Doctoral Thesis

Laser acupuncture compared with oral glucose administration for pain prevention in healthy term neonates undergoing routine heel sticks/lance.

submitted by

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Dedication

For B.M.R.

My Qi, Jing und Shen

氣

精

神

Statutory Declaration

I hereby declare that this thesis is my own original work and that I have fully acknowledged by name all of those individuals and organisations that have contributed to the research for this thesis. Due acknowledgement has been made in the text to all other material used. Throughout this thesis and in all related publications I followed the "Guidelines of the Medical University of Graz on Good Scientific Practice".

Graz, September 2022 Dr. Jasmin Stadler

Disclosures

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Abstract in German

Einleitung: Orale Zuckerlösungen stellen den aktuellen Goldstandard zur Schmerzprävention während kleinen schmerzhaften Eingriffen bei Neugeborenen dar. Nicht-pharmakologische Methoden sind bei solcher Art von Eingriffen stark empfohlen und ein großes Studiengebiet geworden. Akupunktur im Allgemeinen und speziell am Akupunkturpunkt Dickdarm 4 (Di4) zeigten in Studien eine gute analgetische Wirkung und gute Akzeptanz in der pädiatrischen Population. Deshalb soll in dieser Studie untersucht werden, ob bei Neugeborenen Laserakupunktur am Di4 gleichwertig einer oralen Glukoselösung ist.

Methoden: Es wurden 96 gesunde Reifgeborene eingeschlossen, die einen Fersenstich am dritten Lebenstag für das Neugeborenenscreening erhielten, und in zwei Gruppen randomisiert: Akupunkturgruppe (AG) und Glukosegruppe (GG). AG erhielt als Interventionsgruppe Laserakupunktur am Di4 für jeweils eine Minute an beiden Seiten. GG bekam als Kontrollgruppe den Goldenstandard eine 30% orale Glukoselösung zwei Minuten vor dem Fersenstich. Als Hauptziel wurde der Schmerzscore PIPP (Premature Infant Pain Profile) erhoben (observer blinded). Die statistische Analyse erfolgte mit dem Nichtunterlegenheits-Prinzip (non-inferiority, Margin: <1). Als Nebenzielgrößen wurden die Herzfrequenz-Veränderungen (HF) und die Schreizeit gemessen.

Ergebnis: Laserakupunktur verglichen mit oralen Glukoselösungen kann statistisch nicht als nicht-unterlegen angesehen werden [Median (IQR): 12 (10 -14) vs. 12 (9 – 14), p= 0,981, 95%CI: -1,000006 – 1,000059]. Die Nebenzielgröße Schreizeit zeigte vergleichbare Werte in beiden Gruppen [AG 102,0 Sek. (58,0 – 159,0) vs. GG 92,5 Sek. (58,5 – 173,5) p=0,890]. Die HF ergab einen signifikanten niedrigeren Wert (Schläge pro Minute) in der AG verglichen mit GG nach der Intervention [AG 119 (103 - 137) vs. GG 136 (127 – 140), p=0,002] sowie nach dem Fersenstich [AG 125 (114 - 144) vs. GG 138 (129 - 148), p=0,015].

Diskussion: Die Laserakupunktur zeigte im Median gleiche Schmerzscores wie in der Glukosegruppe. Allerdings gab es eine hohe Variabilität in der Studiengruppe,

weshalb Laserakupunktur nicht als gleichwertig betrachtet werden kann. Die HF als Nebenzielgröße jedoch zeigte einen positiven Effekt in der AG. Die HF in der AG wurde nach der Intervention und nach dem Fersenstich niedriger, was als Zeichen der Entspannung interpretiert werden kann. Gleichzeitig stieg die HF in der GG, was wahrscheinlich durch die Glukoselösung und das nicht-nutritive Saugen verursacht wurde.

Konklusion: Laserakupunktur an Di4 zeigte grundsätzlich ein analgetisches Potential bei Neugeborenen. Allerdings wäre aufgrund der großen interindividuellen Variabilität eine höhere Teilnehmeranzahl erforderlich, um eine aussagekräftige Empfehlung aussprechen zu können. Die Daten dieser Studie sollten daher für weitere Laserakupunkturstudien berücksichtigt werden.

Abstract in English

Introduction: Non-pharmacological pain management in neonates is an important research field. Oral sweet solutions are actual standard of care for neonatal pain prevention in minor painful interventions. Recent literature suggests that acupuncture at Large Intestine 4 (LI4) produce analgesic effects in clinical and experimental studies. Therefore, this study aims to investigate, if laser acupuncture at LI4 will reduce pain associated with minor painful procedures non-inferior to orally administrated glucose solution.

Methods: We included 96 healthy term neonates receiving heel lance on 3rd day of life for metabolic screening. They were randomly assigned to either laser acupuncture group (AG) or oral glucose solution group (GG). Laser acupuncture was applied with Soft-laser 10mW at LI4 for 1 min both sides. GG received 30% glucose solution orally 2 min prior to heel lance. Main outcome parameter pain was evaluated using PIPP (Premature Infant Pain Scale) observer-blinded. Secondary parameters were crying duration or heart rate changes (HR). Statistical analysis was performed in non-inferiority concept (margin: <1).

Results: PIPP scores showed a Median (IQR) of AG 12 (10 -14) vs. GG 12 (9 – 14) [p= 0.981, 95%CI: -1.000006 – 1.000059]. Secondary outcomes showed equal crying times (in seconds) between groups [AG 102.0 sec (58.0 – 159.0) vs. GG 92.5 sec (58.5 – 173.5) p=0.890]. HR (bpm) was significantly different between groups after intervention AG 119 (103 - 137) vs. GG 136 (127 – 140), p=0.002] and after heel lance [AG 125 (114 - 144) vs. GG 138 (129 - 148), p=0,015]. Difference between groups resulted due to an increased HR in GG and decrease in AG.

Discussion: We found equal median pain scores between groups. Nevertheless, due to high variability within the studied sample, laser acupuncture could not be declared non-inferior to glucose solution regarding main outcome. However, we found positive effects on crying time and heart rate in AG. Decreased HR in AG can be interpreted as a sign of relaxation, whereas the increased HR in GG was probably caused by the glucose solution and the non-nutritive sucking.

Conclusion: This study indicates clinical relevance of laser acupuncture at LI4 for pain relief in neonates. The present data should be taken into account for further laser acupuncture studies.

Introduction

This doctoral thesis deals with new approaches in non-pharmacological pain management in neonates. These pain therapies are a rising topic and acupuncture provides such a new possibility. The introduction will discuss pain in general and in special in neonates, including pain assessment and the state-of-the-art in non-pharmacological pain management. Furthermore, we will review acupuncture in paediatrics, mechanism of acupuncture in analgesia and will focus on laser acupuncture in special.

Part 1: Pain in General and in Neonates

Pain

The English term "pain" derives from the Latin "poena" and means punishment or penalty. In history, pain was the work of God to strengthen faith or the work of demons to punish the people. The understanding of mechanism of pain may began first in the 17th-century with the philosopher René Descartes (1596-1650), who suggested that the sensory perception is linked to the function of the brain.(1) Science to evaluate the pathway of pain has been born and many studies are conducted to evaluate it.

Nowadays, the definition of pain is changing fast. In 2018, the International Association for the Study of Pain (IASP) recommends a new definition of pain, which tries to combine the subjectivity of the experience of pain, with all its fear and anxiety about the body and existence of an individual, and the objectivity of observable somatic experience.(2)

Neonatal Pain

Pain in neonates is a special matter and moves more into focus since the 1980s.(3) However, these days, pain in neonates is scientifically proven and accepted.(4) The knowledge about it is visibly increasing. There is evidence that neonates can differentiate the intensity or invasiveness of stimuli and that their physiological and behavioral reaction increase the more intense the stimulation is.(5)

Neurobiology of Pain Development in the Neonate

Recent evidence has shown that nociceptive pathways are active after 25 weeks of gestation.(6) By that time, the anatomical maturation is finalized, whereas the pain modulation system is not as accurate and adaptable as observed in older children and adults. The myelination of nerves up to the thalamic levels takes up to 30 weeks of gestation. Additionally, the afferent neurons of the thalamus project axons that migrate into the neocortex and synaptic connections in this area may arise at 24th gestational week.(7) This condition suggests that also preterms feel pain, which evokes a stress response in the neonate. Due to that stress reaction, metabolic acidosis, hypo- or hyperglycemia and electrolyte imbalances may occur. This immediate or delayed stress response is then associated with an increased morbidity and mortality.(8)

All these facts advise a sufficient and effective pain management already in preterm neonates to prevent pain-associated neurotoxic brain injuries. Additionally, due to the plasticity of the neonatal brain, clinicians are forced to reduce painful procedures to a minimum in order to protect the developing central nervous system from damaging effects.

While the behavioral, cardiovascular, respiratory, hormonal, and metabolic responses to pain are the same both in neonates and in older populations, the extent of the pain reaction has been found to be more pronounced in neonates.(9) Fitzgerald and colleagues suggest that the excessive neonatal response is due to the absence of inter-neuronal pathways in the dorsal horn that only develop after birth.(10) Moreover, neonates lack the inhibitory pathway descending from the brainstem not least due to a relative paucity of inhibitory neurotransmitters.(11) Combined with larger dimensions of receptive fields together with the existing synaptic connection between afferents and dorsal horn cells, a single stimulus can lead to a fulminant excitation. This is further amplified by the postnatal changes in the organization of afferent sensory fibers.(6,12) Within a fully developed and functional pain modulation system, A-beta myelinated fibers are responsible for light touch and are confined to laminae III-IV while A-delta myelinated and unmyelinated C-fibers respond to painful stimuli and are located in laminae I-II. Prenatally and at the stage of birth, the A-beta myelinated fibers still spread to the superficial layers I and II, this leading to a more

generalized noxious response. As a result, weak cutaneous inputs may not be discriminated as such and evoke reactions similar to pain responses.(13)

To sum it up, it can be said that it is broadly acknowledged that the anatomical, physiological and biochemical prequisites of nociceptive perceptions are present in neonates. Unfortunately, there is increasing evidence that the local and descending inhibition as well as the discrimination of noxious stimuli is poorly developed at the stage of birth. The neonatal neurobiological pain perception system underlies adaptations within the first two years of life.(14) Subsequently, they are more sensitive and vulnerable to painful procedures in this period.

Procedural Pain in Neonatal Life and its Consequences

Painful procedures are necessary for daily medical care in neonates hospitalized in a Neonatal Intensive Care Unit (NICU). Such painful actions are defined as procedures that harm the skin or mucosal integrity. These events can be for example a tracheal intubation, venipuncture, gastric tube insertion or also a heel lance. An interesting clinical trial by Carbajal et al. evaluated these processes during a regular stay in the NICU. Authors have found that neonates are subjected in median to 10 (range 0-51) painful procedures per day during their hospital stay.(15) Due to this high number of painful events, an adequate and effective pain management is necessary to protect the neonates from short-term and long-term harm.

Moreover, perfectly healthy neonates need to endure indisputable painful interventions within routine care. Obviously, blood sampling for metabolic screening or intramuscular hepatitis B vaccination are necessary interventions that inevitably induce acute pain.(16)

As we can see, there are multiple sources of stress in the NICU. Commonly performed diagnostic and therapeutic procedures contribute to a high overall allostatic load. The concept of allostasis was designed to give an idea of the synergistic effects of different stress sources. The physiological effort to maintain homeostasis regardless of internal and external stress integrates the response of the hypothalamic-pituitary-adrenal axis (HPA-axis), the autonomic nervous system, and the cardiovascular, metabolic and immune systems. Repeated procedures result in an elevated demand on the

allostasic system and subsequent consumption of the entire network over time.(17) Short-term events in the allostasic system include release of catecholamine, hypertension and hypoxemia leading to a decreased blood flow in the brain and a loss of brain-blood-volume up to 50%.(5,11,18)

Because of these unstable and immature physiological systems, neonates are more vulnerable to repeated invasive procedures. However, it is important to realize that procedures do not have to be invasive in order to be harmful for neonates. Even tactile stimulation can potentially lead to the activation of noxious pathways and consequentially contribute to the allostatic load.(19)

Due to plasticity of our brain, pain can cause severe long-term damage in the neonate.(5,20) There are associations between an increased exposure to stressors with a decreased brain size, especially in the frontal and parietal regions, as well as alterations in brain microstructure and functional connectivity within the temporal lobes.(21) Additionally, the long-term consequences of an excessive demand on the allostasis can lead to heightened ongoing stress, or perhaps even result in chronic pain.

To avoid these repercussions, health care workers are faced with the challenge to balance the demand of necessary tests and treatments as well as reasonable monitoring versus keeping the pain and stress of the neonate to a minimum.

Approaches to Prevent Pain

Reducing the number of interventions is probably the most effective method to prevent neonatal pain. Hall and colleagues propose that planned 'cluster care' is a successful approach to reduce the frequency of bedside procedures. By combining routine medical interventions (e.g., physical examination) with other care procedures (e.g., diaper change), the bedside disruptions could be minimized.(22)

Additionally, when ordering blood tests with forethought, several tests are able to be conducted on one single sample, and as a result multiple blood draws can be avoided. If there is a need of more than 3 blood samplings a day, the establishment of a

permanent vascular access (e.g., arterial catheter or central venous line) should be considered.(23)

However, the topic of cluster care is controversial. While some see the systemic reduction of pain, especially when a cluster is followed by comforting events (e.g., feeding), others emphasize the prolonged period of pain and stress accompanied with grouped procedures.(24) Objectors fear that the allostatic load of clustered interventions could cause more harm than good. In clinical practice, a strict only cluster care can be very challenging given the ward circumstances, or the instable condition of the neonate. However, the efforts to maintain a cluster care seem meaningful to offer the neonates an adequately long break or sleep phase between procedures.

Pain Assessment in Neonates

Normally, to measure the severity of painful procedures, in most cases the patient can be asked to rate their complaints. Unfortunately, to query neonates and to expect a response is of course out of the question. Therefore, alternate assessment strategies had to be developed. There has been huge progress over the last years, and research is constantly striving to improve the existing methods as well as implement new technologies altogether.

There is emerging data on pain assessment using near-infrared spectroscopy, amplitude-integrated electroencephalography, functional MRI, skin conductance, and heart rate variability.(21,25) New findings within this field give hope for a neurophysiological approach, which allows to evaluate the pain behavior not only going by its consequences but also straight from its origination. From observing the cortical level, it can be suggested that pain assessments are implementable also in sedated or anesthetized neonates.(26) At the moment, neurophysiological assessment is still in its infancy, and the clinical relevance limited for the time being.

Next to neurophysiological assessment tools, recent literature has promised new approaches in neonatal pain monitoring based on genetic changes. Provenzi and colleagues, for example, have shown that age and exposure to stress could have an

influence on the telomere length (TL).(27) This hypothesis might indicate that sufficient neonatal pain management could increase TL and therefore, TL might be used as a marker for painful procedures in preterm neonates.(27)

Uni- and Multidimensional Pain Scales

Despite great effort to develop a variety of innovative pain monitoring tools, currently used neonatal pain scales are still mainly based on observations of the neonate's behavioral, physical, and physiological reactions.

The origin of such scoring tools can be found in the 70s and 80s of the last century, followed by the 1990s as the most flourishing period for the advance of uni- and multidimensional pain measurements.(28–30)

Ever since, the concept of scoring instruments has been a divided topic. There are those who are convinced that only one parameter is satisfactory to evaluate pain response in neonates, and there are those who want to avoid an over-interpretation of a single parameter (e.g., body movement changes). Pain is a very complex and complicated phenomenon, showing itself in manifold ways.

On the one hand, clinicians often utilize a combination of various parameters to shape a multidimensional pain assessment. On the other hand, the interpretation of several parameters seems to be problematic as there appear to be discrepancies between the diverse approaches. To name some examples, Hartley et al. have detected the existence of nociceptive brain activity without any indication of pain in facial expression.(31) Additionally, Morison and colleagues have found a discordance between the neonatal facial expression and change in heart rate.(32) Slater et al. have revealed that "pain may be processed at the cortical level without producing detectable behavioral changes." (25)

As a result, it could be interpreted that facial expression alone should not be assessed but could rather be seen as one component of several clinical pain assessment tools since, regardless of a low score based on behavioral measurements alone, a neonate may feel pain. Moreover, not only in neonates but also in infants this aspect seems to be relevant and should be taken into account.(33)

Due to the apparent dissociation between behavioral and physiological responses to pain in neonates, others criticize that multidimensional tools may fail to specify the effects of procedures on each system.(28) However, this independent measurement seems to be meaningful when evaluating the effects of non-pharmacological and pharmacological events. For example, Johnston et al. have found that Kangaroo care has reduced the behavioral manners during painful interventions but have shown little effect on the heart rate or on oxygen saturations.(34)

In conclusion, a systematic review by Duhn et al. about infant pain assessment tools has revealed that one half of the included trials has generated unidimensional scales (n=18) while the other half has revealed multidimensional ones (n=17). Authors have concluded very provocatively that "none of the existing instruments fulfilled all criteria for an ideal measure; many require further psychometric testing."(28)

Neonatal pain assessment may therefore include both behavioral observations and physiological measures.(26,35) Physiological responses to pain include increased heart rate, increased respiratory rate, elevated blood pressure and decreased oxygen saturation.(20) Behavioral or physical observations comprise leg or arm movements and diverse facial expressions (e.g., brow bulge, eye squeeze or nasolabial furrow).(25,36)

Clinical guidelines on neonatal pain prevention and management recommend to use pain scales with proven validity and reliability.(9,37,38) Internationally, the two validated neonatal pain scores used most widely are the Premature Infant Pain profile (PIPP) and the Neonatal Infant Pain Scale (NIPS).(39,40) Overall, Olsson and colleagues have shown in a systematic review that PIPP had been used in 48% and NIPS in 23% in included trials.(41)
Since the PIPP score considers a combination of behavioral and physiological changes as well as the gestational age, we have chosen this score for our recent trial.

Premature Infant Pain Profile

The PIPP is a validated acute pain score for preterm and term neonates. (36,39) It has high inter-rater (reliability coefficients of 0.93-0.96) and intra-rater reliability

(reliability coefficients at 0.94-0.98) as well as good construct validity, differentiating pain from non-pain and baseline events ($p < 0.001$).(42) This pain score combines different parameters to assure a multidimensional pain assessment.

There are seven categories, and in each category 0 to 3 points can be allocated.
The PIPP evaluates the following categories:
1) Gestational age: the lower the gestational age, the higher the score.
2) Behavioral state before painful stimulus: the more awake the neonate, the lower the score.
3) Change in heart rate during painful stimulus: the lower the increase of heart beats per minute, the lower the score.
4) Change in oxygen saturation during painful stimulus: the lower the decrease of oxygen saturation, the lower the score.
5) Brow bulge during painful stimulus: the fewer brow bulges, the lower the score.
6) Eye squeeze during painful stimulus: the fewer eye squeezes, the lower the score.
7) Nasolabial furrow during painful stimulus: the fewer nasolabial furrowing, the lower the score.

The highest achievable PIPP score in term neonates has been found to be 18 and is considered to be directly proportional to the neonate's pain intensity during the painful intervention.
The PIPP score used in this trial is attached in the appendix for further information.

Other Neonatal Pain Scores

The second most common pain scale, NIPS, was introduced in 1993 by Lawrence et al. to develop a behavioral evaluation for the assessment of pain in preterm and term neonates. The NIPS consits of six assessment tools: Facial expression (relaxed: 0 point/contracted: 1 point), Cry (absent: 0 point/mumbling: 1 point/vigorous: 2 points), Breathing (relaxed: 0 point/different to basal: 1 point), Arms (relaxed: 0 point/flexed or stretched: 1 point), Legs (relaxed: 0 pint/flexed or stretched: 1 point) and Alertness (sleeping or calm: 0 point/fussy: 1 point), respectively. Here, the maximum points are 7, while a score of 4 or higher represents severe pain. NIPS has a high inter-rater reliability and internal consistency with good validity.(40)

Next to PIPP and NIPS, other validated pain scores include, for example, the 'Behavioural Infant Pain Profile' (BIIP), COMFORT scale, 'Douleur Aigue¨ du Nouveau-ne´,(DAN), 'Neonatal Facial Coding System' (NFCS) or 'Neonatal Pain, Agitation and Sedation Scale' (N- PASS).(43–48).
They represent uni- and multidimensional scores, including most times facial expression, body activity and physiological changes in a varied version of interpretation and rating. COMFORT scale for a multidimensional and BIIP for an unidimensional assessment tool will be described in more detail below. An overview of selected uni- and multidimensional scoring instruments will be provided in *table 1*.

The COMFORT scale is a 8-dimensional assessment tool to measure distress, sedation and pain in nonverbal pediatric patients. It has also been validated for postoperative pain in infants from 0-3 years old, and in preterm population for acute pain. (44,49) A systematic review by Maaskant et al. has evaluated studies using the COMFORT scale for reporting pain.(50) They have found that in included trials most studied probands used to be ventilated children up to 4 years. In conclusion of the review, the COMFORT scale has demonstrated a overall approbriate reliability in generating data on distress, sedation and pain.

The unidimensional scale BIIP is described as a reliable and valid assessment for acute painful procedures in preterm neonates.(43) It combines behavioral state (deep sleep, active sleep, drowsy or quiet awake: 0 points/active awake: 1 point/agitated or crying: 2 points) and facial expressions (brow bulge, eye squeeze, naso-labial furrow, horizontal mouth stretch: each 1 point) as well as hand activities (finger splay, fisting: each 1 point). This suggests that it is very useful to combine the relatively most particular as well as the individual behavioral parameters in neonates to measure pain for a more specific determination of the effects of interventions on a single system.

Pain score	Dimension	Parameters	Maximum score
Premature Infant Pain profile (PIPP)	mutlidimensional	Gestational age, behavioral state, heart rate, oxygen saturation, brow bulge, eye squeeze, and nasolabial furrow.	+ 21
Neonatal Infant Pain Scale (NIPS)	mutlidimensional	Facial expression, cry, breathing patterns, arm movement, leg movement, and state of arousal	+ 7
COMFORT scale	mutlidimensional	alertness, calmness/agitation, respiratory response, physical movement, mean arterial pressure, heart rate, muscle tone, and facial expression	+ 40
Neonatal Pain, Agitation and Sedation Scale (N-PASS)	multidimensional	crying/irritability, behavior state, facial expression, extremities/tone, vital signs (blood pressure, heart rate, oxygen saturation), and correction for premature gestational age	+ 10, with premature gestational age correction: + 13
Behavioural Infant Pain Profile' (BIIP)	unidimensional	Sleep/wake states, 5 facial expressions, 2 hand actions	+ 8
Neonatal Facial Coding System (NFCS)	unidimensional	Brow bulge, eye squeeze, nasolabial furrow, open lips, vertical mouth stretch, horizontal mouth stretch, lip purse, taut tongue, chin quiver, tongue protrusion	+ 8
Douleur Aigue¨ du Nouveau-ne (DAN)	unidimensional	Facial expression, limb movements, and vocal expression	+ 10

***Table 1*:** Overview of uni- and multidimensional pain scores.

Common Pitfalls in Pain Assessment

Altogether, it is central to point out that the assessment method used should be adapted to the type of pain a neonate is experiencing in terms of acute, prolonged, or postoperative pain. Most pain scores have been developed for acute pain evaluation; however, they are used, inappropriately, also for chronic and postoperative pain, respectively, in study settings. A recent systematic review of 2021 demonstrated that 16% of included studies did, in fact, use a unsuitable pain scale for the type of pain investigated or with regard to the specific neonatal population.(41) Moreover, blinded pain evaluation should be available in order to avoid observer bias. Olsson et al. have shown that a blinded pain assessment could only be found in two thirds of the trials.(41)

Besides the huge variety of good and validated pain scales, a regular training of these scores seem to be of major importance. A clinical trial by Sarkaria et al. has evaluated the competences of NICU's staff based on two pain scores (NIPS and COMFORT scale).(49) They have concluded that a prior training before implementation of a new pain assessment tool at a NICU should be obligatory in order to prevent inadequate pain evaluation. Authors advise the development of a training programme to enhance clinical daily practice.(49)

Part 2: Non-pharmacological Pain Management in Neonates

It was a long process before pain management in neonates and infants was started to beestablished and acknowledged. Over the last few years, huge progress has been made in the developing of an adequate low-risk therapy concept for the neonatal population. Due to this evolution, pharmacological pain management is being pushed further and further into the background due to its adverse events and influence on brain development.(51) Due to their physiological immaturity and inter-individual differences in drug metabolism, neonates respond in their own ways to pharmacological treatment.(51,52) The infant improves the absorption, distribution and clearance of drugs only later, i.e. during the first few years. Also, the liver and kidneys are immature and can only achieve a fraction of their adult capability. Additionally, increased total body water and extracellular fluid have to be taken into account and influence the volume of distribution.(53)

Thus, non-pharmacological pain management is increasingly recommended for the prevention of pain.(54–56) These therapies may optimize the homeostatic mechanisms of the infant, thereby mitigating some of the adverse consequences of untreated pain, as well as facilitating healthy physiological adaptations to stress.(14) It can be distinguished, inter alia, into environmental control, feeding methods and other interventions.(54)

Environmental control includes:
(1) Skin-to-skin care / Kangaroo care
(2) Facilitated tucking
(3) Swaddling
(4) Maternal touch
(5) Therapeutic touch/massage
(6) Music therapy
(7) Positioning

Feeding control contains:
(1) Breastfeeding

(2) Non-nutritive sucking

Physiological control comprises:
(1) Acupuncture modalities
(2) Oral sweet solution.

In conclusion, non-pharmacological methods are straightforward to use, easy to learn and feasible. Since acupuncture represents a relatively new research area in neonates, it is more likely to be compared to other non-pharmacological pain therapies. In most cases, acupuncture has been analogized to oral sweet solutions, but also breast milk, placebo, or routine care respectively. In the next few chapters, we will have a closer look at a selection of common non-pharmacological therapy options to receive an overview of advantages and disadvantages of these treatments.

Environmental control

Kangaroo care or skin-to-skin care

Skin-to-skin care (SSC), also referred to Kangaroo care (KC), means holding the neonate with skin-to-skin contact in a chest-to-chest position, most commonly instituted shortly after birth with the mother, in healthy neonates.(57) The duration of skin-to-skin contact varies between 1 to 3 hours per session. This technique of neonatal care has originally been used in developing countries for warmth and bonding, while decreasing morbidity and mortality, especially in stable preterm neonates, named Kangaroo Mother Care (KMC).(58) A recent RCT conducted in Ghana, India, Malawi, Nigeria, and Tanzania, concluded that also in instable preterm neonates with a birth weight below 1800 grams, immediate KMC can lead to a lower mortality.(59) A Cochrane review on kangaroo care has concluded that healthy neonates receiving skin-to-skin contact results in a higher cardio-respiratory stability.(60)

Next to its positive effects on mortality and morbidity, SSC can be used for pain management as well. A Cochrane review has summarized that the pain-reducing effect during minor painful interventions, such as heel lance or intramuscular

injection, has revealed a decrease of crying time, a improvement of pain scores and a decrease of stress in preterm neonates.(61) One of these studies compared 80 minutes of SSC with 30 minutes with a standard incubator care in preterm neonates.(62) Interestingly, only 30 minutes of KC before painful procedure have shown a reduction in pain score PIPP compared to standard care, while 80 minutes have demonstrated no pain-relieving effect. Also, in comparison to oral sweet solutions, the outcome of trials in the Cochrane review appeared to favor SSC, whereas a combination of SSC and something sweet was found to be more effective than SSC or sucrose alone. Patel et al. have compared SSC with swaddling during Vitamin K injection and have demonstrated a significantly lower pain score of NIPS with SSC compared to solely swaddling [SSC: 1.38 (0.70) vs. swaddling: 2.88 (1.00), $p < 0.001$].(63)

Altogether, study outcomes have been in favor of KC, especially in preterm neonates, for pain relief in minor painful interventions, compared to standard care.(61)

Facilitated Tucking

Facilitated tucking (FT) is defined as placing a hand on the baby's hands or feet and positioning the baby to provide support yet allow them to control their own body, and is similar to providing KC. It has been used to alleviate pain during endotracheal suctioning and heel pricks. However, it may not be as effective as oral sucrose for repeated painful procedures.(22)

Authors have found that a combination of several non-pharmacological methods with facilitated tucking or administered alone can be effective for the pain management of neonates.(64–66) Moreover, a very recent evaluation of facilitated tucking in 2021 concluded that it can be an effective additive pain-controlling management combined with morphine administration during chest tube removal.(67)

Swaddling

This non-pharmacological, environmental control for procedural pain involves wrapping the infant in a special cloth (e.g. cloth diaper or towel). This child-care is carried out in many countries in various implementations due to cultural tradition. There is research evidence that this supports a better neuromuscular progress, less

physiological distress, and more self-regulatory competence in preterm neonates.(68) Swaddling has also been found to support infants suffering from neonatal cerebral injuries in the management of excessive crying, and has been found to decrease the amount of crying significantly compared with massage.(69) A RCT from 2019 demonstrated a significant pain reduction when combining swaddling with expressed breast milk (PIPP 5.2 ± 1.7) in comparison to routine care (PIPP 9.5 ± 3.6), swaddling alone (PIPP 8.8 ± 2.9), FT (PIPP 7.2 ± 3.2), expressed breast milk alone (PIPP 7.9 ± 2.6), and FT + expressed breast milk (PIPP 6.6 ± 2.3) ($p<0.001$). Also in older neonates, a review from 2021 presented that swaddling combined with oral sucrose or music therapy can release pain during a routine male infant circumcision.(70) Nevertheless, it also bares many risks when applied in older infants, or when applied too many times, or in wrong or uncontrolled ways. One systematic review also tables the possible side effects, such as hyperthermia, hip dysplasia, sudden infant death syndrome and respiratory infections, respectively.(68) Limitations of swaddling should be in mind when applied. Therefore, swaddling should be done with caution, but seems to be an important supportive tool for pain management at a NICU.

Feeding control

This chapter includes breast feeding as well as application of expressed milk, and non-nutritive sucking. These methods seem to stimulate a special pathway, which include a neuropeptide called Cholecystokinin (CCK).

CCK seems to play a central role in the pain pathway.(71,72) It can be found in high concentrations throughout the central nervous system, especially in pain-relevant regions (e.g. the hippocampus).(73) Additionally, CCK-8 as a subgroup neurotransmitter, which is involved in the regulation of feeding behavior, works also for the maintenance of the nervous system and has a neuroprotective role.(74)

This neuroanatomical condition leads to the assumption that there is a close link between the opioid and CCK mediated mechanism and the modulation of anxiety or pain.(71) There is an innovative effort to use CCK-2 receptor (CCK2R) antagonists for pain alleviation as a new pharmacological treatment.(72)

This opioid-CCK pathway seems to be one main aspect of an analgesic effect of non-pharmacological feeding controls based on the release of CCK during breastfeeding or non-nutritive sucking (NNS).(75,76)

Breastfeeding

Breastfeeding and mother milk are the most natural and simplest available tools in every country. There seems to be evidence that mother milk, next to many other positive effects, can also induce pain relief in neonates. A Cochrane review of 2012 analyzed 20 trials, of which one half evaluated breastfeeding and the other half supplemental breast milk.(77) Heel lances in 16 and venipuncture in 4 studies have been evaluated as painful procedures. This review has demonstrated that breastfeeding compared to positioning, held by mother, placebo, pacifier use, no intervention or oral sucrose have lead to a reduction in changes in heart rate, percentage of crying times, duration of crying and duration of first cry. In addition, also the PIPP score has been found to be lower in breastfed neonates compared to placebo, positioning or sucrose solution.

As mentioned above, the CCK pathway mechanism seems to play an important role based on the release of CCK due to familiar odors of the mother.(71,75) The analgesic effects of breastfeeding may be potentiated by multimodal stimulation provided by the touch and smell of the mother, the contained positioning of the infant or the sweetness of mother milk due to lactose and other ingredients.(78,79) Also tryptophan as melatonin precursor, which is higher concentred than in formula, may increase the beta endorphin concentration.(80) These mechanisms could be responsible for the nociceptive effects of mother milk.

In conclusion, breastfeeding has a pain-relieving potential, can be easily offered, and adopted and is of low risk factors.

Non-nutritive sucking

NNS means sucking on a pacifier without nutritional benefits and is an often-applied tool for calming and relieving pain in infants. Research revealed a reduced pain response in preterm neonates when a pacifier was combined with sweet tastes during painful procedures, in most cases heel lances.(81,82)

The complete physiological mechanisms for this analgesic response remain unclear, but may be related to non-opioid pathways stimulated by oro-tactile and mechanoreceptor mechanisms and the above mentioned opioid-CCK-linked pathway.(55,83) Within this pathway, NNS may activate sensory nerves that can produce the release of CCK, which leads to an interaction with opioids and conductive analgesic effects.(55) Additionally, there seems to be a link between sweet solutions and NNS; however, it remains unclear whether the effects of sweet tastes and NNS are synergistic or additive. Nevertheless, literature tends to support the combination of these two non-pharmacological techniques when it comes to procedural pain.(82,84)

Physiological Control

Sweet Solutions

Despite a recent review demonstrating that skin-to-skin care and special acupuncture techniques reduced the pain score significantly compared to oral sweet solutions, sucrose or glucose solutions are still the current standard of care in pain management for minor painful procedures.(54) Unfortunately, we don't have a consistent definition for sweet solutions as the term entails non-pharmacological as well pharmacological treatments. In most cases and in our trial, we have defined glucose solution as a non-pharmacological therapy.

A systematic review summarized the beneficial effects of sweet solutions on pain release in comparison to massage, breast milk or music therapy.(54) Further, a Cochrane review has analyzed 44 randomized controlled trials and concluded that sucrose reduced pain score and crying duration during minor painful intervention significantly. Nevertheless, there is much controversy around sucrose because there is still a gap of knowledge in the administration dosage and intervals between administration and painful intervention, as well as in the mechanism altogether. While an update in 2016 of the American Academy of Pediatrics gave a dosage recommendation based on weight (0.2–0.5 mL/kg), the time of administering sucrose 2 min before a painful procedure was no longer clearly recommended.(85)

The mechanism of administering oral sweet solutions has not been sufficiently investigated yet. On the one hand, we have the sweet flavor sensation that stimulates our "reward system" and leads to a release of endogenous opioids resulting in a modulation of the painful experience. On the other hand, these activated endogenous opioids can modulate neuronal transmission of pain stimulus. (86)

Recent mice models have given us an initial insight into the long-term effects of oral sucrose administration. The memory in adulthood was poorer when exposed to pain during first week of life, independent of the pain management (sucrose or water administration). Sugar-exposed pups had a poorer short-term memory in adulthood and a decreased sugar preference.(87) The authors now conclude that sucrose administered for pain prevention may not adequately protect the memory performance, and that a decreased sugar preference may indicate a conditioned memory.

In conclusion, although oral sweet solutions offer a reliable pain prevention in neonates, other safe and feasible non-pharmacological alternatives to oral sweet solutions are urgently needed, to guarantee an effective pain management without short-term or long-term side effects.

Summarization

This section has sought to provide an overview of the most popular methods for pain prevention in neonates so far. There may be even more non-pharmacological pain management techniques, which are not mentioned here. Nonetheless, all of them seem to have a supportive and/or additive effect in pain prevention and only a few seem to be able to cultivate serious side effects when applied inappropriately. Most of the practices support a natural well-being and are in accordance with a biological mother-child rapport. Beyond these usual environmental and feeding controls, acupuncture establishes a very new approach of pain-controlling procedures, uniting physiological responses, various brain activities and ways of relaxation in combination. The next chapter will look into these options more thoroughly, and will explain why acupuncture can be a promising innovative tool for pain relief in neonates.

Part 3: Acupuncture in General and in Neonates in Particular

Acupuncture has been practiced for more than 2500 years and has its currently documented roots in China, although some findings in history suggest an even older knowledge of acupuncture points.(88) Chinese acupuncture means the stimulation of points along defined energy channels (so called meridians) with thin needles or other techniques.(89) The World Health Organization (WHO) meetings in the 1980s, and later in 1991, led to a report of an WHO Scientific Group, which determined a standard nomenclature for body acupuncture points (short form: acupoints).(90) This system consisted of an alphanumeric code, the Pinyin Chinese phonetic name, the Han character for each acupuncture point, and the English translation of the Chinese names for acupuncture meridians. For better reading in this scientific work, abbreviations of English names of most of the acupuncture points will be used. An exception is made for Shenmen and Yintang because their Chinese name are more commonly used. The acupuncture point employed in our recent trial as well as all used points in neonatal acupuncture trials will be described below in more detail.

Acupuncture has been extensively practiced in Europe since the middle of the 20th century, which is also when its scientific career started with pain trials. Analgesia using needle acupuncture was established by Johannes Bischko, who carried out the first surgery solely using acupuncture as analgesic in Vienna in 1972.(91,92) Pain has ever since been a huge research area for acupuncture trials.(93,94)

Next to the known needle acupuncture and with rising technology, other acupuncture modalities have been discovered, for example laser acupuncture or non-invasive electrical stimulation of acupuncture points (NESAP). NESAP is performed using TENS (transcutaneous electrical nerve stimulation), using the acupuncture points. This acupuncture technique represents the benefits of electrotherapy and acupuncture in a non-invasive and gentle way. This strategy was developed based on electroacupuncture (EA), which means the insertion of acupuncture needles that are connected to an electrical stimulator into acupoints. EA is very popular in acupuncture therapy and research due to its possibility to regulate the stimulation frequency, intensity and pulse interval, which is also possible in NESAP.(95)

Laser Acupuncture

Laser acupuncture is, in comparison to the roots of acupuncture, a relatively new technique to stimulate acupuncture points with a low-intensity and non-thermal laser irradiation.(96) First reports of the laser technique came from China and Russia, followed by first publications of usage at acupuncture points in the 1970s.(97) The newest definition was developed at the 12th International WALT (World Association for photobiomoduLation Therapy) Congress in France held in 2018, "Photonic stimulation of acupuncture points and areas to initiate therapeutic effects similar to that of needle acupuncture and related therapies together with the benefits of Photo-Bio-Modulation (PBM)".(98) Commonly used names in clinical practice are laser acupuncture or low-level-laser therapy (LLLT).

The main advantage of laser as compared to needle acupuncture lies in its painless, non-traumatic and infection-free application, as well as in its simple usage accompanied by a short treatment time, making it time-efficient and thus cost-effective.

Unfortunately, there are currently only a few studies comparing needle and laser acupuncture to indicate similar pathways. Laser acupuncture seems to have a similar clinical impact as needle acupuncture, for example when it comes to changes in the local cerebral oxygen saturation investigated in a cohort during stroke rehabilitation, or specific effects in the brain including a significantly increased blood flow velocity in the posterior cerebral artery and a significant increase in the amplitudes of 40-Hz cerebral oscillations.(99,100) A recent study has investigated laser and needle acupuncture at acupuncture point Liver 8 in healthy probands with functional MRI (fMRI) and found that both techniques produced different brain patterns.(101) Further fMRI studies have revealed an activation of specific brain areas after laser acupuncture correlated to the stimulated acupuncture point, predominantly ipsilaterally, compared to a placebo-group with non-activated laser.(102,103) Siedentopf and colleagues have concluded that laser acupuncture provides good placebo conditions for trials, and that fMRI represents an adequate imaging tool for further laser acupuncture trials.

Beside basic research, clinical randomised controlled trials are also in support of a comparable response in needle and laser acupuncture. In 40 women suffering from

cervical spondylosis and neck pain, laser acupuncture has been compared to the traditional needle technique.(104) Authors have found that both methods are effective to combat pain intensity, disability and improve quality of life. However, laser acupuncture has shown an even better response in all categories and even a higher decrease of serum TNFα and increase of beta endorphins. Another study, conducted in 30 patients with degenerative joint or spine pains, is postulating a clinically equivalent effect between laser and needle acupuncture.(105) It has also demonstrated a favour of 9 out of 10 patients for laser application and a greater pain reduction during laser acupuncture, too. This clinically relevant better response with laser acupuncture compared to needle acupuncture may be due to the beneficial effects of PBM.

Specific and unique characteristics of laser therapy have been found in its impact on the acupuncture points inducing different pathways as compared to needle acupuncture and its local effect on the tissue. PBM has a wide treatment field because of its effect on acupuncture points but also on local tissue. Low-level-laser therapy as superficial therapy increases wound healing, reduces inflammation, pain and edema. The photochemical effects of PBM results in an influence of cell metabolism in disturbed or damaged cells and initiates healing. The used laser light for wound healing is monochromatic, coherent and mostly in a wavelength raging between 600 – 1000nm and with a low laser output power (1 – 90mW) resulting in an energy dosage of 1-4J/cm^2. (106) A systematic review in pediatrics with chemotherapy-induced oral mucositis concluded a prophylactic as well as therapeutic effect on severity of mucositis and oral pain.(107) Laser therapy is also used in pediatric burn injuries or in children with chronic rhinosinusitis.(108,109) Most of these local treatments may also be combined with local acupuncture points in clinical practice.

There was a huge progress in understanding of these laser-induced cellular and molecular mechanisms in last years. On cellular level, we know that after laser light stimulation immune cells migrate to the site of tissue injury, fibroblasts and macrophages are activated in proliferative healing phase and the rebuilding of extracellular matrix is triggered at the wound site.(110–112) There are laboratory

and clinical studies showing significant reduction of inflammation and prevention of fibrosis.(107,113–117)

One main molecular biology that is under intensive investigation nowadays may be the effect on mitochondrial chromophores, especially cytochrome c oxidase (CCO) and heat/light gated ion channels.(118–120) These mechanisms generate reactive oxygen species that can activate transcription factors and are unique in laser application, yet not in needle acupuncture. CCO works as intracellular acceptor of photons in the light wavelengths used for PBM. This enzyme is necessary for oxygen consumption in the mitochondrial electron transport chain, which drives oxidative phosphorylation for adenosine triphosphate (ATP) production.(121) Therefore, PBM offers a wide spectrum of CCO mediated effects, for example an improvement of hyperglycaemia and insulin resistance or neurostimulatory effects with expansion of brain functions.(122–124)

Such protective brain effects have been documented in several animal model trials, demonstrating that laser acupuncture at Bai Hui (Governing Vessel 20) decreases the brain infarct volume in cerebral ischemic rats, decreases oxidative stress and alleviates cognitive impairment and motor deficits.(125,126)

Finally, laser stimulation of local tissue or of acupuncture points seems to have a complex mechanism of action and offers a great therapeutic potential. Further basic and clinical research has to be conducted to explore the full potential. After all, we are just at the beginning of research in this special and modern area.

Let us now move on to general acupuncture mechanisms, involving research with all kinds of acupuncture techniques.

Mechanisms of Action in Acupuncture

Acupuncture in general acts in many different ways, but only a few of them have been amply studied so far and are therefore still under investigation.(127) Nevertheless, some possible explanations have been discovered already. This chapter first deals with an explanation of acupuncture points before giving an overview of the main acupuncture-induced mechanisms, namely anti-inflammatory and analgesic ones, respectively.

Acupuncture Points

To understand the mechanism of acupuncture it is important to imagine the texture of acupuncture points. There are 361 points on 12 main channels, as well as several extra-points lying next to the channels and extraordinary channels.(89,128) Next to predefined extra-points, there are so called non-acupuncture points, lying on an extra-channel or equipped with a special function. They are often used in clinical randomized-controlled trials with needle acupuncture as control points.

If we consider the acupoints anatomically, we find abundant nerves, muscles, vessels, and tendon there. When examined more closely under the microscope, we will see various kinds of free nerve endings, receptors, Ruffini corpuscles, Meissner corpuscles, Krause corpuscles, lamellated corpuscles, and muscle spindles.(129) After stimulating acupoints, type II and III fibers of the afferent nerves may convey the transmission of the acupuncture-induced signals, which are then processed in the central nervous system including the spinal cord, brainstem, hypothalamus, thalamus, and cerebral cortex.(129)

In addition, there exists a strong relationship between the acupoints and interstitial connective tissue.(130) The somatic tissues and internal organs may play a crucial role in the understanding between the location and effect of acupuncture points.(131) New insights are gained with peripheral receptive fields (RF), which represent a stimulated skin area that impacts the operation of a spinal sensory neuron.(132) While manipulating acupuncture points, it is assumed that one or more RFs are activated and that therefore several sensory central neurons acting as a link for the peripheral response created by acupuncture stimulation.(133) The somato–autonomic reflex may cause an interaction between the acupuncture points and viscera, based on the segmental innervations and conjunction of the somatic and autonomic nerves at the same spinal segments.(134) This may also explain, why non-acupoints lying near to the real acupuncture points and therefore in the same nerval segment have a certain impact on the studied condition.

This somatosensory autonomic reflex may also explain acupuncture effects over a long distance, compatible with the Chinese idea of channels or so-called meridians. A

recent basic research study from 2021 published in Nature provide innovative concepts of such reflex mechanism. Liu and colleagues speculated that "the somato–vagal–adrenal reflexes are driven by sensory pathways that innervate tissues unique to the hindlimbs compared to the abdomen, such as joints, bones and skeletal muscles."(135) Therefore, they investigated a special pathway, which involves dorsal root ganglia neurons marked by PROKR2^{Cre} ("Cre-mediated recombination marks neurons with persistent or transient expression of prokineticin receptor 2[13]"), which innervate deeper layered tissues of the limb but not superficial ones (e.g., abdominal fascia).(136) Thus, they compared among other things an acupuncture point on the hindlimb (Stomach 36 = ST36) with a superficial abdominal one (Stomach 25 = ST25) on the same meridian (Stomach channel). To highlight one of their experiments, low-intensity EA was applied at ST36 in mice with ablated PROKR2^{Cre}-marked sensory neurons. However, the stimulation missed to initiate hindbrain vagal efferent neurons or to activate catecholamine release in these mice.

In summary of the published data, Liu and team provide new approach of neuroanatomical knowledge and regulation of the vagal–adrenal axis in two ways: On the one hand, their research could explain the presence of acupuncture point selectivity by showing the effectiveness of ST36 (hindlimb point) but not of ST25 (superficial point). On the other hand, they demonstrated an acupuncture point specificity based on an effective ST36 compared to a non-effective non-acupoint in posterior hindlimb muscles.(135)

They have also encouraged a discussion about the definition of point specificity, depending on the intensity of stimulation, the insertion depth of the needle or other acupuncture device and the effects measured.

Further examples show that the acupuncture points Large Intestine 4 (LI4) and ST36 may represent an anatomical and histological correlation to nerve-vessel bundles that have perforated the superficial fascia of the body and therefore have a direct influence on the parasympathetic and sympathetic nervous systems.(137) Referring to one fMRI trial of Siedentopf et al. mentioned above, a relationship has been shown between stimulated acupuncture point Bladder 67 (BL67), known for its usage in ophthalmic diseases, and the activation of visual brain areas compared to placebo acupuncture.(103)

This brief overview of anatomical conditions shows that acupuncture points represent an ensemble of complex structures and tissues, interacting in many ways with the body, making it challenging to conduct high-quality clinical trials.
To get a deeper understanding of acupuncture mechanism, we will continue with the molecular and cellular levels of acupuncture functionality.

Anti-inflammatory Mechanisms

There are several inflammatory functions of acupuncture's mode of action; however, two of them seem to be the key pathways: on the one hand the manipulation of macrophage polarization, and on the other hand the maintaining of the T-cell balance.(138)
Due to these mechanisms, inflammatory diseases are main application areas in acupuncture therapy. In 2002, the WHO recommended acupuncture for over 100 conditions, including about 16 types of inflammatory diseases (e.g., allergic rhinitis, rheumatoid arthritis, bronchial asthma, Hepatitis B, and Herpes zoster).(139) Afterwards, other projects and reviews were conducted to update actual evidence in acupuncture therapy, for example by the Australian Acupuncture and Chinese Medicine Association Ltd (AACMA) in 2017 or by the American TCM Society in 2018.(140,141)

Li and team summarized in a profound systematic review from 2021 the most fundamental anti-inflammatory actions of acupuncture. Included findings have been investigated through needle or EA in rat models using, in most cases, ST36 as examined acupuncture point because of its easy accessibility or other disorder-specific acupoints. The review explains that acupuncture can initiate the transformation of macrophages from M1 to M2 phenotype, can suppress the production of proinflammatory mediators, can inhibit oxidative stress, and can regulate the helper T-cell balance.(138) Additionally, Yang et al. demonstrated an upregulation of anti-inflammatory and tissue-repair factor expression, namely Interleukin-10 (IL-10) and TGF-β (transforming growth factor β), making laser acupuncture also interesting for wound healing, where also a lot of research is being carried out as was shown in the chapter before.(106,142)

Analgesic Mechanism

Since the first study of acupuncture's analgesia was published in the early 1970s, acupuncture treatment has been shown to operate as effective treatments for various types of pain.(91,92) Most of the clinical trials on acupuncture have been dedicated to pain disorders. WHO recommendations include in a great majority several pain conditions, like Dysmenorrhea, facial pain, headache, neck/knee/low-back pain, renal colic, earache, or cancer pain.(139) Due to its safe, feasible and cost-effective features, acupuncture has become a widespread and popular alternative treatment option, especially for chronic pain disorders.(95)

Clinical and experimental evidence indicates that on the one hand the purinergic pathway plays a central role in acupuncture-induced analgesia, and on the other hand the endocannabinoid system.(95,143) Therefore, this chapter will focus on these two pathways, in this way explaining acupuncture's analgesic mechanism.

Newest findings have shown that purinergic signaling can have an influence on acupuncture-induced analgesia through release of ATP which may activate purinergic P2X receptors especially of the P2X3 type situated at local sensory nerve endings.(143) This ATP-release at purinoceptors (P1, P2x, P2y) during needle acupuncture has shown a modulation of chemical neurotransmission of ascending neuronal pathways ending in higher pain centers, as well as modulation of an increase of action potentials to dorsal root ganglia.(144)

Zhao and team analysed the effects of EA on GB20 (Gallbladder 20) and GB34 (Gallbladder 34) in a rat model of migraine caused by dural neurogenic aseptic inflammation.(145) They found out that acupuncture relieves migraine due to the influence on P2Y12R, the inhibition of microglia activation, and impeding of the synthesis of Cox-2-dependent PGE2 (Prostaglandin E2) in the trigeminal ganglion via suppression of the RhoA/ROCK pathway (Rho-associated protein kinase; a protein kinase, which is regulating the shape and movement of cells by acting on the cytoskeleton), ending in a reduction of plasma protein extravasation.(145) Another basic research study revealed also the inhibition of microglial activation and additionally the reduction of Interleukin-1β, P2X7R, and P2Y1R (subgroups of purinergic receptors), as well as increase of Interleukin- 10 in the hippocampus and

prefrontal cortex, resulting in an escapement of P2 purinoceptors-mediated inflammation.(146)

A similar pathway was found in a study from 2020 with laser moxibustion (wavelength 10.6 μm, treatment dosage 1529J/cm^2) at ST35 in prepared rats with simulating knee osteoarthritis. Their results showed a significant pain relief in acupunctured rats compared to sham-laser treatment ($p<0.05$). Li and team exemplified that acupuncture restrained the production of proinflammatory cytokines, which inhibited the microglial activation-mediated neuroinflammation in the next step.(147)

To sum up, these models strongly show an acupuncture-induced inhibition of hyperalgesia through varied mechanisms, including purinergic pathways. Additionally, while explaining the complex acupuncture-induced analgesic pathways, the influence on neuroinflammatory processes cannot be considered separately, but belong to an overall course.

Research has also revealed a potential link between acupuncture and the endocannabinoid system (ECS), thus suggesting that also this endogenous system might play a key role in the therapeutic effects of acupuncture. The ECS consists of cannabinoid receptors (CBR), and their endogenous ligands and enzymes that mediate the production, absorption, and breakdown of endocannabinoids. This endogenous system is involved in complex physiological and pathological processes, including pre- and postnatal development, the purinergic pathway through the stimulation of e.g., P2X7 receptors, activation of the immune system, pain modulation and stress adaption.(148–151)

Acupuncture seems to activate both the central and peripheral endocannabinoid processes including the release of opioids and the activation of μ-, δ-and κ-opioid receptors as well as CCK-8 in the central nervous system, serotonin, and adenosine.(95) A trial in rats using EA at GB30 and GB34 has demonstrated a significantly reduction of thermal hyperalgesia by activating CB2 receptors, while showing increased levels of the endocannabinoid anandamide (N-arachidonoylethanolamine) in irritated skin tissues.(152) Another interesting study on neuropeptide release due to electrical stimulation on acupuncture points has discovered a dependency on the used frequencies.(153) It has been shown that a

stimulation of 2 Hz accelerates the release of enkephalins and endorphins, whereas 100 Hz stimulates the release of dynorphin to produce analgesic effects.
A review by Hu et al. has concluded that there are many trials revealing an involvement of ECS in acupuncture-induced analgesia, while presenting neuroprotective and cardiovascular regulatory effects as well.(95)

Most of the mentioned trials have investigated EA, however, there exist similar results from the application of laser acupuncture.
A Brazilian trial in mice has investigated PBM (660 nm, 30 mW, 0.06 cm2, 50 J/cm2) and has proved a significant reduction of paw edema through a bracing modulation of CBR-1 and CBR-2.(154) When using a CBR-antagonist, the significant anti-inflammatory effect of laser therapy was found to be reversed. Neves and colleagues have also demonstrated a reduction of IL-6 in paw and spinal cord, and an activation of the anti-inflammatory cytokine IL-10. The study team has concluded that ECS seems to play an essential role in the action of laser therapy.(154)
Two other research teams using similar study designs have also arrived at the same conclusions.
A study in 2013 revealed that laser treatment (830 nm, 3 J/cm^2) at ST36 led to a significant analgesic effect against acetic acid- and formalin-induced behaviour in rat models, mediated by the stimulation of opiodergic and serotonergic (5-HT1 and 5-HT2A receptors) systems. (155)
Another rat model evaluating postoperative pain has used different laser dosages. This has revealed for the first time a significant pain reduction with 3 or 8 J/cm^2, but not with 1-2, 4-7, or 9-12 J/cm^2. Measurements of IL-1-β and TNF-α has shown a significant decrease after laser therapy with 3 and 8 J/cm^2. Pereira et al. have also postulated a main acupuncture-induced modulation of ECS.(156)

These fascinating discoveries seem to strongly advocate that ECS might be one of the primary mediators as well as a regulatory factor of all the beneficial effects of acupuncture.

Next to these vast research topics in basic acupuncture studies, other but no less important mechanisms have been found over the last years. Acupuncture has been

found to induce processes also in the neuro-immune system, like cholinergic anti-inflammatory, vagus-adrenal-, medulla-dopamine-, or sympathetic-pathways, and the HPA axis.(138)

Therefore, we can conclude all in all that acupuncture induces a cascade of various supportive pathways, making acupuncture and related techniques an eligible potential therapy option in a variety of disorders and diseases.
Nonetheless, all results are only small pieces in a huge puzzle, where not all mechanisms of the impact from acupuncture are yet fully understood.

Acupuncture in The Pediatric Population

Although acupuncture is a gentle treatment tool, it is a relatively new research field in children and neonates.(157,158) Acupuncture treatment for pain in the pediatric population has been investigated in several studies in children, infants and neonates, including headache, abdominal pain, fibromyalgia, juvenile arthritis, complex regional pain syndrome, cancer pain, as well as perioperative or procedural pain.(159–162) In most cases, acupuncture has demonstrated a pain-relieving effect and has met with a good acceptance by patients and parents, and has been suggested as a safe and cost-effective treatment modality for pediatric pain within standardized guidelines and in compliance with all safety precautions.(163–166) Additionally, there are also studies in neonates and infants suffering from infantile colic. In 2010, a study investigated infantile colic in 2 to 8 weeks old patients and found a reduction in total duration of fussing, crying and colicky crying in the acupuncture group compared to no intervention.(162) Next, Swedish researchers conducted a multicentrer randomized controlled three-armed trial between 2013 to 2015, under the name 'ACU-COL'.(167,168) They investigated 147 infants, randomly allocated to standardized acupuncture at LI4, semi-standardized individual acupuncture or no acupuncture. In their first results, a reduction of time crying in both acupunctured groups was revealed.(167) In secondary analysis and follow-up, more parents of acupunctured persons reported an improvement in feeding and sleep behaviour as well as colic symptoms compared to no intervention.(168)

Concerning the safety of acupuncture in pediatric population, studies have shown a good tolerance in children as well as in neonates for different acupuncture modalities.(169–171) Although the neonatal skin is more vulnerable compared to that of adolescents and adults, laser acupuncture and non-invasive electrical stimulation of acupuncture points seems to be safe in late preterm and term neonates.(165,172–174)

Neonatal Pain Studies

Apart from this doctoral thesis, 6 studies have been conducted since 2010 in total, whereas 3 of them have demonstrated a statistically significant pain reduction in acupunctured groups as mentioned in the Introduction. Alongside to 2 laser acupuncture trials, there were 4 more studies with various acupuncture techniques. As we can see, this research field is relatively new, and numbers of conducted trials are manageable.

Beside laser acupuncture, there exist various different acupuncture modalities in general and especially in neonates. Particularly in neonates, a feasible and painless application is worthwhile. Despite one clinical trial with light needling (insertion of an acupuncture needle for a few seconds), other studies have dealt with acupressure, electrical stimulations of acupuncture points (NESAP) or sticking ear-plasters.

However, these studies have shown not only a multimodal therapy concept but also high heterogeneity in regard to the used acupuncture points. These facts make it difficult to compare them directly. Nevertheless, we can learn which acupuncture modality appears to work better than another as well as which acupuncture points have proved to be easier to use or presented a better outcome. Acupuncture points in detail will be presented in a separate chapter below. All acupuncture trials investigated a heel lance for blood collecting as painful intervention, because of its common usage in neonatal routine care.

Laser Acupuncture

Laser acupuncture has been used in 2 studies, with the first study revealing a better pain relief in acupunctured group, while the second one did not.

In 2010, Gottschling et al. conducted a double-blinded randomized controlled trial and used PIPP for pain assessment in 50 term neonates between 2nd and 5th day of life. The intervention group received laser acupuncture with 30 mW (power density 3,8 W/cm^2, 0.45J/point, 830 nm) soft laser for 30 seconds at LI 4 and Shen Men, and the control group placebo laser acupuncture (not activated laser) prior to heel lance without further pain-relieving supply (e.g., oral sucrose solution, pacifier, or SSC). They found a significant difference between intervention and control group favouring laser acupuncture (PIPP score 6.4±1.9 versus 8.2 ±3.3, p = 0.013).(175)

The second trial by Abbasoglu and colleagues used NIPS to assess pain levels in 42 term neonates between 3rd and 8th day of life. The intervention group received laser acupuncture with a 10 mW laser (0.125 J/point, 905 nm, 10Hz) for 30 seconds at Yin Tang, while the control group was treated with 0.5 ml of 24% oral sucrose solution. The results showed a significantly higher NIPS score in the acupuncture group than in the control group (4.52±0.87 versus 3.66±1.01, p = 0.006).(175,176) Additionally, their secondary outcomes showed a significantly shorter mean procedure time (86.33±23.90 sec vs. 104.76±18.33 sec, p = 0.008), but a longer mean crying time (97.95±34.23 sec vs. 46.66±37.82 sec, p. = <0.001) in the acupuncture group compared to control group.

Light Needling

In 2011, Ecevit and colleagues conducted the first and only neonatal needle acupuncture study for pain relief during minor painful interventions in 20 preterm neonates.(177) The age of the participant was not clearly defined. In their cross-over, randomized controlled trial, they applied light needling for 30 seconds at Yintang in the intervention group. The control group received only routine care consisting of 2ml/kg expressed breast milk before heel lance and a pacifier. The results demonstrated a significantly lower pain score NIPS in the intervention group than in the control group (4.2±1.9 versus 6.1±0.8, p = 0.00).

Although it was an invasive acupuncture method, none of the neonates cried during the application of the acupuncture needle. Additionally, crying duration was also significantly lower in the intervention group. Authors observed a relaxing sleep and a

significant decrease in heart rate. All these factors can be possible sedative effects of acupuncture in general and of the acupuncture point Yintang.(178,179)

Non-invasive Electrical Stimulation of Acupuncture Points

Mitchell and team have designed a double-blinded, placebo-controlled, randomized controlled trial, investigating the TENS application as non-invasive electrical stimulation of acupuncture points.(180) The study has evaluated 4 groups with a total of 121 term neonates between 1st to 3rd day of life and has obtained the following main outcome of PIPP changes from baseline during heel lance: (1) NESAP with 1ml of 24% sucrose solution showing the lowest PIPP score changes (3.6±1.2; $p<0.05$), (2) NESAP with water having the highest PIPP changes from baseline (5.0±4.0; $p<0.01$), (3) Sham-NESAP with 1ml of 24% sucrose solution generating PIPP scores changes of 4.0±1.8 ($p<0.05$) and (4) Sham-NESAP with water with similar results as group 2 (4.9±4.0; $p<0.01$). They have stimulated the following 4 acupuncture points equally: Bl60 (Bladder 60), KI3 (Kidney 3), ST36 (Stomach 36) and SP6 (Spleen 6).

This study is more difficult to interpret because outcome parameters had been defined as pain score changes, while all other trials only assessed the pain score at time of heel lance. Therefore, a direct comparison with literature is not possible.

The results have shown the pain score to be less when applying sucrose solution, additionally with best results when combined with acupuncture. However, acupuncture administered alone and the placebo group have shown comparable high pain score changes.

In general, TENS can be applied using self-adhesive electrodes placed on the skin above acupuncture points without the need of needles to stimulate.(90) This non-invasive method can help to supplement the pharmacological management of pain.(181,182) The therapeutic technique is a new approach in neonatal acupuncture methods. Preliminary studies by Yates et al. have demonstrated its safety and technical parameters.(172)

Because of its very new usage in neonatal patients, experiences with these technical values are just in its infancy and must be evaluated in further studies in more detail. Perhaps applying different frequencies or intensities of TENS could demonstrate a more favorable response.(180)

Acupressure

A further study, carried out by Abbasoglu and colleagues, has researched acupressure at KI3 and BL60 for 3 minutes in 32 preterm neonates with gestational age between 28 and 36 weeks. This randomized controlled trial has used PIPP for pain assessment. The control group has been defined as routine care, but without detailed information. The PIPP score here has not been found to be significantly different between groups with 9.13 ± 1.99 in the intervention and 9.56 ± 1.71 in the control group (p = 0.52). Besides, the mean duration of procedure and resulted in a significantly shorter time in acupressure group (123±60.4 sec) contrasted to control group (184.56±43.09 sec) (p = <0.001). Additionally, significantly shorter mean duration of crying was also observed in acupunctured preterms (103.93±72.87 sec vs.190.31±43.83, p = 0.001).

Positive aspects of this study have been the simple and feasible application of acupressure. This acupuncture method is really one of the oldest techniques and can be utilized at any time by any person. Localisation of selected acupuncture point scan be easily trained to medical staff and applied before minor painful intervention. However, effective acupuncture points for pain relief or other areas must be investigated first.

A critical point for further comparison in the described research is the lack of additional information of the control group. Routine care depends on clinic specific standardized procedures. Therefore, for good scientific practices, a control group must be defined satisfactorily.

Auricular Non- invasive Magnetic Acupuncture

The latest study in this repsect has researched another practicable acupuncture method, namely auricular non-invasive magnetic acupuncture.(183) In the course of it, 40 neonates have been randomized either in a magnetic acupuncture group or a placebo group with magnetic or placebo stickers being placed bilaterally to five auricular acupuncture points following the Battlefield Acupuncture protocol.(184) In this trial, acupuncture has been applied additionally to oral sucrose application. Routine care has been provided for all subjects and predefined as oral sucrose administration before heel lance or further individual analgesia (including nurse-directed sucrose, kangaroo care, breastfeeding and, swaddling). The intervention

group was in mean 34.1 (SD 2.6) weeks of gestational age with a mean age of 9.6 (SD 8.7) days of life and the control group 34.4 (SD 2.9) weeks and 6.5 (SD 7.6) days, respectively. The results have shown a significantly lower pain score PIPP during heel lances in the intervention group compared to placebo group (mean (SD): 5.9 (3.7) vs. 8.3 (4.7), p = 0.04). Secondary results showed no significant difference in heart rate or oxygen saturation between groups before, during and after heel lance.

Utilzations here have been magnetic sticking ear-plasters, which generate a static, magnetic field due to continuous placement of magnets on ear acupuncture points. Analgesic effects can be explained by sensory neurone blockades, changes in local blood flow or remodelling of the local microvasculature.(185)
Authors have concluded that auricular non- invasive magnetic acupuncture seems to be safe and feasible and that it gives a new approach in non-pharmacological pain management.

Chen and colleagues have demonstrated first results of Battlefield acupuncture in neonatal pain management, which seems promising for clinical routine. In further studies, it would be interesting to investigate acupuncture compared to oral sucrose administration, because alternatives to routinely used sucrose application is urgently needed. By way of criticism it must be said that here oral sucrose has been used as routine care. Furthermore, additional analgesia has been allowed individually, which may well have distorted study results due to the unknown impact. Nevertheless, this trial has generated important facts about magnetic ear-plasters in neonatal care and about Battlefield acupuncture.

Summarization

To summarize, we can see a huge variance of used acupuncture techniques of which 3 studies carried out have shown a pain-relieving effect in acupunctured groups conducted by Gottschling and team, Chen et al. as well as Ecevit and colleagues.(175,177,183) Acupuncture enable an individual management based on preferences of the patient and the acupuncturist. Its strengths are the manifold technique options, and a variety of acupuncture points with a low side effect rate.

Acupuncture Points used in Neonatal Pain Studies

In this section, we will have a closer look to used acupuncture points in trials described above. They employed body as well as ear acupuncture points. Body acupuncture points are based on the stimulation of points along defined energy channels (so called meridians). However, ear acupuncture or auricular therapy is based on the idea that the ear is a micro system, which reflects the entire body and can be also stimulated using acupuncture practices. All acupuncture points here are described following the standard of international acupuncture nomenclature, which indicate an English name and abbreviation, the Pinyin name (Chinese name) and its meaning (translation of the Pinyin name).(186) In addition, the acupuncture treatment is described according to the established guidelines of Standards for Reporting Interventions in Clinical Trials of Acupuncture (= STRICTA).(187)

Large Intestine 4

In traditional Chinese medicine, every acupuncture point has its own application area. One of the most "powerful" and frequently used points is called Large Intestine 4 (LI 4), in Chinese "He Gu", which means "Union Valley".

LI 4 is located on the dorsal side of the hand in the middle between 1st and 2nd metacarpal bones, in direction to the middle of the 2nd metacarpal bone (see *figure1*). Its main field of use is any kind of pain.(89)

We have used this acupuncture point in our trial because of its general indication in traditional Chinese medicine and its well-studied effect on infantile and neonatal pain. The Swedish trials mentioned above have used LI4 in infantile colic to reduce crying and pain.(162,188) Reinthal et al. have used also light needling acupuncture at LI 4 in infants with colicky crying, showing that gastrointestinal disorders and the crying duration could be reduced significantly.(160) Moreover, the neonatal study carried out by Gottschling et al. has also utilized LI4 combined with an ear-acupuncture point.(175)

Lastly, clinical neuroimaging trials have shown that EA at LI4 and LI3, both used for its analgesic effects, can influence the functional connectivity between brain regions

in the pain matrix, thus modulating the pain perception of healthy volunteers under evoked experimental pain.(189)

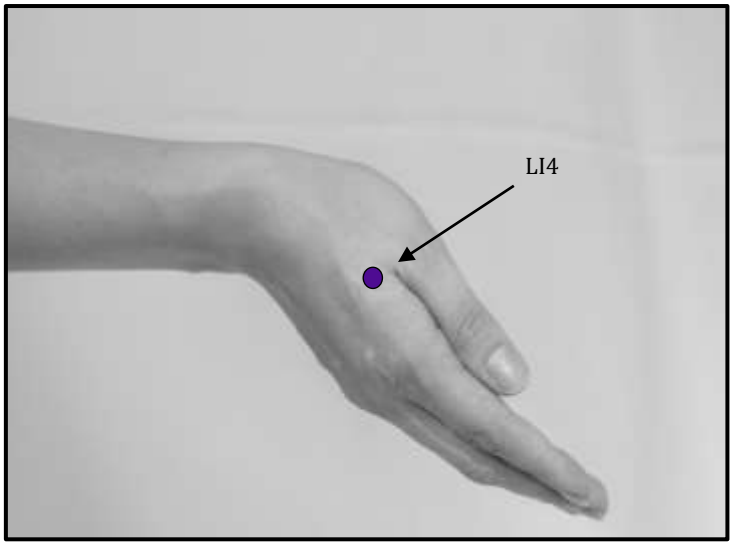

Figure 1: Acupuncture point Large Intestine 4 on the right hand.

Yintang

Yintang is the Chinese name, also known as *the Hall of Impressions*, and classified in English acupuncture nomenclature as EX-HN3 (Extra - Head and neck point 3).(90) It is located on the front head between the eyebrows, also known as the third eye area, and is generally acupunctured for local issues like headaches, allergies, sinusitis, vertigo, heavy sensation in the head, eye disorders, but also anxiety, insomnia or stress.(89) The location is shown in *figure 2* (red point). Its pain-relieving effect is more locally limited (e.g. headache) than for general pain management. Nevertheless, it can have a beneficial influence on reducing stress and anxiety during painful procedures. This effect can be observed as reduction of the pain score in the trial carried out by Ecevit et al, who has needled this point.(177) Otherwise, Abbasoglu et al. have applied laser acupuncture at Yintang, reporting a higher pain scores in acupunctured group.(176) In this case, it can only be suspected that the energy dosage used has been insufficient for pain reduction in the intervention group.

An interesting study by Wang et al. has reported decreased pre-procedural anxiety and reduced intra-procedural Propofol requirements when applying acupressure at

Yintang in a group of pediatric patients 30 minutes before the patients underwent general anesthesia for gastrointestinal endoscopic procedures. (190)

To summarize, the utilization of the acupuncture point Yintang in neonates seems to be limited because of its location and application area and should be avoided especially for laser acupuncture.

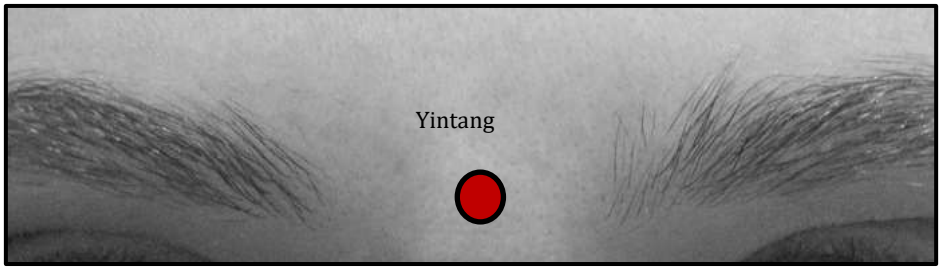

Figure 2: Acupuncture point Yintang (red point) between eyebrows.

Bladder 60 and Kidney 3

In one of the studies by Abbasoglu et al., they have applied acupressure for 3 minutes at BL60 and KI3, and also in Mitchell's trial these points have been used in combination with 2 others. BL60 is called Kunlun in Chinese, which is a name for *the mountain belt that runs across the centre of China*. The area of application includes pain in the shoulder, back and arm, swelling and pain of the heel, also headache, blurring of vision and neck rigidity, and is one of the main points to induce labor.(89) The point can be found behind the outer ankle joint, in the depression between the prominence of the lateral malleolus and the Achilles tendon (see *figure 3* or *4*, yellow point).

In combination with KI3, which is the mirror point on the inside the ankle, it represents an arrangement between yin (KI3) and yang (Bl60), as principal concept of traditional Chinese medicine, resulting in a powerful way to enhance our essence (jing). Therefore, in acupuncture treatments, it is very often combined.

KI3, which is located between the medial malleolus and the Achilles tendon, is called Taixi and symbolizes the great ravine stream (location see *figure 3* or *4*, orange point). This acupuncture point is employed for chronic back pain, local pain of the

heel, chronic weakness, gynecological or urogenital disorders or psychosomatic conditions.(89)

In the study by Abbasoglu et al., these points have been selected because there are nociceptor-rich areas at the ankle near the site where heel lancing is done.(191) They have inferred that applying acupressure activates the analgesic system, resulting in release of serotonin, which has also a vasodilatory and capillary permeability effects.(192–194) That effect could be the reason why authors have found a shorter procedure time for heel lancing after applying acupressure.

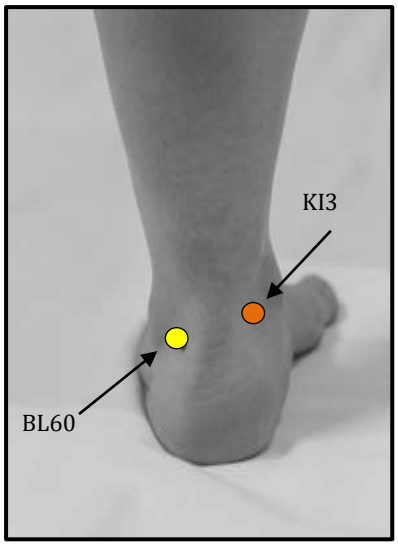

Figure 3: Acupuncture points Bladder 60 (yellow point) and Kidney 3 (orange point) on the left leg.

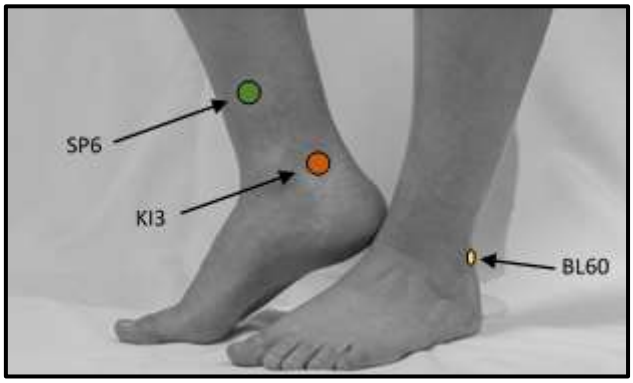

Figure 4: Acupuncture points Spleen 6 (green point), Kidney 3 (orange point) and Bladder 60 (yellow point) on both legs.

Stomach 36 and Spleen 6

Mitchell and colleagues have expanded the couple Bl60 and KI3 with another very popular one, namely Stomach 36 (ST36) and Spleen 6 (SP6). They have applied TENS electrodes at all these points bilaterally before painful procedure. ST36 is called Zusanli, which symbolize *to go three villages further* and is therefore often stimulated by moxibustion in elderly before long walks. It is said to balance our energy, relieve pain (especially in the lower limb), and treat a number of health issues and chronic diseases.(89) Its pain relief is focus of a whole series of trials and can be acupunctured in diabetic neuropathy of the lower limb, visceral pain, acute angina pectoris and chemotherapy-induced peripheral neuropathy.(195–198) Zusanli is located about a hand-wide below the knee on the outer side of the leg (see *figure 5*, blue point).(137)

Its couple, SP6, is termed Sanyinjiao and stands for *three Yin intersection*, because the Yin meridians "spleen", "liver" and "kidney" are crossing this point. It is medial on the lower leg, 4 fingers wide above the medial malleolus, on the posterior border of the medial aspect of the tibia (see *figure 4* or *5*, green point). The main areas of indication are all gynecological disorders, urogenital diseases, chronic dysfunction of the gastrointestinal tract and psychosomatic conditions.(89) It is additionally used in painful dysfunction of the lower leg and ankle joint. There are several trials that have proved the pain relieving effect of SP6 during labour and dysmenorrhea.(199,200)
ST36 and SP6 in combination is basic for regulating the function of our stomach and indigestion as well as general weakness.

To summarize our knowledge, a combination of these 4 points, Bl60 + KI3 + ST36 + SP6, can be a powerful ensemble for painful conditions in general and especially of the lower limb. Therefore, trial setup with these points seems to be thought-out and effective. Nevertheless, Mitchell et al. have found a higher pain score in the acupunctured group as compared to the control group with the examined parameters. It is discussable, if pain reduction with other parameters of TENS therapy (for example different frequencies or intensities) would have been more successful.

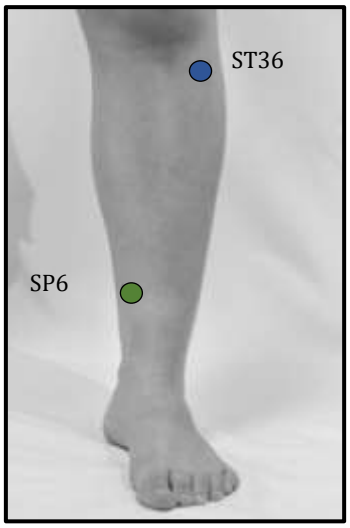

Figure 5: Acupuncture points Stomach 36 (blue point) and Spleen 6 (green point) on the left leg.

Ear acupuncture points

Auricular therapy has been used by Gottschling and team combined with LI4 as well as by Chen et al. with the so-called Battlefield acupuncture protocol (BFA).(183)
The laser acupuncture study has applied Shenmen as auricular point, which is called point 55 in auricular medicine. Its position is as shown in *figure 6* (violet point). Shenmen is the main auricular point in pain therapy, corresponding to LI4.(201) Therefore, it is one of the main points in the NADA protocol or Battlefield protocol.(184,202) NADA means 'National Acupuncture Detoxification Association' and is a protocol which includes 5 ear acupuncture points for withdrawal.

The study with magnetic ear plasters has employed the BFA procedure. This protocol has been developed for the effective management of acute pain.(184) Functional MRI has shown modulatory effects on neuronal immunohistochemistry, neurotransmission and essential cortical structures (for example orbitofrontal or limbic regions).(203) The BFA consists of 5 auricular points shown in *figure 6* and has been stimulated sequentially in the following order on each ear: 1 – Cingulate Gyrus (presented on figure 12 in green), 2 – Thalamus (yellow), 3 – Omega 2 (mangan), 4 – Point Zero (red) and 5 – Shenmen (violet). Additionally, the used magnetic acupuncture is postulated to generate a static magnetic field, which results from the continuous placement of magnets and induces analgesia by causing sensory neurone blockades, alterations in auricular blood flow and transformation of the local microvessels.(185) Advantages of ear acupuncture in general and especially with

magnetic plasters seems to be feasibility and simple application in neonates. Magnetic plasters can be stimulated several times a day not only by doctors but also by different medical staff. Additionally, this concept can be modified in various application fields, for example in neonatal abstinence syndrome.(204) Ear acupuncture is in its infancy, but the knowledge is rapidly becoming more sophisticated.(205)

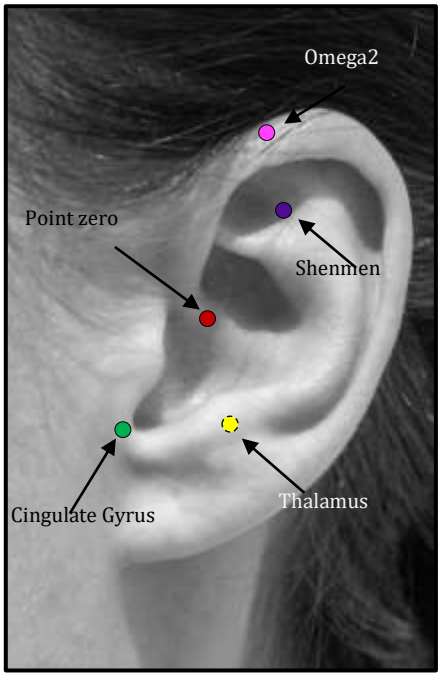

Figure 6: Ear acupuncture points Cingulate Gyrus (green point), Thalamus (yellow point, on the inside), Omega 2 (mangan point), Point Zero (red point) and Shenmen (violet point) on the left ear.

Summarization

All used acupuncture points have been selected due to analgesic and/or local effects and have shown good results in 3 studies. LI4 combined with Shenmen, Yintang with light needling and Battlefield ear acupuncture represent the most successful acupuncture points.

Part 4: Hypothesis

To summarize, pain in neonates can lead to short-term and long-term consequences and therefore, an adequate pain management is required. Non-pharmacological treatments, like swaddling, sucrose solutions or skin-to-skin contact, are preferred in preterm and term neonates. Acupuncture also seems to be an innovative non-pharmacological option due to its analgesic effects. The majority of the acupuncture research focuses on pain management in different areas and is also undergoing investigation in the pediatric population.

Nevertheless, there are only a few trials that have so far considered acupuncture in neonatal pain relief and therefore, more studies are required in this research field. Moreover, only two studies have explored laser acupuncture regarding this specific subject. Furthermore, there is good evidence that the usage of laser acupuncture is safe in neonates without harming the neonatal skin.

We have conducted a laser acupuncture trial for non-pharmacological pain management in neonates and have shown a relatively new acupuncture technique regarding the current standard of care. We have chosen an oral glucose solution for the control group and attempted to compare it by way of a non-inferiority concept.
In conclusion, we have been striving to prove that laser acupuncture at LI4 can actually reduce pain associated with minor painful procedures in healthy term neonates in a way non-inferior to orally administrated glucose solutions.

Material and Methods

Study Design

This single center, observer-blinded, non-inferiority, randomized, clinical controlled trial started on March 14th, 2016, and ended on April 27th, 2018. The trial was conducted at the Division of Neonatology, Department of Pediatrics and Adolescent Medicine, Medical University of Graz, Austria, Europe.
This trial was registered in German Clinical Trials Register with the registry number DRKS00010122 on March 2nd, 2016.

Participant enrolment and eligibility criteria

Inclusion criteria were:

- Healthy term neonates (> 37. gestational age).
- Without clinical and/or congenital complications and/or need for intensive care
- After spontaneous delivery or Caesarean section.
- Receiving heel lance for metabolic screening between 2nd and 5th day postnatal.
- Written informed consent signed by the parents.

We informed parents of neonates matching the inclusion criteria on the first days after delivery. After requiring a signed written informed consent, we included them for a heel lance for metabolic screening on the 3rd - 4th day of life. We didn't enrolled participants, if listed inclusion criteria were inapplicable or if there was current pain therapy in the participant or breastfeeding mother.

Study Groups

The intervention group received laser acupuncture and was therefore called Acupuncture Group (AG). The control group received as standard of care an oral glucose solution and was named Glucose Group (GG).
AG: Neonates underwent laser acupuncture using LABpen MED 10 (10mW Laser, Behounek, Graz, Austria), applied at Large Intestine 4 (LI 4) for 60 seconds at the right and left hands (*figure 7*). We obtained an energy dosage of 34J/cm^2 (0.6J/point).

GG: Participants received 30% glucose solution orally (Glux® from Pharma Stulln, Germany) over a period of 30 seconds.

Figure 7: Laser acupuncture with LABpen MED 10 at Large Intestine 4.

Randomisation and Allocation

Participants were randomly assigned to AG or GG in a 1:1 ratio, using the Randomizer for Clinical Trials tool (http://www.randomizer.at/) developed at the Medical University of Graz.(206) The randomization block size for participants was 6.

Intervention

Included neonates on 3rd to 4th day of life were brought to a specially prepared room, which was safe for laser acupuncture. We followed a strict treatment protocol to guarantee a similar treatment manipulation regarding intervention times and surrounding conditions (e.g. room temperature, light condition, warmed heels for an easier blood draw).

Preparation time

Neonates were placed in supine position on examination table without being swaddled or receiving a pacifier (*figure 8*). All participants were monitored continuously with a pulse oximeter (IntelliVue X2, Philips Medicin System, Boeblingen GmbH, Boeblingen, Germany) across the right wrist to measure heart rate and oxygen saturation.

All of them received an eye protection (Natus Biliband Eye Protector, Natus Medical, San Carlos, California). Heels were warmed for an uncomplicated blood sampling. When preparation was finished, we randomized them in AG or GG.

Figure 8: Preparation time. Neonate was placed in supine position, monitored with a pulse oximeter and eyes were protected. Video was recorded as shown in the picture.

Intervention time

Intervention time took 150 seconds (2.5 minutes) in total in both groups. Interventions were carried out by the same medical doctor to guarantee similar manipulation.

AG: We acupunctured in total 2 minutes (1 minute per side) and waited 30 seconds until we performed heel lance.

GG: We applied oral glucose solution over 30 seconds. Then we waited 2 minutes until heel lance.

The eye protector was removed in both groups 30 seconds before heel lance.

Heart rate, oxygen saturation and state of arousal were documented just before heel lance.

Procedure time

Prior to heel lance, video recording of the participant started. The skin was prepared using a disinfectant (kodan®forte farblos, Schülke&Mayr GmbH, Norderstedt, Germany) and cellulose swabs (Profümed®, Profümed GmbH, Grimmenstein, Austria). Afterwards, heel lance was carried out with an automatic lancet

(gentleheel®, Alleset Healthcare Solution B.V., Netherlands) by the same medical doctor.(207) During procedure time, we documented heart rates and oxygen saturations as well as crying time. After standardized blood sampling of 1 capillary tube (correspond to 0,15ml blood) for metabolic screening, the procedure was completed.

Video recordings and participation in the study ended after blood sampling. *Figure 9* shows an overview of the study course.

Video recordings

The videos started 15 seconds prior to heel lance and lasted until blood sampling was completed. A certified pediatric nurse analyzed these video recording and determined PIPP scores for each participant blinded to group allocation (observer blinding).

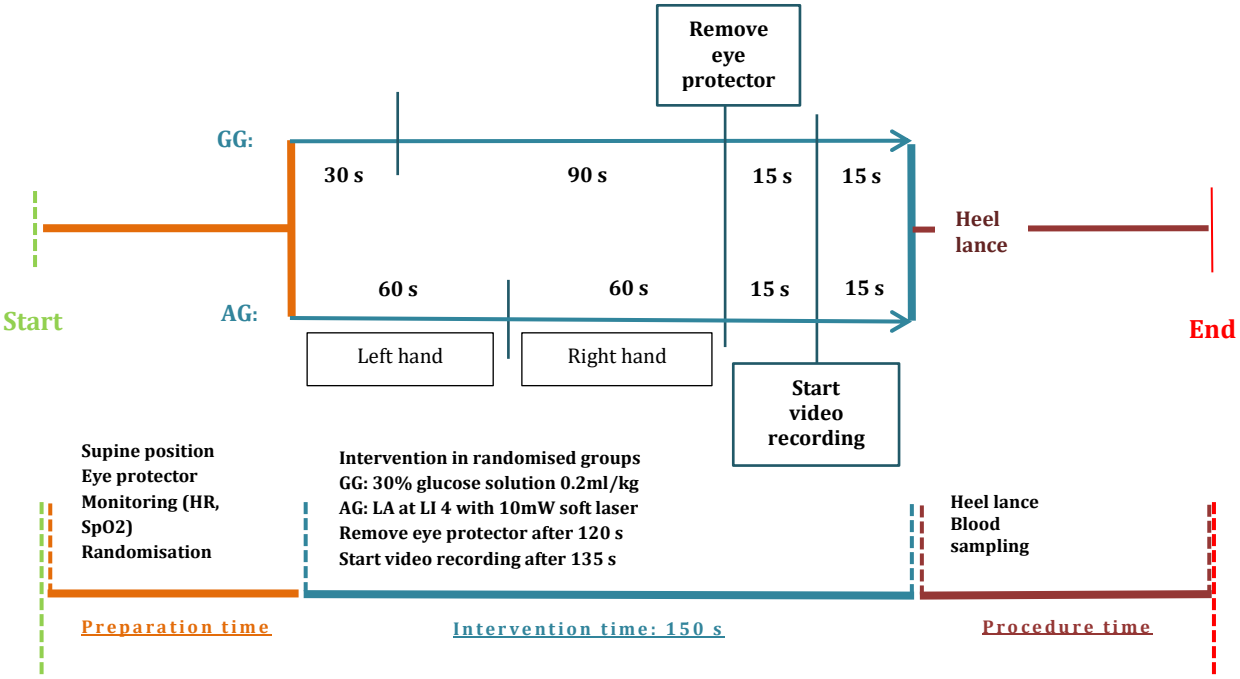

Figure 9: Study course of intervention. AG, acupuncture group; GG, glucose group; LA, laser acupuncture; LI 4, Large Intestine 4; s, seconds.

Outcome parameters

Primary outcome was the difference in PIPP score between groups.

Secondary outcomes were:

- Difference in heart rate change from baseline
- Difference in oxygen saturation change from baseline
- Difference in crying time (in seconds)
- Duration of blood sampling (in seconds)
- Adverse events during study course

Minor Painful Intervention

We have chosen a common minor painful intervention carried out in every neonate between 2nd and 5th day of life in Austria: the metabolic screening. The National Austria Newborn Screening Program started in 1966 and identifies inherited metabolic and endocrine disorders.(208)

Oral Sweet Solution

Glux® is a routinely used oral glucose solution in our NICU and is recommended for minor painful interventions as described in the Austrian interdisciplinary guidelines for perioperative pain management.(209) Application should be done 2 minutes prior to a painful intervention using a dosage of 0.2ml/kg body weight.

Technical Parameters and Safety Precautions of Laser Acupuncture

We used a continuous wave semiconductor GaAs (= Gallium Arsenide) laser with wavelength of 675 nm, an output power of 10 mW and diameter of 1.5 mm.(210) The low-level laser was classified to the European Norm (EN 60825-1) with class 3R. That meant that laser radiation could cause serious eye damage. Therefore, safety precautions were necessary to protect therapist and probands. The therapist and any other person in the treatment room wore specific protective glasses during laser acupuncture. Neonates received an eye protector that is usually used during phototherapy for neonatal jaundice, which was approved by the Department of Technical and Organizational Safety of the Medical University of Graz also for low-

level laser usage.(211) We decided to apply this eye protector to all included neonates to insure an equal manipulation in both study groups.

Sample Size

Sample size calculation was based on a study by Gottschling et al., who reported a PIPP of 6.4 ± 1.9.(175) Computation was performed using the one-sided t-test. To reject the null hypothesis that the study groups are not equivalent expecting a difference in means of 0, assuming a clinically important difference of ≥1.0 (Δ) PIPP, we calculated a sample size of 90 probands. This sample size would yield a power of 80%. Including a dropout rate, 95 probands had to be randomized.

Statistical analysis

We performed our statistical analysis with IBM SPSS (Statistical Package for the Social Sciences, Inc. New York, USA for Windows Version 24.0) and SAS 9.4 (state-of-the-art statistical analysis software; 2002–2012 by SAS Institute Inc., Cary, NC, USA) as statistic programs.

We used t-test or Mann Whitney U-test for continuous variables and chi-square test or Fisher's exact test for categorical variables, as appropriate for comparison of baseline characteristics.

PIPP-scores were compared with the one-sided t-test. Intention-to-treat principle was applied with no imputation for any missing data. Non-inferiority was achieved if the upper boundary of the two-sided 95% confidence interval (CI) for the median difference between AG and GG was less than the margin, Δ = 1. Hodges-Lehmann estimation of location shift with asymptotic Hodges-Lehmann confidence limits was used to calculate CI.

The comparison of secondary outcomes was done with t-test and chi-square test or Fisher's exact test, as appropriate. Not normally distributed continuous data was analyzed with non-parametric tests.

Results

Enrolment and Allocation

We explained our study to 188 parents and obtained 102 signed written informed consents. Of these 102 approvals, 6 were not realizable due to organizational difficulties.

Finally, 96 participants were randomized and received either acupuncture or oral glucose solution as intervention. One participant was excluded from statistical analysis as parents withdrew approval (randomized to AG). In 4 cases (AG: 3 and GG: 1), technical problems resulted in lack of feasibility of statistical analysis of outcome parameters: in 1 case the video recording was insufficient and in 3 cases the pulse oximeter didn't work.

Consequently, we included 91 participants in statistical analysis of primary and secondary outcome parameters (AG: 44, GG: 47). However, baseline characteristics were evaluated in 95 participants.

Consort flow diagram shows this workflow of enrollment, allocation, and analysis(212), *figure 10*:

Consort flow diagram

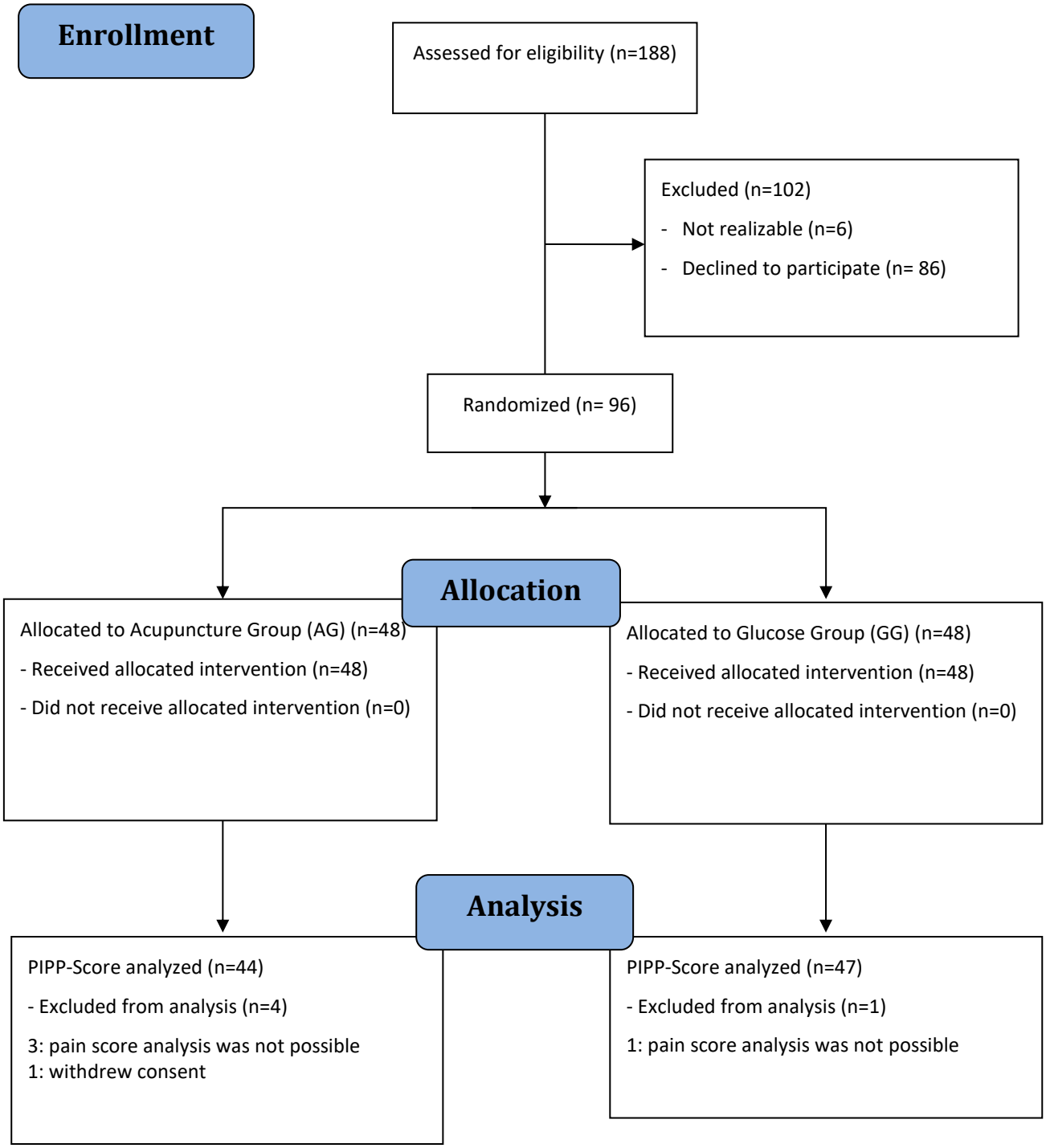

Figure 10: Consort flow diagram. *Reproduced from (212) with permission of SAGE publisher.*

Baseline Characteristics

If we have a closer look on our baseline characteristics in *table 2*, there were no significant differences between groups. In all neonates (n = 95) sex was balanced (48 male and 47 female) and gestational age was in median 38.9 gestational weeks (IQR 39.9 – 40.9).

The mean of the birth weight was 3310g (± 362g) and therefore in an average range [mean 45th percentile (15th -50th)]. Birth height [51cm (49-52)] and head circumference [35cm (34 – 35.5)] were also in an average range in all participants.

We had more spontaneous birth than Cesarean section (spontaneous: Cesarean section = 61 : 34), but well balanced between groups: (31:16 in AG and 30:18 in GG). The intervention took place in median on the 3rd day of life (IQR 3-4) in both groups (p=0.894) as planned.

	All (n=95) Median (IQR) or mean±SD	Groups AG (n=47)	GG (n=48)	p-value
Gender (m/w)	48/47	24/23	24/24	Pearson Chi-Square: 0.917
Gestational Age (weeks)	38.9 (39.9 – 40.9)	40.1 (38.9 - 40.9)	39.4 (38.6 - 40.8)	0.29
Day of life	3 (3 – 4)	3 (3 – 4)	3 (3 – 4)	0.894
Birth weight (g)	3310 ± 362	3368 ± 373	3253 ± 345	0.123
Percentile birth weight (g)	45th (15th -50th)	45 (10th – 60th)	40 (20th – 50th)	0.395
Birth height (cm)	51 (49-52)	51 (49 - 52)	50 (49 - 52)	0.446
Percentile birth height (cm)	50th (35th – 80th)	50th (25th – 75th)	50th (40th – 80th)	0.628
Head circumference	35 (34 – 35.5)	35 (34 - 36)	35 (34 – 36)	0.312
Percentile head circumference	50th (10th – 70th)	50 (10th – 80th)	48 (10th - 60th)	0.305
Birth mode:				
- Spontaneous	61	31	30	Fisher's exact: 0.988
- Cesarean section	34	16	18	
Apgar 5 min	10 (10 - 10)	10 (10 - 10)	10 (10 - 10)	0.649

***Table 2**:* Baseline characteristics in participants. *Partial reproduced from (212) with permission of SAGE Publishing.*

Primary Outcome

As we can see in *figure 11*, the median PIPP score in both groups was 12 with an interquartile range of 10 – 14 in AG and 9 – 14 in GG (p=0.981). Although we had equal PIPP scores in both groups, the upper bound of the 95% confidence interval of the location shift in PIPP score comparing AG and GG was 1.000059 and therefore slightly above the predefined upper margin of <1 (see *figure 12*).

The common standard deviation of the sample was 3.4 and hence higher than expected. Basically, as mentioned in chapter 'sample size', our calculations were based on results of Gottschling and colleagues, who reported a standard deviation in PIPP score of 1.9.(175). Because of a higher common standard deviation in our sample, we only reached a power of 40% to detect non-inferiority. Additionally, this higher common standard deviation widened the 95% confidence interval. Therefore, a higher number of participants would be needed to decrease the width of the 95% confidence interval and reach a non-inferior result according to the predefined margin of upper 95% confidence interval of the location shift in PIPP score <1.0.

Consequently, we could not state that laser acupuncture is non-inferior to oral glucose solution. Figures 4 and 5 are reproduced from (212) with permission of SAGE publisher.

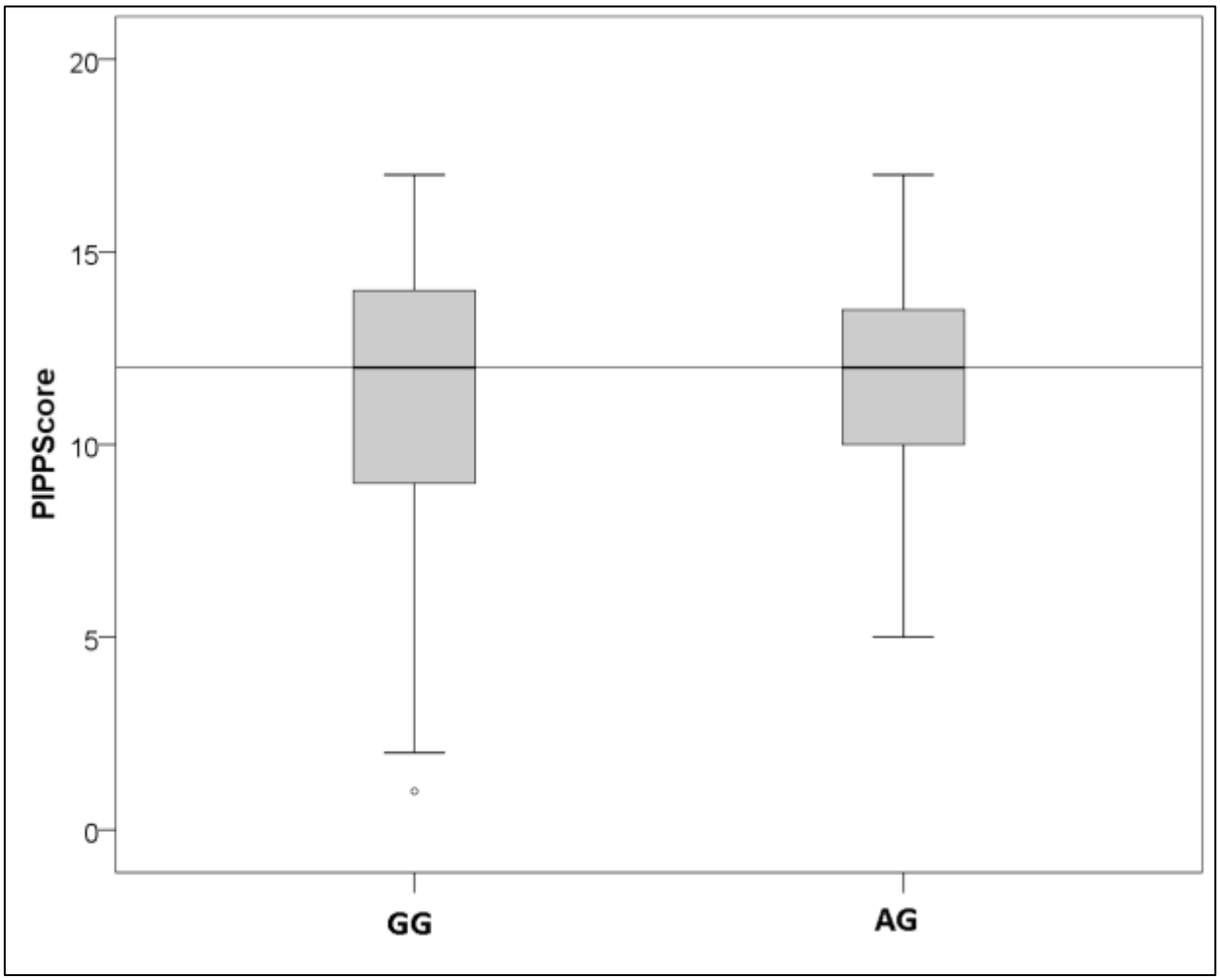

Figure 11: PIPP boxplot. *Reproduced from (212) with permission of SAGE publisher.*

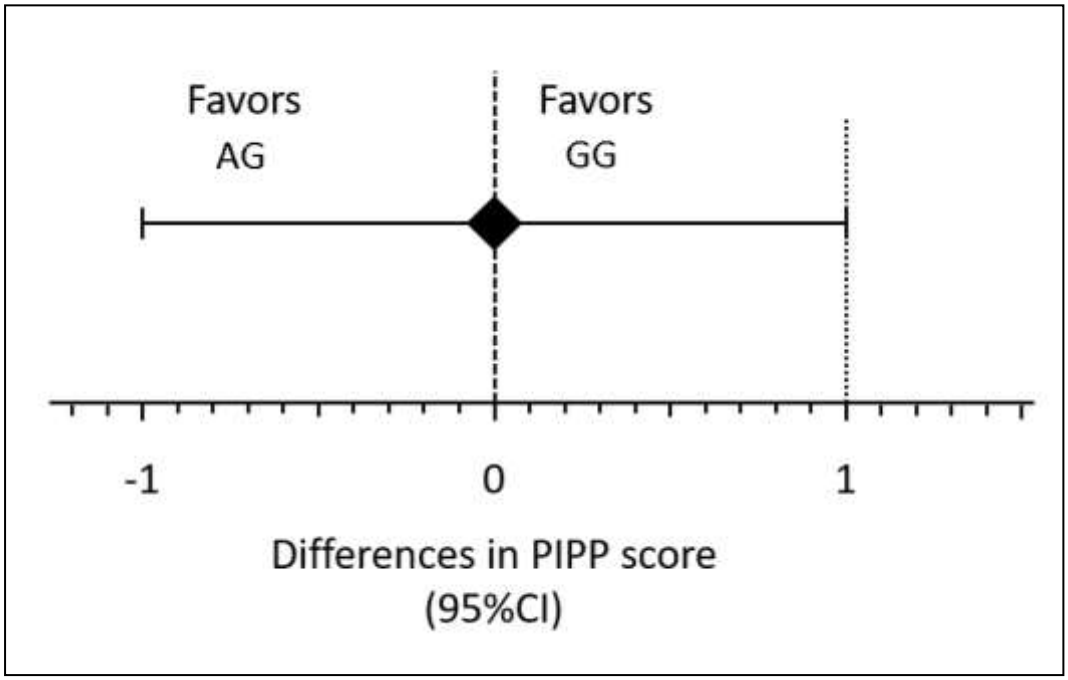

Figure 12: Differences in PIPP score (95% CI). *Reproduced from (212) with permission of SAGE publisher.*

Secondary Outcome

Secondary outcomes are listed in *table 3*.

In all 95 cases, no adverse events were documented in both groups.

Oxygen saturation

The oxygen saturation was in median above 96%, the lowest value was 93% after blood sampling. There were no differences between groups before (p=0.371) and after heel lance (p=0.669) and after blood sampling. (p=0.388).

Blood sampling duration

The blood sampling duration lasted in median 159 seconds (IQR 101-225) in all participants and showed no differences between groups (p=0.443).

Crying time

Furthermore, the crying time was similar and showed no statistical significance between groups: AG 102.0sec (58.0 – 159.0sec) vs. GG 92.5sec (58.5 – 173.5sec) p=0.890.

Heart rate

The median of heart rate [Median (IQR), values are represented with beats per minute (BPM)] was equal before intervention [AG 125 (110-136) vs. GG 126 (116-142), p=0.326] and after blood sampling [AG 176 (153 - 188) vs. GG 175 (148 - 188) p=0.970]. However, we observed significant lower heart rate in AG after intervention [AG 119 (103 - 137) vs. GG 136 (127 – 140), p=0.002] and before heel lance [AG 120 (111 - 139) vs. GG 133 (122 - 144), p=0.048], as well as after heel lance [AG 125 (114 - 144) vs. GG 138 (129 - 148), p=0.015], as shown in *figure 6*.

Table 3. Secondary Outcomes				
	All (n=91) Median (IQR)	Groups AG	GG	p-value
Heart rate (bpm):				
- Before intervention:	126 (113 – 138)	125 (110 - 136)	126 (116 - 142)	0.326
- After intervention:	130 (113 – 144)	119 (103 - 137)	136 (127 - 146)	**0.002***
- Before heel lance:	130 (115 – 142)	120 (111 - 139)	133 (122 - 144)	**0.048***
- After heel lance:	133 (121 – 147)	125 (114 - 144)	138 (129 - 148)	**0.015***
- After blood sampling:	176 (152 – 188)	176 (153 - 188)	175 (148 - 188)	0.970
Oxygen saturation (%):				
- Before heel lance:	97 (96 – 99)	97 (96 - 98)	98 (96 - 99)	0.371
- After heel lance:	97 (95.5 – 99)	97 (96 - 98)	97 (96 - 99)	0.669
- After blood sampling:	96 (93 – 98)	95 (93 - 97)	96 (93 - 99)	0.388
Crying time (sec)	95 (58 – 164)	102.0 (58.0 – 159.0)	92.5(58.5 – 173.5)	0.890
Blood sampling duration (sec)	159 (101 – 225)	157 (100 - 212)	163 (104 - 238)	0.443
Adverse events	None			

Table 3: Secondary Outcomes. P-values marked with * are statistically significant. *Reproduced from (212) with permission of SAGE publisher.*

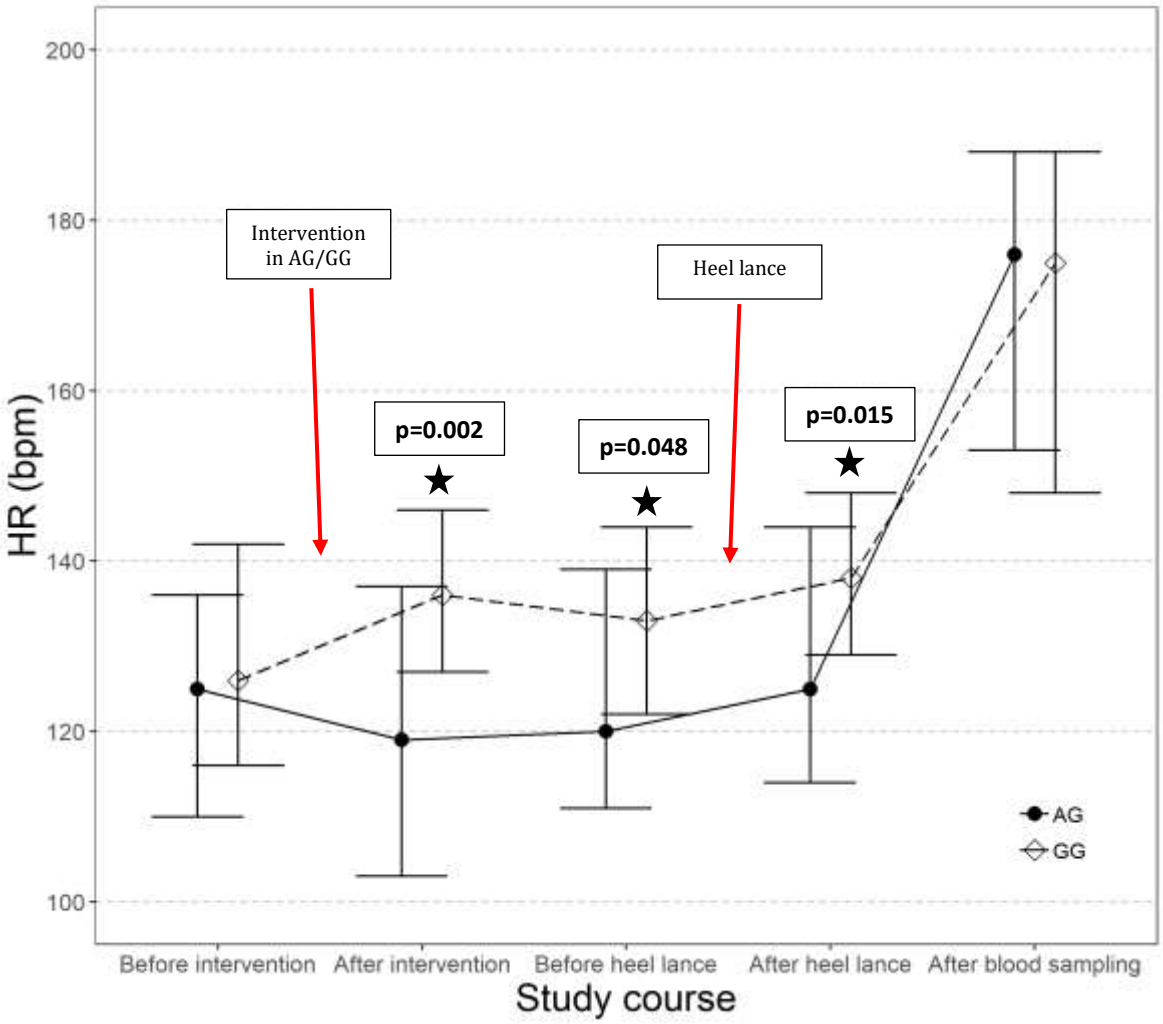

Figure 13: Heart rate changes during study course. Values marked with a star are statistically significant. *AG, acupuncture group; GG, glucose group; HR, heart rate.*

Discussion

To this date, there are just a few studies about acupuncture in neonates for pain prevention. Controversially, they are quite heterogeneous with their approaches, resulting in both positive and negative results. Non-pharmacological pain management in neonates is a relevant and current topic with a multitude of different therapy options although investigation is difficult due to the challenging trial setup in the neonatal population. Nevertheless, we have had the wish to add a new study, i.e. to investigate if laser acupuncture at LI 4 is non-inferior to oral glucose solution during minor painful intervention in term neonates.

Main outcome

Statistical Interpretation of Main Outcome Parameters

Our study has been designed by way of a non-inferior design. This means that we have predefined a margin corresponding to a range that is considered to be compable. Pain scores within this range do not differ in a clinically relevant way. In order to designate an intervention as equivalent, one must be sure that it does not only lead to non-inferior results in the study, but also in the underlying population. Therefore, the whole range of the 95% confidence interval has to remain below this predefined margin in order to establish if laser acupuncture is clinically equivalent (statistically not inferior) to orally administered glucose solution or not. However, in this study our sample has been unexpectedly heterogeneous resulting in a wider 95% CI of differences between the groups, and then crossing the predefined margin of 1. Therefore, the main results are inconclusive and although the median PIPP scores were equal to start with in both groups, we cannot state that laser acupuncture is non-inferior to oral glucose solution.

This is due to the fact that our sample size calculation was based on results of Gottschling and colleagues, who reported a standard deviation in PIPP score of 1.9.(175) In our sample, the common standard deviation was 3.4 and therefore, a higher number of participants would have been needed to decrease these discrepancies.

With our standard deviation of 3.4, our sample only had 40% in order to detect non-inferiority. New sample size calculation with a standard deviation of 3.4 has shown that we would need 288 participants in total to reach 80%.(213)

To summarize, although the PIPP pain score was 12 in both groups, we cannot state here that laser acupuncture is statistically not inferior to oral administered glucose solution, this being due to the fact that we did not find a clinically relevant equivalence. Unfortunately, our main outcome parameters are therefore inconclusive; nevertheless we believe that this provides valuable data for further studies to be carried out.

Comparison to Recent Literature

As far as recent literature is concerned, there are only two neonatal studies that have used laser acupuncture for pain relief during minor painful interventions. In laser acupuncture trials, it is important to describe several parameters to improve the comparability of study outcomes and the repeatability of studies.(210) Such parameters should include wavelength (in nm), power (in mW), irradiation time (in seconds or minutes), beam area (in cm^2 or mm^2), radiant energy (in Joule) and radiant exposure (for example in J/cm^2).(214,215) There is no relevant literature about the effective energy dosage in neonates or infants. All calculations are based on experiences in adults or children and use reduced radiant exposure, respectively. Nevertheless, as mentioned in the introduction, our preliminary safety studies of laser acupuncture in neonates have proved to be safe in late preterm and term neonates.(165,172–174)

In this study, the laser had a wavelength of 675 nm, an output power of 10 mW and diameter of 1.5 mm. We have calculated an effective energy dosage of 0.6 J/point as appropriate. As we stimulated one acupuncture point for 60 s, we received an energy dosage of 34 J/cm^2. Hence, we have used a higher energy dosage compared to the previous literature.

The above-mentioned study carried out in the past by Gottschling et al. used a 30 mW laser at LI4 and Shenmen for 30 seconds. They received an energy dosage of 0.45 J/point. As the diameter of the used laser is unknown, a calculation of J/cm^2 is not

possible, for comparison. Gottschling and colleagues showed by using this energy dosage a significant pain reduction compared to sham acupuncture. Besides LI4, Shenmen is an ear acupuncture point that is mainly used in pain therapy.

The second study conducted by Abbasoglu et al. in the past used laser acupuncture with 10 mW at Yintang for 30 s. The calculated energy dosage (0.11 J/point) here is much lower than in the other trial, by Gottschling et al., as well as in our study. Authors have assessed the pain score in term neonates. The control group obtained 0.5 mL of 24% oral sucrose solution. In this study, the intervention group showed a significantly higher NIPS score than the control group (4.52±0.87 vs. 3.66±1.01, p = 0.006).

We suggest that this lower energy dosage led to an ineffective pain reduction in the intervention group. Additionally, the acupuncture point Yintang is not a routinely used point for pain compared to LI4 or Shenmen. It remains in the field of speculation if a sedative effect of Yintang could have been reached with a higher energy dosage of laser acupuncture.

As we compared the results of these two studies with our actual trial, a combination of two acupuncture points for pain relief – although the used energy dosage was a little bit lower – seems to be more effective than only using one acupuncture point. Nevertheless, the studied sample by Gottschling et al. proved to be very low, includeing only 50 term neonates in total. Also, they did not use an oral glucose solution for a control group, but a placebo group with an inactivated laser. Oral sweet solutions represent the golden standard in neonatal pain management in minor painful procedures. Additionally, the use of placebo groups in neonatal trials given the current state of technology regarding neonatal pain processing and effective pain therapy, seems to be ethically debatable. Therefore, we decided against using a placebo group in our trial.

Comparing acupuncture point Yintang with LI4 and Shenmen, Yintang does not seem to be an appropriate point for pain management. Relaxation and sedation should always only be an additional positive side effect but not the first choice in pain therapy. It is also debatable, if a point between the eyebrows should be used for laser acupuncture altogether. In any case, participants should wear a safe eye protection to

avoid accidently occurring retina damages. If the neonate is not calm and relaxed during therapy, laser acupuncture can be very difficult to handle. Consequently, points near to the eyes should be avoided to guarantee a highest standard of safety for the neonate.

Overall Comparison of Neonatal Acupuncture Studies

Existing studies in neonatal procedural pain have been described in the Introduction in detail (see table 4). The table below is meant to provide an overview of existing trials and the new data from this doctoral thesis. As we can see from the overview, all studies have been performed in preterm or term neonates and investigated heel lances as a painful procedure. However, high heterogeneity can be observed in the chosen acupuncture method, the point and control. Most of the studies assessed pain through the PIPP score, while some others evaluated using NIPS.

The most successful acupuncture modalities used were laser acupuncture applied with 30mW at LI4 and Shenmen for 30 sec, light needling for 30 sec at Yintang, and auricular non- invasive magnetic acupuncture of both ears according to the Battlefield protocol, respectively. In conclusion, it is not possible to claim one acupuncture procedure as right and more effective than another one. We must always interpret them in a clinical context and see which acupuncture method is the most feasible and practicable one in neonatal routine care. Besides that, studies have demonstrated that the impact on the chosen acupuncture points must also be taken into account.

Additionally, no adverse events have been documented in all these studies and thus the applied acupuncture techniques seem to be safe in neonates.(171)

Author (year, country)	Sample size	Painful Procedure	Acupuncture method	Acupoints	Control	Pain score	Main Results Acupuncture	Control	p value
Gottschling et al. (2010, Germany)	50 term neonates	Heel lance	Laser acupuncture 30 mW for 30 sec (0.45 J/point)	LI4, Shenmen	Placebo laser acupuncture	PIPP (mean±SD)	6.4±1.9	8.2 ±3.3	0.013
Ecevit et al. (2011, Turkey)	20 preterm neonates	Heel lance	Light needling for 30 sec	Yintang	2ml/kg breast milk + pacifier	NIPS (mean±SD)	4.2±1.9	6.1±0.8	< 0.001
Abbasoglu et al. (09/2015, Turkey)	32 preterm neonates	Heel lance	Acupressure for 3min	BL60, KI3	Routine care	PIPP (mean±SD)	9.13 ± 1.99	9.56 ± 1.71	0.52
Abbasoglu et al. (12/2015, Turkey)	42 term neonates	Heel lance	Laser acupuncture 10 mW for 30 sec (0.11 J/point)	Yintang	0.5mL 24% oral sucrose solution	NIPS (mean±SD)	4.52±0.87	3.66±1.01	0.006
Mitchell et al. (2016, USA)	121 term neonates	Heel lance	NESAP+TENS 3.5mA, 10Hz for 10 min combined with sucrose or water	ST36, SP6, KI3, BL60	Sham-NESAP combined with 24% oral sucrose or water	PIPP changes (mean±SD)	NESAP+Sucrose: 3.6±1.2 ShamNESAP+Sucrose: 4.0±1.8 NESAP+Water: 5.0±4.0 ShamNESAP+Water: 4.9±4.0		p<0.05 p<0.05 p<0.01 p<0.01
Chen et al. (2017, Australia)	40 preterm and term neonates	Heel lance	Magnetic ear plaster	Gyrus cinguli, Thalamus, Omega 2, point zero, Shenmen	Placebo plasters	PIPP (mean±SD)	5.9 ± 3.7	8.3 ± 4.7	0.04
Stadler et al. (2021, Austria)	96 term neonates	Heel lance	Laser acupuncture 10mW for 60 Sec (0.6 J/point)	LI4	30% oral glucose solution	PIPP median (IQR)	12 (IQR 10 – 14)	12 (IQR 9-14)	0.981

Table 4: *Overview of neonatal studies.*

Laser Acupuncture in General

Against all odds, we can conclude that laser stimulation does seem to have a clinically relevant reducing effect on pain in neonates that should be taken into account for upcoming neonatal pain studies. Laser acupuncture as compared to needle acupuncture or other modalities is painless, easy to handle and fast to applicate. It also seems important to apply an adequate energy dosage ranging between 0.45 and 0.6 J/cm^2. With higher output power (mW), irradiation time will be shorter and therefore, more feasible in everyday clinical practice.

Acupuncture with laser or by another technique can also be used by trained nurses and is not only restricted to experienced medical doctors. Device and acupuncture protocol training for pain prevention should be substantial. If the nurse is trained to use laser devices and the commonly used acupuncture points (for example LI4), laser acupuncture does not depend on the presence of a medical doctor and would become even more feasible for a more general application. Such protocols are for example used in the National Acupuncture Detoxification Association (NADA) protocol.(202) This protocol includes only 5 ear acupuncture points for withdrawal and can be learnt by any medical staff.(204) This could and should be established as an example for acupuncture-trained medical staff. It also shows that it is possible to establish new acupuncture protocols in different areas of application and could be possible to apply also in neonatology and pediatric medicine.

Safety of Laser Acupuncture

Additionally, we have generated more data about the safety of laser application in neonates. We have observed no adverse events and hence, laser acupuncture has a high safety profile within safety precautions. This result is conclusive to other preliminary studies.(165,175,191)

Our research group has investigated the safety of laser application with a laser needle that can be used for acupuncture therapy. These preliminary studies have revealed a safe laser application over 5 and 10 minutes with 10 and 15 mW concerning the integrity of laser on neonatal skin and heat development on the lasered area.(166,216) There has been found to be a mean increase in local skin temperature about 1-2 °C after 5 and 10 minutes of application, respectively. Both studies have implied that laser application is safe in compliance with the right irradiation time and

energy dosage. Clinical laser acupuncture studies should all be aware of these results. In conclusion, usage of low-level lasers can be recommended for application in neonates.

PIPP Score

The PIPP score is validated for preterm and term neonates during a painful intervention with an inter- and intrarater reliability of >0.89 and >0.95.(39,42,217) Therefore, one skilled pediatric nurse specialized in pain management in neonates has performed the PIPP assessments.

We used a median pain score of 12, this indicating severe pain. Neither glucose solution nor laser acupuncture have been found to reduce pain sufficiently compared to the literature. One factor for higher pain scores could be the assessment by a nurse. Simons et al. have reported that nurses tend to score different procedures as more painful than physicians.(218) One additional bias could be the absence of a second blinded observer and assessor. Because neonatal pain assessment is not commonly standardized, practitioners may assess pain not in a consistent way although using the same criteria to start with.

Most of our participants were asleep after intervention and therefore reached a higher score in the behavioral stage. It is described in the literature that neonates in a quiet sleep state respond less strongly to a painful stimuli and therefore get a higher pain score.(219–222) Additionally, Ahn et al. have speculated that awake neonates can express pain more apparently than ones asleep.(223) Contrary to that, interventions using laser acupuncture or oral glucose solution induce not only a pure pain reduction comparable to administering drugs, but also a more comfortable situation for the neonate and the following soothing of the neonate can be brought about. The validated Bernese pain scale (BPSN) for preterm and term neonates, for example, includes the sleep, consolation, and posture. A sleeping neonate with relaxed posture will be rated less as being in pain than one that is awake and agitated. A recent study recommends a modified version of BPSN that includes facial expression, crying, posture and heart rate for acute pain assessment.(224) Additionally, COMFORT scale or NIPS rate a relaxed neonate with a lower pain score than an agitated one.(44,225)

Altogether, it is discussable, if a pain score including the behavioral stage is validated enough for non-pharmacological pain management.

Furthermore, facial expressions seem to have the strongest association with nociception-specific brain activity while physiological signs, like heart rate or oxygen saturation, have only little to no associations at all.(213)
In 2016, Shabani et al. conducted a cross-over clinical trial on music therapy for pain reduction in premature infants during blood samplings.(226) They used facial expressions measured by the neonatal facial coding system (NFCS scale) and the sleep–wake states (coded as deep sleep, active sleep, drowsy, quiet alert, active alert and crying) for determining neonatal pain response. Authors have concluded that music therapy is effective to decrease the heart rate, sleep–wake state scores and facial expressions of pain. That means, that this study indicated a lower sleep-wake state as expression of relaxation during painful intervention.

We have chosen PIPP in our trials because it is well studied and commonly used in preliminary studies. Nevertheless, further usage of PIPP for assessment in non-pharmacological pain management trials should be under discussion.

Secondary Outcome Parameters

Additionally, laser acupuncture also has effects on pain-related symptoms, which can be used as surrogate parameter. Nevertheless, as mentioned in the introduction, Porter et al. have reported a missing correlation between behavioral and physiological parameters and thus, isolated surrogate parameters should be considered with caution.(5) However, it could be of medical interest and relevant in clinical everyday life for the treated individual. Therefore, these surrogate parameters should be more closely looked into.

Crying Times

The crying time is one of the most analyzed parameters in pain preventing trials. The interpretation of trial results should be done with caution because the duration of crying can be biased due to measuring techniques and definition of crying. Even so, it

could be an adequate value in clinical routine for interpreting the comfort or discomfort of the patient.

Studies concerning pain prevention in neonates have analyzed the duration of crying, percentage of time crying or the duration of first cry.(77,228) This represents a certain variance of measuring techniques. Preliminary acupuncture trials have used crying duration and have demonstrated reduced crying periods when applying needle acupuncture at Yintang or acupressure at BL60 and KI3.(177,191) In these cases, reduced crying times could be due to a lower stress level owing to the used acupuncture points. Yintang can have a good advantageous impact on decreasing stress and anxiety during painful procedures.(190) BL60 and KI3 in combination may have a local impact on blood vessels and therefore lead to a smoother blood sampling with reduced crying of the neonate.(191)

In our study, the duration of crying was similar in both groups with 102.0sec (58.0 – 159.0sec) in AG and 92.5sec (58.5 – 173.5sec) in GG with a p-value of 0.890. This secondary result shows a potential clinical effect of laser acupuncture at LI4 for pleasant comfort during painful intervention comparable to orally administered glucose solution.

Heart Rate and Heart Rate Variability

A physiological surrogate parameter of pain evaluation is the heart rate or the heart rate variability (HRV). The HRV is a special method based on the measurement of QRS-distances and different parameters calculated therefrom. Resulting values involve a so called low-frequency power (0.04–0.15 Hz), which characterizes an increased sympathetic activity, or a high-frequency power (0.15–0.4 Hz), which is indicative of the parasympathetic nervous system.(230) Further, the low-frequency/high-frequency ratio might reflect the global sympathetic-vagal balance.

HR and HRV are often used as markers of the autonomic nervous system. There are speculations that acupuncture can stimulate changes of the function of the autonomic nervous system and thereby lead to a reduction of the heart rate and therefore can be an expression of relaxation.(229) Nevertheless, the HRV seems to be more sensitive

than the heart rate alone, although it is non-specific in early diagnostic analysis.(157,230)

HRV analysis has evidence in different neonatal diseases like asphyxia, neonatal seizure, prolonged pain in neonates with pneumothorax or necrotizing enterocolitis.(231–234) Nevertheless, HRV should be considered with caution in preterm neonates, due to their immature nervous system.(235) Kaar and colleagues also conclude that HRV is not suitable in acute stress or pain situations and might only be validated for prolonged pain monitoring.(236) Additionally, a trial by Padhye et al. has shown that low-frequency HRV response to pain increases with higher postmenstrual age.(237)

Preliminary pediatric acupuncture studies used heart rate as surrogate parameter and demonstrated a significant decrease after acupuncture.(157) Only 2 trials on pain prevention in neonates have analyzed the heart rate and one trial HRV as secondary outcome parameter.(177,180,183) There have been no differences in heart rate in both trials between intervention and control group, although Ecevit et al. have described a heart rate decrease 3 minutes after needle acupuncture, but these results were not contained in the outcome table. Mitchell et al. have analyzed HRV in the low-frequency/high-frequency ratios from baseline to heel lances or recovery phases and have shown no significant differences among the treatment groups.(180) The authors believe that the time of measuring (2 to 5 minutes) might have been too short for validate significant changes. This would be consistent with what we have found in the literature described above.

We have used heart rate also as secondary outcome and documented a significant difference between groups after intervention, before heel lance and after heel lance. Before intervention and after blood sampling, the heart rate was found to be similar in both groups. If we take a closer look, we see that significant differences have resulted from an increase of HR in GG and a decrease after LA (as shown in Figure 6). An increase of HR after glucose solution may be caused by physiological reaction to glucose and non-nutritive sucking. These alterations have been investigated in preliminary trials. They showed a significant increase in HR, if only glucose solution

was administered without any intervention followed.(238,239) However, none of the above mentioned acupuncture studies has described likewise effects whereas a decreased HR after acupuncture seems to be appropriate and can be interpreted as easing and less irritation during intervention.

Salivary Cortisol Level

Another surrogate parameter we have found in the literature is the salivary cortisol level, which has been used in various neonatal studies. However, it does not appear to be practicable in daily routine or in neonatal research for acute pain assessment because it is difficult to obtain.(240) For example, Mitchell et al. have reported a success rate of obtaining adequate saliva for analysis of only 46%.(180) Therefore, we decided not to use this additional surrogate parameter.

Oral Glucose Solution

Oral sweet solutions, sucrose, and glucose, also have a high impact on heart rate and oxygen saturation and show a beneficial effectiveness in pain reduction. It seems to be safe in short-term application, but is still under investigation.(22,241–243) Consequently, sweet solutions were used in preliminary studies for control groups alone as well as combined with acupuncture. These trials resulted in both positive and negative results for glucose solution on pain reduction.(180,183,191)
Since oral sweet solutions represent the golden standard, we have still decided against using a placebo group. Thus, the absence of a placebo group can be interpreted as a limitating factor in this study.

Preterm or term neonates admitted to a NICU often require repeated painful procedures and have therefore a higher demand of pain management.
Whereas a single dose of oral administered glucose solution is secure and effective, frequent doses of glucose solution can have potential side effects such as necrotizing enterocolitis, hyperglycemia, and fluid overload in premature infants.(242,244) There are concerns about neurodevelopmental retardation with repeated sucrose exposures.

We suggest that acupuncture in general –independently of which technique is applied – could be a safe alternative non-pharmacological procedure that could be used for repeated minor painful interventions in neonates compared to oral administered glucose solution.

Limitations

Neonatal trials are always very challenging because of the special study population and their needs. As described above, a missing placebo group is one of our limitations. However, we decided against a placebo group right from the beginning due to ethical reasons.

Besides, appropriate handling of probands in both groups is necessary to reduce the bias of external influence. In our case, an equal manipulation throughout was guaranteed due to standardized protocol. Nevertheless, minimal variation in handling between groups was inevitable due to intervention procedure. During intervention, it was essential for example to hold the hand of the neonate during laser acupuncture or holding the head during oral glucose application.
Furthermore, swaddling as additional pain prevention was not possible because laser acupuncture was performed on both hands. Neither was a pacifier able to be used since this would have influenced facial expression regarding the PIPP score analysis. However, swaddling, and non-nutritive sucking are already accepted methods to enhance comfort of the patient during a painful intervention. These points should be born in mind for future trials to minimize discomfort. This could also be taken into account when using a different pain score or study concept.

Another limitation in methodological procedure is the heart rate assessment as described above. For further studies to be carried out, a standardized documentation of heart rate and oxygen saturation values should be required.
At this point, the impact of the immature nervous system is also a debatable aspect in providing an adequate pain management. The characteristics of a neonatal and therefore not completed development of inhibitory pathways could be a further explanation for a higher variability in pain score or ineffective pain reduction with some acupuncture techniques or stimulations.(20,223)

Implications for clinical everyday life

There exist many acupuncture methods which are still under investigation for clinical application in neonates. Nevertheless, these techniques are promising tools for painless and non-invasive therapy options in children and neonates.

Especially laser acupuncture offers a feasible and simple way in therapeutic treatments. Additionally, acupuncture techniques in general are easy to learn and can be provided at low costs. Laser acupuncture devices are inexpensive over time because they can be used many years to treat a variety of diseases. Also acupuncture needles or magnetic ear plasters are fair in acquisition and are therefore often used in crisis areas and poorer countries, as we can see in Battlefield acupuncture.

Moreover, non-invasive acupuncture techniques are simple to learn and can be applied by any medical staff who is trained by a medical doctor and expert in acupuncture. NADA ear acupuncture or Battlefield acupuncture provides good examples, where defined acupuncture protocols are trained to medical staff. Although acupuncture and therefore traditional Chinese medicine come under individualized medicine, predefined protocols are possible in certain diseases or application areas.
There exist also official guidelines from WHO (World Health Organization), which provide a guidance for acupuncture administration, the minimum infrastructure necessities and important safety precautions.(245)

Perspective and Suggestions for Future Trials

In future clinical laser acupuncture trials, the focus should be on finding the sufficient energy dosage in the pediatric population. However, especially in neonates, a huge number of probands can be challenging.
We believe that further laser acupuncture trials should be more transparent in presenting technical details. It is necessary to describe certain parameters to enhance the comparability and repeatability of trials.(210) Required parameters include: (1) wavelength (in nm), (2) power (in mW), (3) irradiation time (in seconds or minutes), (4) beam area (in cm^2 or mm^2), (5) radiant energy (in Joule) and (6) radiant exposure (for example in J/cm^2).(214,215) Laser acupuncture studies including these mandatory laser parameters are conducted in a good scientific way.

Moreover, study methods must address a scientific way of acupuncture point choice based on an appropriate explanation. Unfortunately, a scientific explanation of single acupuncture points proved in randomized controlled trials or in basic research is missing and difficult to achieve. However, a two-thousand-year-old experience of tratogether wiht the WHO benchmarking of acupuncture are able to provide an overall recommendation for an accurate usage of acupuncture points.

The perspective should be the involvement of acupuncture techniques in clinical routine, for example laser acupuncture, acupressure, NESAP or magnetic plasters. In these disciplines, also trainings for medical staff should be provided.

Next to acupuncture parameters in future trials, non-pharmacological pain management should be further investigated because of lacking alternatives to oral sucrose solution. Safe and effective pain relief which can be offered repeated times on one day or during a long hospital stay is urgently needed at the NICU. Acupuncture seems to be a promising tool because of the lack of side effects and a potential positive effect in long-term usage. However, these circumstances must be evaluated in clinical trials first.

Regarding acupuncture trials for minor painful interventions, a validated pain score is implied. Despite this, secondary results can grant more detailed interpretation of study outcomes and should include crying time, heart rate and salivary cortisol levels if easy to collect. HRV is not suitable in acute stress or pain situations and might only be validated for prolonged pain monitoring.(236) Especially, heart rate is an interesting outcome parameter in acupuncture studies. As shown in this recent trial, we managed to document an immediate acupuncture response of LI4, which can be relevant in clinical daily life. Therefore, vital signs during acupuncture studies are needed and should be made compulsory.

Conclusion

This randomized clinical trial has been conducted with a view to evaluating the effect of laser acupuncture at LI 4 on pain response in neonates. Therefore, we have compared laser acupuncture with the actual standard of care of administering oral glucose solution, in a non-inferiority setting. Although we had equal medians in the PIPP score in both groups, we were not able to prove non-inferiority. We reached unexpected high variations in the PIPP scores resulting in inconclusive results.

The secondary outcome parameter crying time showed similar values in groups and therefore laser acupuncture does seem to have the same potential clinical effect for pleasant comfort during painful intervention comparable to orally administered glucose solution. Additionally, heart rate has been found to reveal partially better results for laser acupuncture, this generating new data about immediate acupuncture response of LI4.

In comparison to recent literature, only one acupuncture modality alone cannot be stated to be more effective than another one. Different acupuncture techniques can be appropriate in a variety of clinical applications. Hence, it should be interpreted in a clinical context which acupuncture method is the most feasible and practicable one in neonatal routine care. Next to the right acupuncture tool, appropriate acupuncture points should be selected. Three studies have presented a significantly better pain relief in acupuncture group. Consequently, LI4 combined with Shenmen, Yintang with light needling and Battlefield ear acupuncture have proved to be the most successful acupuncture points.

We suggest that acupuncture in general should be seen as a safer alternative non-pharmacological technique that could be used for repeated minor painful interventions. The sedative and calming effects on neonates of acupuncture should be contemplated in neonatal care.

Our results achieved indicate clinical relevance for pain relief in neonates. They should be considered for generating further hypotheses. On the grounds of this present study, continued studies are urgently warranted. Additionally, we can conclude that we see huge advantages in laser acupuncture regarding its safe, easy, and painless usage.

Abbreviations

AACMA:	Australian Acupuncture and Chinese Medicine Association Ltd.
ATP:	Adenosine TriPhosphate
BFA:	Battlefield Acupuncture Protocol
BL:	Bladder
BPSN:	Bernese Pain Scale of Neonates
BPM:	Beats Per Minute
CBR:	CannaBinoid Receptor
CCK:	CholeCystoKinin
CCK2R:	CholeCystoKinin-2-Receptor
CI:	Confidence Interval
DAN:	Douleur Aigue¨ du Nouveau-ne
EA:	ElectroAcupuncture
ECS:	EndoCannabinoid system
EX-HN3:	Extra - Head and neck point 3
fMRI:	Functional Magnetic Resonance Imaging
FT:	Facilitated Tucking
HPA:	Hypothalamus-Pituitary-Adrenal axis
IASP:	International Association for the Study of Pain
IL-10:	InterLeukin-10
IQR:	Interquartile Range
KC:	Kangaroo care
KI:	Kidney
KMC:	Kangaroo Mother Care
LI:	Large Intestine
LLLT:	Low-Level-Laser Therapy
NADA:	National Acupuncture Detoxification Association
NESAP:	Non-invasive Electrical Stimulation of Acupuncture Points
NFCS:	Neonatal Facial Coding System
NIPS:	Neonatal Infant Pain Scale
NICU:	Neonatal Intensive Care Unit

NNS: Non-Nutritive Sucking

NPASS: Neonatal Pain, Agitation and Sedation Scale

PBM: PhotoBioModulation

PGE2: Prostaglandin E2

PIPP: Premature Infant Pain profile

$PROKR2^{Cre}$: Cre-mediated PROKineticin Receptor 2

ROCK: Rho-associated Protein Kinase

SAS: State-of-the-art statistical Analysis Software

SD: Standard Deviation

SP: Spleen

SPSS: Statistical Package for the Social Sciences

SSC: Skin-to-Skin Care

ST: Stomach

TENS: Transcutaneous Electrical Nerve Stimulation

TGF-β: Transforming Growth Factor β

TL: Telomere Length

WALT: World Association for photobiomoduLation Therapy

WHO: World Health Organization

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Appendix

Premature Infant Pain Profile: working sheet

Premature Infant Pain Profile (PIPP)

Adjusted for clinical trial.

Baseline heart rate: _____beats per minute (=bpm) Baseline SO_2: _____%

Indicator	Points (Please tick the appropriate box)			
1. Gestational age	0 >= 36 weeks	1 32 weeks to 35 weeks 6 days	2 28 weeks to 31 weeks 6 days	3 < 28 weeks
2. Behavioural state	0 active/awake eyes open facial movements	1 quiet/awake eyes open no facial movements	2 active/sleep eyes closed facial movements	3 quiet/sleep eyes closed no facial movements
3. Heart rate maximum	0 0-4 bpm increase	1 5-14 bpm increase	2 15-24 bpm increase	3 ≥ 25 bpm increase
4. Oxygen saturation minimum	0 0 to 2.4% decrease	1 2.5 to 4.9% decrease	2 5.0 to 7.4% decrease	3 ≥ 7.5% decrease
5. Brow bulge	0 none (≤ 9% of time)	1 minimum (10-39% of time)	2 moderate (40-69% of time)	3 maximum (≥ 70% of time)
6. Eye squeeze	0 none (≤ 9% of time)	1 minimum (10-39% of time)	2 moderate (40-69% of time)	3 maximum (≥ 70% of time)
7. Nasolabial furrow	0 none (≤ 9% of time)	1 minimum (10-39% of time)	2 moderate (40-69% of time)	3 maximum (≥ 70% of time)

In total: _____ points

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Case report form: working sheet

Case Report Form

Laserakupunktur am Di 4 zur Schmerzprävention bei Neugeborenen.

Patienten-ID: _____ Datum der Untersuchung: _____

Unterschriebene Patientenaufklärung liegt vor: ja

Angaben zum Patienten:

Geburtsgewicht: _____ g Perzentile: _____%

Geburtsgröße: _____ cm Perzentile: _____%

Kopfumfang: _____ cm Perzentile: _____%

Mikrocephalie: ja nein

AFD (Appropriate for Date) SFD (Small for Date) LFD (Large for Date)

Geburtsart: Spontan Per Sectio: in LN (Spinalanästhesie) in AN (Allg.narkose)

SSW: _____ APGAR-Score: ____ /____ /____

Lebenstag: _____

Fehlen von klinischen und/oder kongenitalen Erkrankungen: ja nein

Erhält der/die Patient/In derzeit eine Schmerzmedikation? ja nein

Angaben zur Mutter:

Erhält die stillende Mutter derzeit eine Schmerzmedikation? ja nein

Angaben während der Untersuchung:

Schreizeit: _____ Sekunden

Komplikationen: _____

Unerwünschte Nebenwirkungen: Keine

Übelkeit Erbrechen

Rötung der Haut über Di 4

Sonstiges: _____

	Herzfrequenz	SO_2	Bemerkungen
Behandlungsbeginn Akup/Gluc (mit Maske)			
Behandlungsende Akup/Gluc (mit Maske)			
Blutabnahme Beginn (direkt **VOR** Fersenstich)			
Direkt NACH Fersenstich			
Blutabnahme Ende			