approved by the Swedish Health Authorities and Ethics committee). Two Clinical trials amendments were submitted to extend the Shelf life of our investigation medicinal product a first time from 12 to 24 months and a second time to 24 to 36 months.

"Child friendly formulations" of Fentanyl

Further to the approval of the fentanyl PIP in October 2010, the strategy had to be modified, as the PDCO did not agree to have clinical studies conducted with Fentanyl diluted commercially available product, but instead wanted to have the study performed with final formulation designed for neonates. We therefore develop the formulation before initiating the first clinical study. We develop a solution for injection of fentanyl citrate 5mcg/ml in 2ml and 10 ml glass sealed ampoule and we chose APL as contract manufacturer. We provided them with the formula, manufacturing process, quality controls, release and stability specifications for our fentanyl child friendly presentation. The formulation used for clinical trials is also the final commercial formulation, that is the reason why corresponding two first batches were included in our 36 months real and accelerated-time stability studies conducted following ICH guidelines.

We have collected real time stability data on 2 batches, showing that the formulation, as anticipated, is stable over time.

In order to have a pharmaceutical dossier completed, we would need to manufacture additional batches. These batches would be clinical trial samples for the 2nd efficacy and safety clinical trial requested by the PDCO, in the approved PIP. However, the recruitment of neonates being very slow, our clinical program is taken more time than initially anticipated, and is the reason why the quality development has been also delayed.

We could only submit a PUMA when all clinical trials described in the PIP will be completed, and that is the reason why we are not yet in a position to submit any regulatory application.

We also selected insurance for fentanyl clinical study and the subscription of this insurance was coordinated for all clinical sites. We also worked on the final practical aspect of the Fentanyl and Morphine clinical study protocols to ensure compliance with the approved PIP.

Work package 5: Europain survey

EUROPAIN SURVEY (Hypothesis, objectives, Methodology)

European Pain Audit in Neonates (EUROPAIN) European survey of sedation and analgesia practices for ventilated newborn infants)

The EUROPAIN study was an epidemiological study,based on the following hypothesis:

- Most newborn ventilated infants receive continuous sedation and analgesia.
- Non ventilated babies are not sedated.
- Morphine, fentanyl and midazolam are the most frequently drugs used in this setting.
- Infrequent use of validated pain assessment tools to monitor sedation and analgesia occurs in ventilated newborn infants.
- Most units have developed written local guidelines for sedation and analgesia in ventilated neonates, but huge variability exists among practitioners in the same unit, across different units in the same country, and across different countries in Europe.
- Development, dissemination, and regular updates of common European standards will improve the care and clinical outcomes of ventilated newborn infants.

Main objectives

- To determine the current clinical practices regarding the use of sedative and analgesic drugs for ventilated newborns in different countries in Europe.

Principal criteria

- The frequency of ventilated neonates receiving sedation and analgesia in different European units
- The medications used for sedation and analgesia in ventilated neonates across Europe.
- The length of use of medications administered for sedation and analgesia in ventilated neonates
- Similarities and differences in sedation and analgesia practices among European countries

Secondary objectives and criteria

- To determine the proportion of neonatal units that have developed and implemented local written guidelines to provide continuous sedation and analgesia in ventilated newborn infants as well as to prevent and treat procedural pain.
- To document the published guidelines for neonatal analgesia and sedation in different European countries and develop consensus for common European standards that can be applied in all medical settings.
- To determine the frequency of use of pain assessment tools in ventilated newborn infants and evaluate their impact on pain management practices.
- To determine practices to assess and prevent withdrawal syndromes.

Secondary criteria

- Variations across European countries of the proportions of units that have developed and implemented local written guidelines for sedation and analgesia in ventilated neonates
- Identification and description of national guidelines for sedation and analgesia in neonates in all participating countries. Identification of recommended drugs.

Type of study

- Epidemiological observational study.

Study plan

The EUROPAIN STUDY was observational and therefore it did not interfere with routine practices of participating units. No changes in diagnostic, therapeutic or any managing strategy of patients were imposed by the participation in this study. This epidemiological study will only collect data on clinical practices in each unit.

The inclusion criteria were:

- All neonates up to a corrected age of 44 weeks post conception. That means, for example, that a baby of 40 weeks gestational age could be included up to 28 days (4 weeks) of post natal age or that a baby of 32 weeks gestational age can be included up to 12 weeks of post natal age.

Actions at the unit level

- No modifications of current managing protocols or strategies were required by the participation in the EUROPAIN STUDY. The unit coordinators only provided data on local protocols to manage procedural pain and sedation and analgesia in neonates as well as on general statistics of the unit. All treatments were authorized for included neonates since this study did not include any intervention
- A nurse and physician coordinator as well as a data quality manager were designated for each unit.

Actions at the national level

- The country coordinator provided data on national guidelines to treat or prevent procedural or continuous pain in neonates.

Data collection

- The duration of data collection for every included infant was 28 days. However, data collection was stopped before 28 days if the infant leaves the unit (discharge, death, or transfer to another hospital).
- Data were collected on individual data collection forms. These forms included demographic data, modes of respiration, continuous or intermittent sedative, analgesic or neuro-blocking drugs, pain assessment and drug withdrawal practices.
- Paper patient data collection forms were written in English with a subtitled translation in the country language. The web-based databases displayed questionnaires in the country language.
- The data collection forms were be completed by the unit nurse or physician coordinator or another person that they have designated.
- For each center, the duration of the inclusion period was one month.
- Data was entered on a secure web-based questionnaire.

Justification of number of patients

Regarding the number of neonates to include, in order to show possible differences in sedation and analgesia practices among the participating European countries, we considered a scenario where differences were small. Thus, we chose an effect size (W) of 0.1. We also assumed that 15 countries would participate. Using NCSS-PASS 2008 software, we found that a total sample size of 2303 neonates would achieve 90% power to detect an effect size (W) of 0.1 using a 14 degrees (15 centers) of freedom Chi-Square test with a significance level (alpha) of 0.05. Therefore, we aimed at including an average of 154 neonates per participating country.

Main Actions carried out during the study

- In each country, the National Principal Investigators (NPI) sent invitations to join the study to all level three neonatal units of the country. The NPI then communicated the names, emails and telephone numbers of the units that accept to participate, to the EUROPAIN STUDY principal investigators
- The NPI was responsible for the coordination of all units in the country and to ensure communication with the EUROPAIN STUDY principal investigators
- The NPI collected demographic data about the participating country.

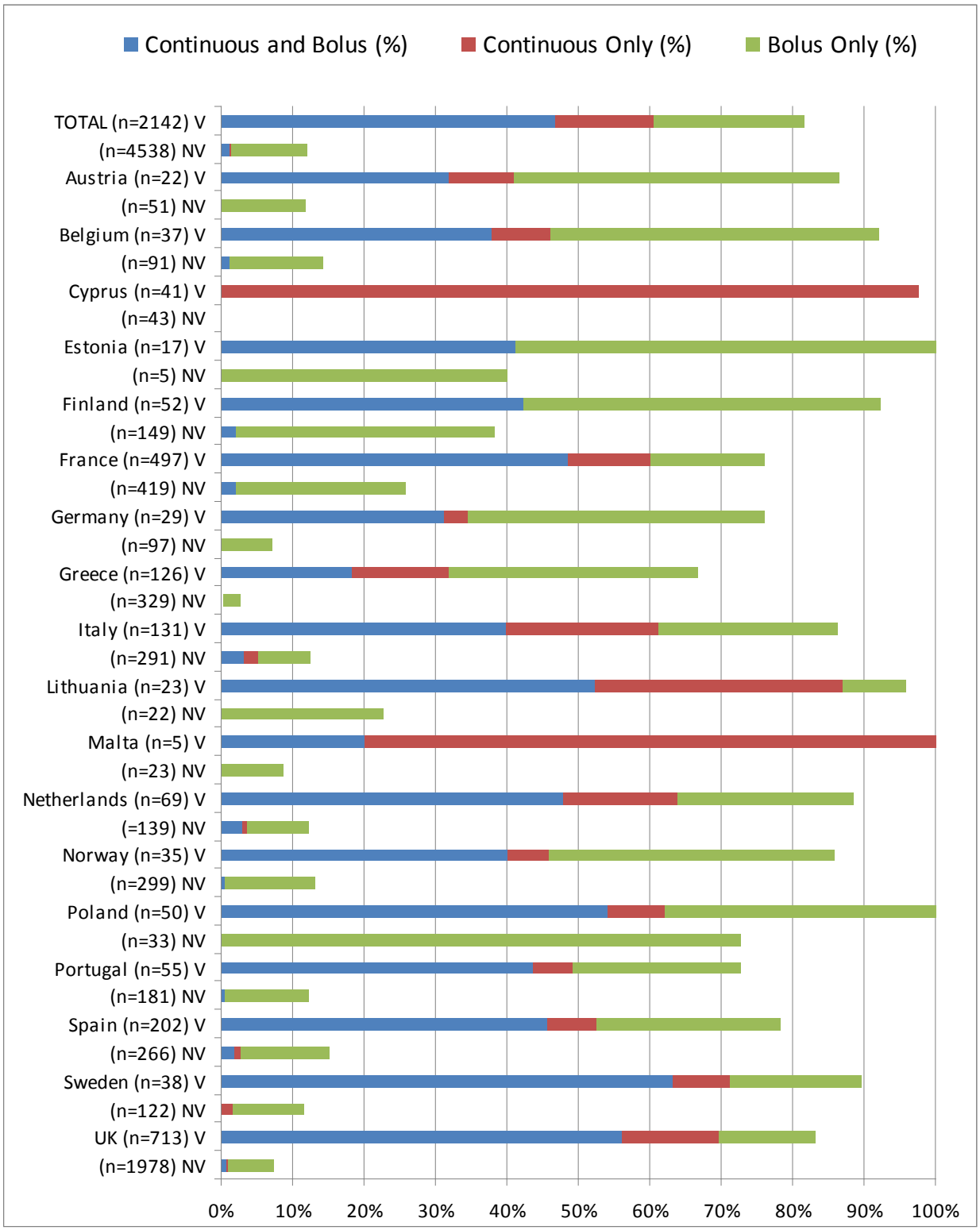
- A nurse and physician coordinator, as well as a data quality manager were designated for each unit. The nurse and physician coordinator were responsible for informing all the unit staff about the study.
- The EUROPAIN STUDY principal investigators prepared a specifically designed web-based database for data entering. Data could be entered directly from the patient's file. A paper copy of the database was distributed, to allow centers that preferred, to perform a preliminary entry on paper forms before entering data on the web-based database. The medical coordinator or a person that he (she) will choose will enter the data into these specifically designed databases.
- The physician coordinator reported general statistics of the unit such as number of beds, number of admission, year ventilator-days etc to the Europain study principal investigators.
- Every unit will also report existing local guidelines on sedation and analgesia in ventilated infants, including routines for withdrawal and on the use of pain assessment tools.
- **MONITORING PANEL**. A monitoring panel was created to monitor the progress of the study. This panel ensured communication with all the participating units. The monitoring panel was constituted by two persons working full-time during the study period. They were stationed in Paris and working under the responsibility of EUROPAIN STUDY principal investigators.

Results

Pain and stress induced by mechanical ventilation, invasive procedures, or painful diseases supports the use of sedation/analgesia (S/A) in newborns admitted to Neonatal Intensive Care Units (NICUs). To date, these practices have not been studied at a large scale.
The main objective of the Europain survey was to determine current clinical practices regarding the use of S/A drugs in NICUs across Europe.
This was an epidemiological observational study on clinical practices with data collected at the bedside. Data was collected for all neonates in participating NICUs from admission until the infant left the unit (discharge, death, transfer) or for up to 28 days. Data collection occurred via an online database for one month at each NICU. All neonates up to 44 weeks gestation were included.
We summarize hereafter two of the main results of this study regarding the use of S/A as well as the frequency of pain assessment scales.

1) Use of Sedation and analgesia

From October 2012 to June 2013, 243 NICUs from 18 European countries collected data on 6680 eligible neonates. Of these, 2142 received tracheal ventilation (ventilated) and 4538 had spontaneous breathing or non-invasive ventilation (non-ventilated). The median (IQR) gestational age of ventilated neonates [32.1 (28.1-37.4)] was less than non-ventilated neonates [36.6 (33.6-39.1), $p<0.001$]. Overall, more ventilated neonates [81.5% (n=1746)] received S/A drugs than non-ventilated neonates [12.1% (n=548); $p<0.001$]. Fig. shows the rate of S/A use by country; table shows S/A drugs used in ventilated and non-ventilated neonates.



V=Ventilated, NV=Non ventilated

Table. Main drugs used for sedation/analgesia and muscle paralysis in ventilated and non ventilated neoantes in NICUs from 18 European countries

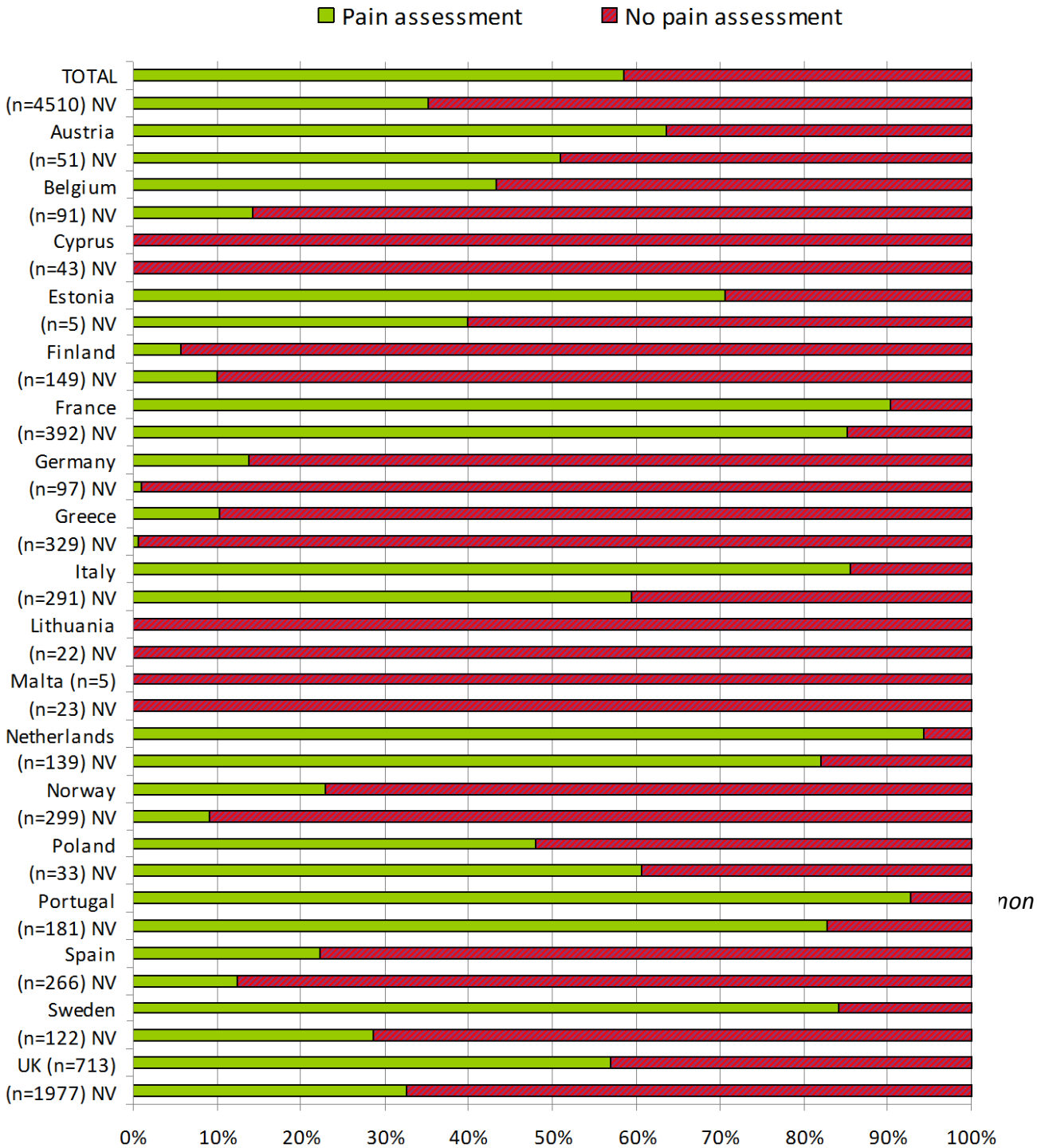
	Ventilated, n=1746	*Non-ventilated, n=548*
Morphine	*52.9%*	*17.0%*
Fentanyl	*36.0%*	*11.9%*
Sufentanil	*12.6%*	*1.3%*

Midazolam	*30.7%*	*7.3%*
Neuroblocker	*31.0%*	*0*
Paracetamol	*4.2%*	*55,8%*

We can conclude that most ventilated but few non-ventilated neonates receive S/A therapy in European NICUs. Rates of S/A for ventilated neonates vary from 66 % to 100 %, in different countries. Wide variations in the drugs used and mode of administration (continuous, bolus, or both) exist among countries

2) Pain assessment

From October 2012 to June 2013, 243 NICUs from 18 European countries collected pain assessment data in 6680 neonates. Of these, 2142 received tracheal ventilation (ventilated) and 4538 had spontaneous breathing or non-invasive ventilation (non-ventilated). The median (IQR) gestational age of ventilated neonates [32.1 (28.1-37.4)] was less than non-ventilated neonates [36.6 (33.6-39.1), p<0.001]. Overall, 58.5% of ventilated neonates and 35.2%% of non-ventilated neonates received bedside pain assessments (p<0.001). Fig. shows pain assessments by country.



Conclusions: *Over half (58.5%) of ventilated neonates and about one third (35.2%) of non-ventilated neonates had pain assessments performed in European NICUs. Wide variations in the methods used and rates of pain assessment exist among countries.*

4.3 Use and dissemination of foreground

Potential impact

This study has shown that although 82% of ventilated neonates in European NICUs receive some form of sedation/analgesia (S/A), important variations in the rates of S/A occur among countries. Moreover, different drugs are used to among countries and in most countries S/A is performed without a formal pain assessment.
We believe that some of the implications of these results are:
- The creation of a well motivated network of NICUs across Europe capable of making important improvements in the management of neonatal pain
- The development of a benchmarking process by units to improve their S/A practices in view of the results of other units.
-The need to develop European up-to-date recommendations to serve as a guide to S/A practices in ventilated and non ventilated neonates admitted to NICUs across Europe.
-The need to promote the use of validated pain scales in NICUs whenever a S/A is used or deemed necessary
-The need to continue the improvement of pain assessment tools for ventilated and non ventilated neonates
- The need to study the long term effects of S/A used in neonates admitted to NICUs

Dissemination Activities

The aim of this project was to provide evidence for a better use of analgesics in newborn infants, and to improve care of newborn infants. Dissemination, implementation, exploration and knowledge transfer and education to develop child analgesic friendly formulation are important parts of the project.

A website: www.neoopioid.eu is in the air since the beginning of the project and this website has been updated throughout the progress of the project. In addition, a website for the EUROPAIN survey has been created www.europainsurvey.eu

Neonatal intensive care units all over Europe have been encouraged to participate in the EUROPAIN survey. A total of 243 units in 18 countries all over Europe have registered data from 6680 newborn infants. Our hope is that this has laid the ground for a fruitful network that facilitate other collaborations in the future.

The investigators involved in the NeoOpioid project have arranged workshops and held oral presentations at scientific and medical conferences. Several reviews and scientific articles have been written.

The clinical and pharmacokinetic analyses have not been finalized. However, based on our pilot studies we propose provisional guidelines using Fentanyl for procedural pain and Morphine for chronic pain. We recommend intermittent bolus administration based on the scoring of pain.

Continuous administration is not recommended. Results from the method development studies and pilot studies have been presented in scientific publications and reports.

The main studies within the project have been registered in publically accessible databases (clinicaltrials.gov and/or clinicaltrialsregister.eu).

Preliminary results from the Europain survey have been presented at scientific conferences in some of the participating countries. The final results will be presented in scientific peer review journals and at scientific conferences. It will also be available at the project websites, and press releases will be distributed.

Section A (public)

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