UNIVERSITÄTSKLINIKUM HAMBURG-EPPENDORF

KLINIK UND POLIKLINIK FÜR KINDER- UND JUGENDMEDIZIN

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PREDICTABLE FACTORS FOR EFFECTIVE USE OF DOXAPRAM IN EXTREMELY LOW BIRTH WEIGHT PRETERM NEONATES AND NEUROCOGNITIVE OUTCOME: RETROSPECTIVE SINGLE-CENTRE STUDY

DISSERTATION

ZUR ERLANGUNG DES GRADES EINES DOKTORS DER MEDIZIN
AN DER MEDIZINISCHEN FAKULTÄT DER UNIVERSITÄT HAMBURG

VORGELEGT VON:

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GEBOREN IN WLADIWOSTOK

HAMBURG 2023

Angenommen von der
Medizinischen Fakultät der Universität Hamburg am: **6.05.2024**

Veröffentlicht mit Genehmigung der
Medizinischen Fakultät der Universität Hamburg.

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1. Introduction

Over the last three decades, perinatal care has improved greatly, increasing the survival rate of extremely preterm infants (Luregn J et al, 2012), with extreme low birth weight (ELBW). The extreme prematurity impacts disease pathophysiology, which, as a result, forces to a modification of patient management and treatment. While the incidence of major neonatal diseases such as necrotizing enterocolitis (NEC), bronchopulmonary dysplasia (BPD), periventricular leukomalacia (PVL), and intraventricular haemorrhage (IVH) remain constant despite increased numbers of ELBW infants, the prevalence of apnea of prematurity (AOP), strictly related to gestational age and birthweight, has been risen. Moreover, its therapy became even more challenging. The incidence of AOP approaches up to 90% for those weighing less than 1000 g, causing prolong hypoxic episodes and even invasive ventilation. This plays a crucial role in development of poor short- and long-term outcomes. Being born at a phase of extensive brain growth and the undergoing profound processes involving neuronal migration, synaptogenesis, myelination, and cytological maturation, extreme preterm infants are extremely vulnerable to hypoxia, ischemia, and inflammation. Each of these factors affects the brain development differently. Understanding the types of injury and how each impairs neurodevelopmental outcomes can assist in its further prevention. Therefore, modern neonatology focuse on predictable factors of effective therapy, as well as its impact on long-term outcome and factors, which might amplify a possible adverse effect.

1.1. Apnea of the premature

1.1.1. Classification of apnea of the premature and chronic intermittent hypoxia

Apnea of prematurity (AOP) is a developmental condition. Most likely, its presence reflects ‘‘physiological’’ rather than ‘‘pathological’’ immaturity of respiratory control and is strongly correlates with maturation of the respiratory system (Abu-Shaweesh, 2008). During pregnancy, the fetus develops a stable respiratory pattern for successful gas exchange and sustained breathing at term gestation. This requires full maturation of all elements in this integrated respiratory network such as sensors, receptors, the control centre, and the effectors. Due to extreme prematurity, these infants lack the main structural and functional components for successful breathing. Fur-

thermore, AOP is the most common clinical phenomenon among extreme preterm infants. Apnea-related hypoxic episodes in the majority require various pharmacological intervention or even prolonged ventilatory support and accompany an increased rate of retinopathy of prematurity (Di Fiore et al., 2010). An observational study reported that preterm infants with the most severe apnea often develop severe lung disease (Eichenwald et al., 1997). Hence, the harmful impact is not from apnea per se, but rather the association of chronic, intermittent hypoxia, hypoxemia, and/or bradycardia that limits oxygenation and perfusion to vital organs (Di Fiore JM et al 2013), which may result in impairment of long-term neurodevelopment (Miller MJ et al, 2004, Martin et al., 2011; Pillekamp et al., 2007). The frequency and severity of AOP and chronic, intermittent hypoxia peaks in the first weeks of life, a period when the system is maximally vulnerable (Di Fiore et al., 2010). Large trials demonstrated higher incidence of intermittent hypoxic episodes among extreme preterm infants particularly (Di Fiore et al., 2012). Therefore, understanding the pathophysiology of hypoxemia during AOP could be the key in the management of extreme preterm infants.

There are three types of apneas: central, obstructive, and mixed. Central apnea is characterised by total interruption of respiratory efforts without any evidence of obstruction. Obstructive apnea is associated with ineffective respiratory movements while the upper airway is closed or obstructed. Lastly, mixed apnea consists of obstructive and central components. Usually, its initial phase is caused by loss of central respiratory drive, leading to deficient recovery. A second phase involves activation of upper airway muscles even though the upper airway is closed (Gauda et al., 1987). In preterm infants, mixed apnea is the most abundant, usually accounting for 50% of long apneic episodes, followed by central apnea (Barrington KJ, et al, 1990). AOP is widely defined as the cessation of breathing in excess of 15 seconds (20 seconds, according to the American Academy of Pediatrics), typically accompanied by desaturation and bradycardia (Miller MJ et al, 2004). However, shorter episodes of apnea—and even periodic breathing—may be accompanied by bradycardia or hypoxemia. Experts disagree on what constitutes clinically significant bradycardia or desaturation in such infants. In ventilated preterm infants, episodic desaturation is a common concern; it is almost invariably preceded by ineffective ventilation due to loss of lung volume, lower airway closure, or bronchospasm (Bolivar JM, 1995; Dimaguila, 1997). Responses to hypoxia in preterm infants is biphasic and depends

on impaired chemosensitivity and characterised by centrally mediated suppression. Another consequence of immature brainstem control and the influence of arterial peripheral chemoreceptors is periodic breathing. Periodic breathing is an important part of apnea in preterm infants. Rigatto et al. showed that prolonged apnea was almost always preceded by short apneic pauses like those observed during periodic breathing (Rigatto et al. 2003). Initially, it results in overcompensation of slight hypoxia or hypercapnia by tachypnea, causing relative hypocarbia. However, continuing this leads to apnea in a cyclic pattern. This breathing pattern is characterised by oscilliatory or ventilatory cycles of 10–15 seconds with pauses of 5–10 seconds. Periodic breathing may result in the critical condition, intermittent hypoxia. Extreme preterm periodic breathing is negatively influenced by even small ventilation volumes and is accompanied by significant oxygen desaturation (Rigatto et al, 2003). The incidence of this breathing pattern in extremely preterm infants increases dramatically over the first 4 weeks, then progressively declines after 6–8 weeks postnatal (Di Fiore et al., 2010).

1.1.2. Physiology of apnea: and Integrated respiratory and neuronal network

Apnea of prematurity appears because of compromised intrinsic and extrinsic connections that affect the central respiratory network, peripheral and central chemoreceptors or mechanoreceptors. Finally, this leads to a reduced impulse to the muscles of respiration organs (Di Fiore et al. 2013).

A stable respiratory performance is based on a fine balance between the activation and inhibition of inputs from higher brain structures and centres (the frontal cortex, insular cortex, hypothalamus, reticular activating system, and amygdala), mechanoreceptors within the respiratory tract, peripheral chemoreceptors in the carotid body, and central chemoreceptors on the ventral medullary surface organs (Di Fiore et al. 2013). The function of integrated respiratory and neuronal network depends on the connection of synapses between premotor respiratory neurons, respiratory motoneurons and the diaphragm. Its modulatory influence on the breathing pattern is most profound during early postnatal development (Gauda et al. 2012). Therefore, due to the immaturity of the respiratory network it is characterised by a postponed

and impaired ventilatory reaction to hypoxia, hypercapnia, and an amplified inhibitory response to stimulation of airway receptors (Miller et al. 1992).

In contrast to adults and term neonates, preterm infants have a remarkable ventilatory response to hypercapnia; without increasing their respiratory rate, they extend their expiratory duration. This is associated with central mediation from the brainstem (Dreshaj et al. 1998). Additionally, some have considered that the sensitivity of central CO2 chemoreceptors in the ventrolateral surface of the medulla and the immaturity of higher brain centre that regulates ventilatory rhythm generation underlie apnea of prematurity (Martin et al. 2005). The ventral medullary chemosensitive area plays a crucial role in regulating activity and respiratory responses to CO2. Initiation of inspiration appears in the pre-Bötzinger complex, then moves to the premotor inspiratory neurons and the diaphragm, the external intercostals and upper airway muscles (pharyngeal and laryngeal). Finally, expiration is generated by retrotrapezoid nucleus/ parafacial respiratory group, followed by premotor neurons which innervate the muscles which are active in expiration (Feldman et al. 2012; Feldman et al. 2011). Furthermore, Henderson-Smart *et al*. found decreased numbers of synaptic connections and poor myelin in the preterm brain (Henderson-Smart et al, 1983). This results in impaired auditory-evoked responses in infants with apnea, specifying a delay in brainstem conduction time. These data indicated functional rather than anatomical immaturity in the pathophysiology of apnea.

Peripheral chemoreceptors, such as the glomus cells, carotid body, and its maturity are also important for respiratory response to hypoxia, hypercapnia and acidosis, being able to increase ventilation. Intrauterine oxygen sensitivity of the main peripheral chemoreceptor, the carotid body, is adapted to rather low PaO_2 (levels of PO_2 are around 20 mmHg), while gas exchange is performed by the placenta and regular breathing is unnecessary. This explains that hypoxic respiratory depression is physiological for this gestational age (Williams et al. 1991). Therefore, preterm infants continue to respond to hypoxia postnatally by a late depression (Martin et al. 1998). About two weeks postnatal, peripheral arterial chemosensitivity resets after its maturation is completed. During this time, though, hyperoxia can silence the carotid body. The immaturity of the peripheral chemoreceptors could be one of the reasons for hypoxia-induced respiratory depression in preterm infants with decreased ventilatory frequency, while tidal volume is sustained (Martin et al. 2005).

Chemical or mechanical stimulation of upper airway afferents can also induce apnea and bradycardia. Changes of airflow, transmural pressure, or phasic respiratory muscle activity affect pressure, flow, or respiratory drive receptors, respectively (Sant'Ambrogio et al. 1983; Widdicombe et al. 1988). The innervation of laryngeal afferents is mediated through the nucleus of the solitary tract, which can initiate inhibition of phrenic motor neurons. The sympatic part of innervation, the superior laryngeal nerve (SLN), is connected to the cardiac vagal neurons (CVN) in the nucleus ambiguus. The airway protective laryngeal chemoreflex (the triad of apnea, bradycardia, and hypertension) associated with laryngeal closure and swallowing movements, is innervated by the superior laryngeal nerve afferents. This reflex is exaggerated among preterm infants. As a result, they react with prolonged apnea.

Apnea of prematurity can often be accompanied by bradycardia as a response to hypoxemia and decreased saturation from hypoxic incitement of the carotid body chemoreceptors. On the other hand, if the heart rate decreases without decreased blood oxygen saturation, it is not necessarily triggered by hypoxemia. This indicates strong involvement of the vagus and central mechanism followed by the triggering of laryngeal receptors sustained (Martin et al. 2005; Gauda, et al. 2012). The involvement of the central respiratory and cardiovascular centres as well as their synergy in the production of bradycardia during AOP requires further investigation.

1.1.3. Role of Inflammation

Lung inflammation impacts the respiratory process and impairs apnea of prematurity. The cellular injury due to the initiated inflammatory cascade in the lungs begins with higher oxygen exposure postnatal. Furthermore, despite advanced management in neonatology, extreme preterm children are still frequently exposed to infection or other inflammatory processes both intrauterine and postnatal. Even though chorioamnionitis has been emphasised by reduced incidence of respiratory distress syndrome and minimisation of oxygen requirements at birth, its correlation with chronic lung diseases remains significant (Shimoya et al., 2000; Watterberg et al., 1996). Interestingly, there may be a relationship between progressive inflammation within premature lungs and increased frequency of chronic intermittent hypoxia episodes, which are primarily observed during the first two weeks postnatal. However, it is still unclear if this is a cause or an effect.

Clinically, increased frequency and increased severity of apnea are important and well-known signs of acute infections in premature infants (Hofstetter et al. 2008). On newborn animal models, Abu-Shaweesh, et al. (2008) demonstrated hypoxic ventilatory response and enhanced inflammatory cytokine gene expression in the medulla oblongata after application of LPS in the trachea (Balan et al. 2011). The exact role, which systemic or lung inflammation plays in typical preterm, predominantly vagus-mediated reflexes, effect on lung volume or in rapid adaptation of receptors, is still unknown. However, during systemic infection, inflammatory cytokines do not pass through the blood brain barrier, but inflammatory cytokines and modulators (prostaglandins, IL-1) are still upregulated in the brain, which can inhibit respiration (Olsson et al. 2003). Gauda, et al. reported that the structure and function of carotid body can be impaired due to inflammation; this can intensify apnea for at least 1 week after the acute inflammatory episode. Moreover, there was a significant correlation between acute systemic inflammatory conditions (NEC, LOS) and increased frequency and duration of apnea in the 24h period before diagnosis (Hofstetter et al., 2008, Fairchild et al. 2016).

In summary, systemic inflammation (sepsis, NEC) has a tremendous impact on the frequency of apnea and is widely considered as a trigger for apnea of prematurity. Inflammation during early development also impairs development of the airways and vasculature. Arrested vasculogenesis can cause the progression of persistent pulmonary hypertension in infants as well as increase hypoxic pulmonary vasoconstriction (Rey-Parra et al., 2008). The adverse effects of inflammation on the developing respiratory network and lungs create the perfect conditions for continuing chronic, intermittent hypoxia. This, in turn, impacts short- and long-term co-morbidities in premature infants.

1.1.4. Clinical Associations of Immature Breathing and BPD in Preterm Infants

Some studies have data on the reduced response of peripheral chemoreceptors (dysregulation) in preterm children with bronchopulmonary dysplasia. However, intermittent hypoxia under BPD can cause the peripheral chemoreceptors to be excessively sensitive, potentially destabilising breathing patterns and blood oxygen saturation. Most studies on the subject provide information about the higher frequency

of apnea among extreme preterm infants with BPD (Eichenwald et al. 1997, Lorch et al. 2011). Nevertheless, the recent study could not find a statistically significant association of incidence of central AOP with severe ROP, BPD, or severe IVH (Fairchild et al. 2016). This can be explained by expectedly prolonged respiratory support and medication with caffeine that masked the apnea. It is relevant to mention, that children with BPD may later become prone to obstructive apnea.

1.1.5. Therapeutic interventions

Since apnea-related hypoxic episodes vastly impair neurodevelopment and may increase comorbidity as well as prolonging the infant's hospital stay, the management of apnea in extreme preterm infants plays an important role at the neonatal intensive care unit. There are different approaches to management, depending on the severity of AOP. The most common therapy includes continuous positive airway pressure (CPAP). For over 40 years, CPAP has proven to be a safe and effective therapy. A few RCT and Cochrane studies found that CPAP was safe at 4-8 cm H2O. Two main functions provide this effectiveness: stabilisation of lung volume and improvement of airway potency due to limitation of upper airway closure. CPAP is especially effective for apnea with an obstructive component. CPAP also benefits apnea by increasing functional residual capacity, thereby improving oxygenation status (Di Fiore et al. 2012; Martin et al. 2005). Despite the effectiveness of CPAP, there are still some disadvantages to be considered such as nasal trauma and low compliance. Few studies and meta-analyses could provide results revealing similar rates of treatment failure between CPAP and HFNC, nasal trauma and preumothorax were significantly lower with HFNC. Recently the new approaches of respiratory management have been investigated as possible additional treatment for apnea for AOP. One of them is noninvasive high frequency oscillation (nHFO). Clinical observation studies show an improvement due to the application of nHFO, where the positive effect has been explained by the oscillatory art of respiration and induction the sensitivity of afferent neurons. Additionally, it may increase MAP, improving oxygenation and ventilation. Another new approach in ventilation considered in therapy of AOP is neurally adjusted ventilation assistance (NAVA), which provides even more precise control of invasive and noninvasive respiratory support, optimises synchronisation with device and changes in the infant's own respiratory drive. An important advantage of this method is backup ventilation during periods of apnea. A single study indicated a significant

improvement in apnea under ventilatory support via non-invasive NAVA compared to therapy with CPAP (Firestone et al. 2020). Another study with extreme preterm infants also represented an improvement of apnea and bradycardia under ventilatory support via NAVA (Tabacaru et al. 2019). Moreover, automated oxygen titration systems are beginning to come into use in some NICUs following recent work highlighting their safety (Dargaville et al. 2021).

The xanthine therapy as prevention of apnea was established in the1970s. Since then, it has become widespread in neonatology, although their precise mechanisms of effect are not clearly understood. Caffeine reduces the number of apneic episodes as well as the duration of mechanical ventilation (Kumar et al. 2019). Additionally, according to various studies, treatment with caffeine significantly decreases incidence of BPD and improvement of neurodevelopmental outcome at 18-21 months (Davis et al. 2010; Schmidt et al. 2006, 2007, 2012). On another hand, xanthine therapy has well-documented acute adverse effects such as tachycardia, cardiac dysrhythmias, feeding intolerance, and, rarely, seizures. These seizures are mostly the result of toxic levels of xanthine, but some occur while on a normal dosage. Treatment with xanthine enhances metabolic activity, boosts oxygen consumption, and increases caloric demand—which can be crucial for extreme preterm infants (Meilan et al. 2016). Xanthine therapy improves minute ventilation and CO2 sensitivity, reduces hypoventilation, decreases hypoxic depression of breathing, and enhances diaphragmatic activity. Although, the mechanism of action of xanthine in the perinatal and early postnatal period remain unclear, it may block both adenosine receptors A1 and A2A (Herlenius et al. 2002). Inhibition of these receptors stimulates the respiratory neural output through decreased GABA release. Simultaneously, adenosine receptors affect inflammation and neuroinflammation; this may explain pulmonary improvement in premature infants treated with caffeine by interaction with xanthine (Brothers et al. 2010.). In their study, Valdez et al. demonstrated an association between serum caffeine levels and changes in the pro- or anti-inflammatory cytokine profiles (Valdez et al. 2011). This could also explain the positive effect of caffeine. Adenosine is to inhibit neuroregulation (via activation of adenosine A1 receptors) and during hypoxia can mediate the negative development of hypoxia-induced loss of brain white matter and ventriculomegaly (Turner et al. 2003). Therefore, the effect of caffeine, adenosine antagonist, on the hypoxic brain has been found to improve my-

elination and prevent ventriculomegaly as well as perinatal white matter injury (Back et al. 2006). Our understanding of the role of xanthines may be enhanced by future studies correlating physiological observations with the localisation of adenosine receptor subtypes in respiratory-related regions of the developing brainstem. However, their precise pharmacological action is still under investigation.

Nevertheless, there are additional alternative ways which have been successfully implemented such as mechanosensory inputs through optimising nursing care, greater parental involvement, and physical contact (Kangaroo care) or stochastic mechanosensory stimulation which has been introduced by Bloch-Salisbury et al. (2009), olfactory stimulation may diminish apnea. The previously widespread anti-reflux medication is not evidence-based and no longer recommended. Nowadays, there is no evidence of a significant correlation between GER and cardiorespiratory events (Di Fiore et al, 2012; Martin et al, 2015),

1.2. Doxapram

When extreme preterm infants are afflicted by chronic ischemic episodes and persistent apnea despite common treatment protocols, the invasive ventilation is necessary. However, further intubation and invasive ventilation might increase the risk of secondary lung injury and subsequent bronchopulmonary dysplasia, which, in the long run, impairs neurodevelopment and is associated with an increased risk of death or disability (Poets et al. 2015). In this case, doxapram may be a suitable alternative. Doxapram is an off-label analeptic and second-line treatment for severe apnea of prematurity when caffeine and noninvasive ventilation have failed. Doxapram was first synthesised in 1962. It was found to have a strong, dose-dependent respiratory stimulant action in mammals. The overview of main features of doxapram, including its pharmacokinetic, metabolism, adverse effects, current recommendations, and clinical established benefits is represented in Table 1.

Pharmacology	• **The chemical formula**: 1-ethyl-4-(2-morpho- linoethyl)-3,3-diphenyl-2-pyrrolidinone. • **Initial metabolisation**: in the liver by CYP3A4 and by CYP3A5 • **active metabolite:** keto-doxapram. • **Enzyme oxidation pathway**: unclear.

	• **Stimulating effect on the respiratory drive**: through activation of central respiratory regulation in the brainstem and peripheral chemoreceptors in the carotid and aorta. ◆Molecular level: primarily, direct inhibitory effect on the K+ channels, specifically TASK-1 and TASK-3 (TWIK-related Acid Sensitive K channel), which are basic cellular elements mediating the response to respiratory stimuli: changes in pH and oxygen levels. Doxapram enable to act as a respiratory stimulant through the same molecular mechanisms (inhibition of TASK channels): release the Ca-dependent neurotransmitter by inhibiting baseline membrane K+ conductance in a dose-dependent manner. ◆Peripheral ventilatory response: While hypoxemia (PaO2 < 55) and acidosis drive oxygen sensing in the carotid glomus (type I) cells and increase phrenic nerve activity, doxapram is able to increase carotid body afferent activity equivalent to that produced by severe arterial hypoxemia (PaO2 = 35–40 mm Hg), improve peripheral sensation to hypoxemia and central sensation to hypercarbia. It also has an additive effect on the carotid body response to hypercapnia. ◆Central ventilator response: Acidemia increases also the drive potential of respiratory neurons due to blockade of TASK channels in motor neurons in the spinal cord, the brainstem, the ventral medullary, and the preBötzC, target for neuromodulation, especially in motoneurons. Doxapram has the similar stimulatory effect on activity in the PreBötC and additionally intense effect on XII motor output, improving their drive potential and counteracting the inhibitory effects of the normocarbia or hypocarbia on respiratory rhythm, independent of peripheral chemoreceptors. Central actions of doxapram in respiratory rhythm generation are implemented by increased drive potentials in inspiratory and expiratory neurons and enhanced respiratory activity in the brainstem–spinal cord. These drives increased depth and rate of respiration. Doxapram leads to a more robust increase in motor output rather than a strong facilitation of the frequency of the respiratory drive. • C**eiling effect:** because the central activities of the PreBötC and XII are not affected by doxapram during severe hypoxia, no further benefit can be expected from doxapram treatment while exposed severe or chronically hypoxia. • C**affeine:** no interactions between these drugs, although, the importance of taking into account PCA and birth weight in caffeine and doxapram clearance, though doxapram does not affect the pharmacokinetics of caffeine***.***

Metabolism	• After intravenous administration metabolised very rapidly. • Effective blood concentrations approximately 2 µg/mL. • Immediate onset within minutes of its effects on increased minute ventilation and tidal volume. • P**reterm infants:** the pharmacokinetics and metabolites in preterm infants verify large variability between individuals: ▪ Gender ▪ Post-menstrual age (PreBötC have clear functional differences within the first two weeks postnatal). ▪ Enzyme maturation and conditions of renal excretion. Especially, for SGA or IUGR infants. ▪ the oral bioavailability of doxapram in preterm neonates is 74%, requiring a 33% higher dosage orally than intravenously. ▪ the effects in different areas of the respiratory control system appear to be concentration-dependent.**
Brain	• Enhance recovery and facilitation of phrenic motor neurons after spinal cord injury. Doxapram primarily influences respiratory drive and not respiratory muscle activity. • Ability of doxapram to trigger neuroplastic processes. • The additive effect of doxapram in caffeine-treated preterm infants with persistent AOP. ***
Adverse effects	• Relatively minor • **Adults:** in 5% of patients, cough, dyspnea, tachypnea, headache, dizziness, anxiety, hypertension, flushing, sweating, nausea, vomiting, diarrhoea, urinary retention, and muscle spasticity. CNS excitation—especially in cases of liver insufficiency. • **Preterm and newborn:** cardiovascular side-effects like atrioventricular heart block and tachycardia (probably also caused by K+ channel inhibition). Gastrointestinal disturbances, excessive crying, irritability, increased agitation, jitteriness**,** hypertension**,** hypokalaemia**,** increased electrographic seizure activity and less sleep–wake cycling than in control groups*. C*oncerns about its possible long-term effects on mental development were not confirmed in a multi-central cohort study**.******
Recommendation	• Oral or iv. • Doses at recommended infusion rates of 1-2–2.5 mg/kg/h. • Loading doses is possible. • Current recommendations for doxapram dosing based on bodyweight. • Regulation of dosage adjusted for GA and PNA could minimise these risks. Yet there are still no clear dosage recommendations based on factors other than bodyweight alone. *****

Established benefits	• Declined frequency of hypoxic events, decreased duration of mechanical ventilation, and decreased rate of bronchopulmonary dysplasia**.** • Reduced oxygen requirements. • Prevents endotracheal intubation (effectiveness up to 60%) • increased success of extubation**.** • **Predictors of success:** immediate response after application with increase of the SpO2/FiO2 ratio after therapy and decreasing the number and duration of hypoxic episodes**. *******

Table 1: Main pharmacological characteristics of doxapram.

* Ogawa et al. 2015; Talley et al. 2000; Ogawa et al. 2015; Yost 2006; Engbers et al. 2021
** Ramirez et al. 1996; Ogawa et al. 2015; Flint et al. 2021; Allegaert K, et al, 2007; Greze et al. 2016)
***Sandhu et al. 2013
**** Fischer et al. 2013; Yost 2016; Poets et al. 1999, Czaba-Hnizdo et al. 2014; Ten Hove et al. 2016
***** Flint et al 2021.
******Prins et al. 2013; Flint et al. 2017; Vliegenthart et al. 2017; Poets et al.1999; Henderson-Smart et al. 1983; Davis et al. 2010.

2. Aims of this Study and Hypotheses

The first aim of this retrospective, single-centre study is to analyse and compare the clinical data of extreme preterm infants who were treated or not treated with doxapram. Additionally, we aim to describe the “typical” patient treated with doxapram.

The second aim is to analyse the effectiveness of doxapram treatment in extreme preterm infants with extremely low birth weight and possible indicators of a successful usage of doxapram by an evaluation of different factors (prenatal, intranatal, and postnatal clinical short-term data).

The third aim is to evaluate the possible effects of doxapram on long-term neurodevelopmental outcomes.

3. Methods
3.1. Patients and settings

We performed this retrospective cohort study at the Altona Perinatal Center in Hamburg. The Altona Perinatal Center is classified as a Level I facility. It provides care for high-risk pregnant women and newborns, including extreme prematurity, critically ill newborns, and surgical repair of complex congenital or acquired conditions.

The initial cohort included all extreme preterm infants born from January 2013 through December 2018 with a gestational age < 27 weeks and a birth weight < 1000 g (N= 124 infants) that were treated at the neonatal intensive care unit in the Altona Children's Hospital. We excluded infants who died before 7 days of life or who had various syndromic disorders. From this cohort, we selected patients treated with doxapram (N=40) and compared them to those without doxapram (n=84). Forty-six patients (37%) were lost to follow-up, forty-seven (64.4%) non-treated patients were given a neurodevelopmental follow-up. Among those treated with doxapram, patient follow-up data were available by 29 (78.4%).

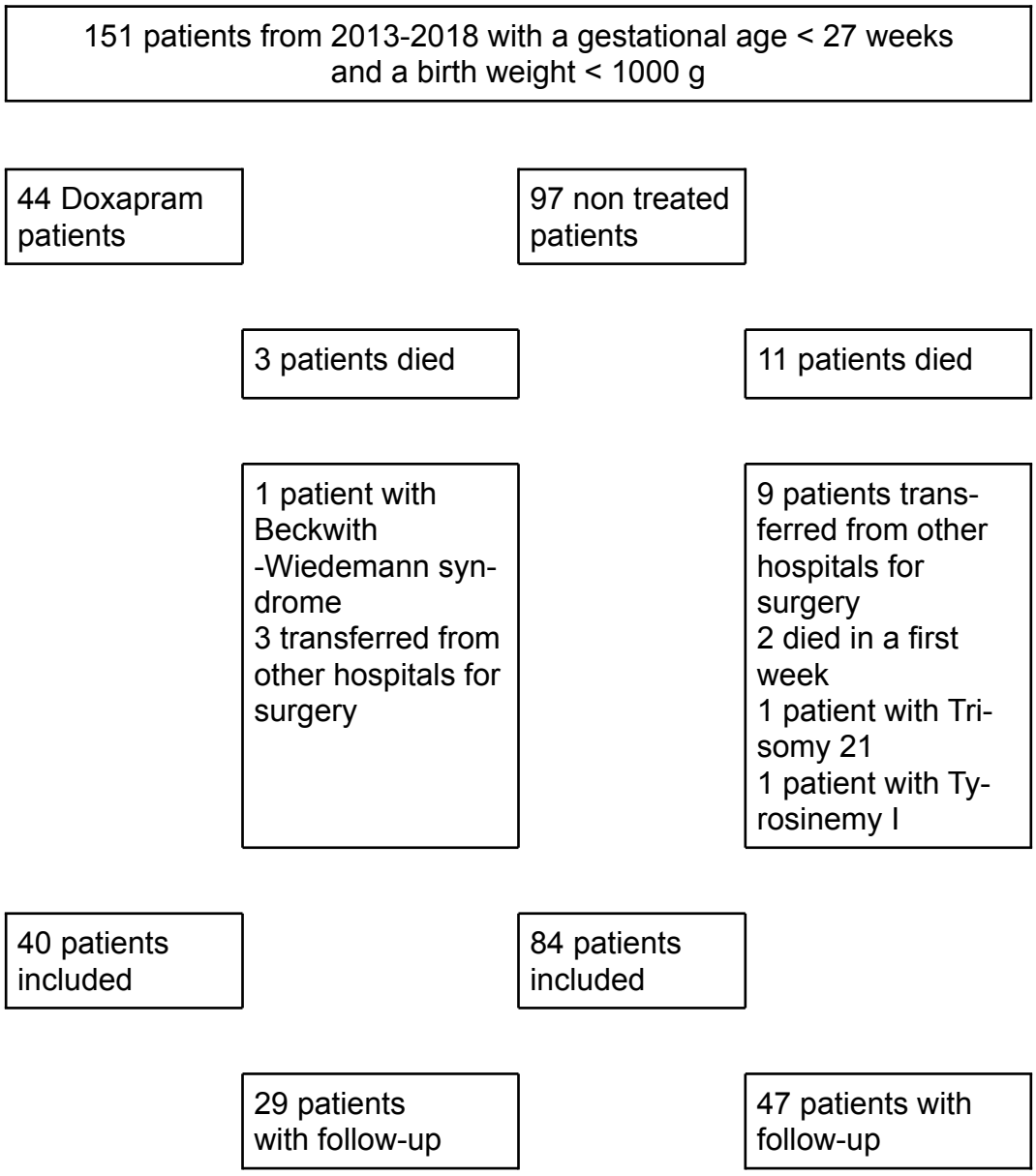

Figure 1: Population flow sheet: doxapram-treated versus non-treated patients

3.2. Data collection

All data were obtained from bedside medical records and extracted from the NEOAT 5 Database (Paedsoft, Tübingen). Follow-up data were collected from electronic medical records from Soaring Clinical (University Hamburg UKE, Siemens AG). We analysed multiple demographic and short-term clinical data, including prenatal and intranasal complications, medication, duration of invasive and non-invasive mechanical ventilation or other respiratory support, duration of the oxygen therapy, number of intubations, and number of sepsis (Table 3). Data on the administration of doxapram,

dosages, duration of therapy, fraction of inspired oxygen concentration (FiO2), and occurrence of AOP were also collected. Data were collected until death or discharge from the NICU. Bronchopulmonary dysplasia (BPD) was classified as mild with a requirement for supplemental oxygen not longer than 28 days of life, moderate for supplemental oxygen or CPAP at 32 weeks post-conceptional age, and severe BPD when supplemental oxygen or any respiratory support was required at 36 PMA. Infants who were transferred to an outside hospital on oxygen prior to 36 weeks' PMA were not included in the BPD analysis. Early onset sepsis (EOS) was defined as signs of sepsis occurring at ≤72 h of life, positive blood culture, increased CRP or IL6, leucopenia or leucocytosis, and antibiotic treatment for at least 5 days. Sepsis occurring after 72 h up to the age of <90 or 120 days was considered late-onset sepsis (LOS). Persistent ductus arteriosus was identified by serial echocardiography, including the flow measurement in A. cerebra media or A. mesenterica. It was treated with Ibuprofen or Paracetamol intravenously or surgical via PDA-Ligature. Intraventricular haemorrhage (IVH) grade I-IV or periventricular leukomalacia were identified by serial head ultrasounds. Diagnosis of necrotising enterocolitis by abdominal clinical and radiograph signs showing pneumatosis, portal venous air, or pneumoperitoneum (Bell Stage IIb- IIIa) required surgery.

All extreme preterm infants treated in Altonaer Children's Hospital were enrolled in a routine follow-up program for standardised neurological examination of mental and psychomotor development using the Bayley Scales for Infants. Every examination was performed by developmental specialists, neuropaediatricians, and physiotherapists. The following tests were required: EEG, MRI, ophthalmologist, hearing test. Within 24 months, the program was completed. We collected all data about children who attended the program.

The neurodevelopmental assessment at 24 months consisted of Bayley Scales of Infant development (BSID-II), Mental Development Index (MDI), and Psychomotor Development Index (PDI) scores with <–1 standard deviation (SD) below the mean (<85 points) were defined as abnormal. Cerebral palsy (CP) was diagnosed according to international standards via tests such as Prechtl's Qualitative Assessment of General Movements and medical resonance imaging. All levels of CP were included. Neurological delay (abnormality) was defined as one or more of the following out-

comes: MDI and/or PDI score <85, CP, hearing loss despite amplification, and visual impairment leading to blindness or only light perception in at least one eye.

3.3. Cohort characteristics

Our retrospective study involved 124 preterm infants with gestational age under 27 weeks and birth weight less than 1000g that were admitted to our NICU at Altonaer Children‘s Hospital. Forty of them (32.2%) received doxapram. The data from these patients were included in the statistical analysis and their characteristics are represented in Tables 3.1, 3.2 and 4.1. and 4.2. The median gestational age was 25.1 (IQR 23.3-26.6) weeks, their median weight was 691.3g (IQR 295-990 g). During the study, the total mortality rate of this population 11.2% (14 individuals). Only 67 (54%) were exposed to a complete cycle of antenatal corticosteroids. In our cohort 31 (25%) patients were discharged without diagnosed BPD. The overall incidence of infections was by 64,5%, moreover, the rate of LOS is relatively high was observed in 58 (48.4%) patients and recurrent sepsis in 12 (9.7%) children. From this cohort, 40 infants were treated with doxapram.

		% (n=124)
Gestational Age, Weeks		25.1 (23.3-26.6)
Birth weight, g		691,3 (295-990) ± 167,9
Birth weight, P		30,7 (1-75) ± 20,7
Head Circumference, cm		22,7 (17,5-31,5) ± 1,96
Female gender		49,2 (61)
Multiple birth (yes)		23,4 (29)
Mortality		11,3 (14)
Antenatal corticosteroids:	no	8,9 (11)
	not complete	37,1 (46)
	complete	54,0 (67)
Rupture of Membranes		50,8 (63)
AIS		45,2 (56)

		% (n=124)
Pathological Doppler		14,5 (18)
Pathological CTG		23,4 (29)
Preeclampsia		9,7 (12)
Antepartum bleeding		18,5 (23)
Premature Labor		60,5 (75)
Birth Modus: Sectio		89,5 (111)
Cardio- pulmonary resuscitation in delivery room		21,8 (27)
LISA in delivery room		69,4 (86)
Intubation in delivery room		29 (36)
LISA secondary		13,7 (17)
Intubation, average N per patient		1,62 (0-10) ± 1,92
Average duration of invasive ventilation, d		10,1 (0-107) ± 15,87
Average duration of non-invasive ventilation (CPAP), d		42,62 (0-90) ± 16,79
Average duration O2-Therapy, d		52,1 (0-143) ± 35,76
Doxapram therapy		32,2 (40)
BPD	none	25 (31)
	light	45,2 (56)
	moderate	9,7 (12)
	severe	13,7 (17)
Infection	no sepsis	35,5 (44)
	EOS	24,2 (30)
	LOS	48,4 (58)
	Recurrent Sepsis	9,7 (12)
hsPDA	none	60,5 (75)
	drug-treated	29 (36)

		% (n=124)
	surgically treated	9,7 (12)
FIP		15,3 (19)
NEC		9,7 (12)
Neurology:	IVH II-III	14,5 (18)
	PVL	3,2 (4)
	Seizures	5,6 (7)
	Hydrocephalus	4 (5)
	Ventriculitis	1,6 (2)
	Neurological complication	25 (31)

Table 2: Baseline characteristics of the study population. Values are presented as means ± SD, % (n) or median (IQR) (min–max)

3.4. AOP treatment policy

Our unit policy maintains non-invasive or minimally invasive respiratory support, including LISA in the delivery room and respiratory support primarily with CPAP. As an escalation, nHFO, NIPPV was used. According to the infant's physical condition, further escalation with invasive mechanical ventilation may have been necessary. In addition, our protocol for the AOP treatment for this group of preterm neonates requires an immediate start with caffeine following the loading doses in the delivery room and increasing dosage to 10 mg/kg/d. The decisions to treat with doxapram were based on our internal guidelines. Doxapram treatment started after all non-invasive respiratory support failed or for successful extubation with expected excessive AOP to avoid possible endotracheal intubation.

We collected data at the beginning of the treatment with doxapram as well as recording the treatment duration, maximal dosage, and cumulative dosages. This includes the retrospective analysis of the clinical situation and the medical decision to treat with doxapram.

Mainly, doxapram was initially prescribed by continuous intravenous infusion without loading dose. The starting dose was 1,5-2 mg/kg/hour. If it was effective, the doxapram dose could be decreased stepwise and switched from intravenous to gastroenteral administration for preterm, fully enteral feeding, on the judgement of the attending physician. Doxapram treatment was stopped if the patient required endotracheal intubation for mechanical ventilation or if apnoea subsided with low doses of the drug.

Outcome: Our definition of treatment success with doxapram was avoiding endotracheal intubation and mechanical ventilation. We defined treatment failure as the need for intubation due to the persistence of apnoea despite doxapram treatment.

The secondary aim was to evaluate the potential predictors of the success of doxapram treatment, which included prenatal conditions, gestational age, postnatal age at the start of doxapram, gender, bodyweight, non-invasive respiratory support, infections, and number of intubations. Additionally, we considered the short-term outcomes, BPD, duration of ventilation, hospitalisation, and the long-term outcome of neurodevelopmental delay.

3.5. Statistical analysis

We used antenatal, perinatal, and clinical characteristics for frequency and descriptive tables. Continuous variables were represented via medians with interquartile ranges (IQR). Categorical variables were represented as frequencies with percentages. We collected bivariate data between the doxapram-treated group and non-treated group. Then, we analysed discrete/categorical data by performing chi-squared test ($\chi 2$ test)/Fischer test, contingency tables for categorical data, and a Mann-Whitney U test. We elaborate on continuous data such as the duration of respiratory support, oxygen delivery, duration of doxapram treatment, cumulative doxapram doses, and duration of hospitalisation. A generalised binomial linear model was applied to compare doxapram-treated and non-treated groups to identify possible short-term outcomes or adverse effects. For example, these include the duration of respiratory support, BPD, duration of hospitalisation, IVH, and NEC. Then, we analysed the responding/non-responding groups to identify the predictors of successful doxapram treatment by binary logistic regression. We also performed multiple logistic regression analysis; unfortunately, the model was incorrect because of the limited

numbers of children in the follow-up. We considered statistical significance two-sided ($p<0.05$). All statistical analyses were conducted in SPSS (IBM SPSS Statistics, Version 27).

4. Results

4.1. Cohort description, non-treated and treated with Doxapram

The median gestational age of the non-treated cohort (N=84) was 25.5 weeks, and the mean birth weight was 707.3 g. (Table 4.) To compare this cohort to the cohort of children treated with doxapram we could observe that these preterm infants were more immature—their mean gestational age was one week less, 24.5 (IQR 23.3-26.3). The mean birth weight was also lower: 623.7g (IQR 300-960 g). The mortality rate after 7 days of life and before discharge was at 21.4 % (N=11) of non-treated children compared to 7.7% (N=3) in doxapram cohort, however, not significantly relevant. Analysis of management in the delivery room (LISA, ITN, CPR) showed no significant differences between these two cohorts. Nevertheless, the number of multiple intubations per patient was greater in the group treated with doxapram. The mean duration of invasive ventilation is longer at 18,3 (IQR 1-73) days compared to the mean 9,6 (IQR 1-56) days in the non-treated cohort. In the cohort without doxapram 43.4% did not require any invasive ventilation during their stay at our NICU and only 12,5% (N=5) in doxapram cohort (P=0,05). The number of patients treated with doxapram suffered sepsis significantly frequently compared to children who did not receive doxapram (82,5% vs. 54.9%, p=0,02). Children treated with doxapram were later discharged. The mean duration of hospitalisation was 128 (IQR 82-476) days in contrast to mean median 93 (IQR 52-166) days for non-treated children. The weight percentile at discharge was also lower in the doxapram cohort with a median of 18 (6-30) P and a median of 50 (1-90) P among non-treated patients. The neurodevelopmental delay in follow-up showed a significant distinction in doxapram-treated children with higher incidence of CP or severe mental delay in the doxapram cohort (n=2, 4,2% & n=7, 24,1% p=0.01; n=1, 2,1% & n=2, 6,9% p=0.003, respectively). No adverse effects were observed in this study.

		Patients without Doxapram (n = 84)	Patients treated with Doxapram (n = 40)	p value*
Gestational Age, weeks		25.5 (23.5-26.6)	24.5 (23.3-26.3)	
Birth weight, g		707,3 (295- 990)	623,7 (300-960)	
Mortality		13,1 (11)	7,7 (3)	0,29
Antenatal corticos-teroids:	no	7	12,5 (5)	0,32
	not complete	34	42,5 (17)	0,32
	complete	58	45 (18)	0,32
No complications antenatal		17,9 (15)	0	0,01
Prenatal infection		40,5 (34)	35 (14)	0,01
Placental minder perfusion		27,4 (23)	40 (16)	0,01
Infection associated with placental minder perfusion		14,3 (12)	25 (10)	0,39
Respiratory management	Exclusive NIV	43,4 (36)	12,5 (5)	0,05
	Invasive ventilation from 1.day	20,5 (17)	17,5 (7)	0,05
	Number of intubations more than 3	11,9 (10)	47,5 (19)	0,05
	Mean duration invasive ventilation, d	9,6 (1-56)	18,3 (1-73)	
	Mean duration of O2-Therapy, d	41,4 (0-143)	65 (5-131)	
	O2 supply at 36. GW	18,1 (13)	40,5 (15)	0,01
	O2 supply at discharge	8 (6)	24,3 (9)	0,02
	Hydrocortisone therapy	11,1 (9)	25,6 (10)	0,04
	Diuretic therapy	44,4 (36)	80 (32)	*
BPD	none	37,7 (29)	6,5 (2)	*
	light	46,8 (36)	51,3 (20)	*
	moderate	9,1 (7)	12,8 (5)	*
	severe	6,5 (5)	30,8 (12)	*

		Patients without Doxapram (n = 84)	Patients treated with Doxapram (n = 40)	p value*
Postnatal infection	no sepsis	45,1 (37)	17,5 (7)	0,02
	EOS	23,2 (19)	17,5 (7)	0,14
	LOS	25,6 (21)	45 (18)	0,14
	EOS+LOS	4,9 (4)	7,5 (3)	0,14
	Recurrent Sepsis	1,2 (1)	27,5 (11)	0,14
Neurological complications	IVH II-III	13,3 (11)	17,5 (7)	0,35
	PVL	0 (0)	10 (4)	0,01
	CMV postnatal	3,6 (3)	15 (6)	0,03
	Neurological complication, overall	19,3 (16)	37,5 (15)	0,02
Mean duration of hospital stayed, d		93 (52-166)	128 (82-476)	0,001
Home monitor		11,5 (9)	40,5 (15)	*
Follow up		64,4 (47)	78,4 (29)	*
CP		4,3 (2)	24,1 (7)	0,01
Mental Retardation	no	76,6 (36)	44,8 (13)	0,003
	light	14,9 (7)	24,1 (7)	0,003
	moderate	6,4 (3)	24,1 (7)	0,003
	severe	2,1 (1)	6,9 (2)	0,003
Microcephalic		8,5 (4)	37,5 (12)	0,002
Neurological Abnormality		31,8 (14)	65,5 (19)	0,008

Table 3: Characteristic of cohorts with children non-treated and treated with Doxapram. p value from the Mann-Whitney U test, Fisher's exact test, and $\chi 2$ test.

4.2. Doxapram-Cohort Description

Doxapram treatment was started via intravenous infusion in 40 (32.2%) patients. Their median postnatal age at start of the first administration of doxapram therapy was 23 (IQR 7-100.) days. The cumulative median duration of therapy with doxapram lasts 6 (1-28) days. The median cumulative dose was 144.24 (15.4-820.56) mg/kg. The treatment was successful in 52.5% of patients.

Postnatal age at initiation of first cycle, days	23 (IQR 7-100.)
Total number of applications	59
Total number of applications with success	28 (47,4%)
Total duration of therapy, days	6 (IQR 1-28)
Average dosage per hour, mg/kg/h	1,84 (IQR 0,5- 2,34)
Total cumulative dosage per kg, mg/kg	144,24 (IQR 15,4-820,56)

Table 4: Characteristics of doxapram therapy

Further analysis via applied generalised binomial linear model also verify significance followed factors among patients received doxapram: gemini, lower birth weight, exposure of antenatal corticosteroids, various prenatal complications (especially antepartum bleeding and AIS), increased number of intuitions, diagnosed ureaplasma, sepsis and need of catecholamine.

	p value	odd ratio	95% CI
Multiple birth (yes)	0,019	0,023	0,001-0,54
Birth weight, g	0,045	0,986	0,972-1,0
Antenatal corticosteroids	0,020	0,130	0,023-0,729
Prenatal complications	0,020	11,223	1,469-85,743
Antepartum bleeding	0,019	0,016	0,001-0,515
AIS	0,005	4,9	4,69-6748
Number of intubations	0,034	12,105	1,200-122,14
Ureaplasma	0,034	0,038	0,002-0,77
EOS	0,017	0,000	7,155-0,2
Sepsis	0,001	2,5	5,41-198686
Recurrent sepsis	0,101	0,001	8,172-4,22
Administration of catecholamine	0,033	0,068	0,006-0,807

Table 5: Baseline characteristics of the patients received doxapram. Generalised Linear Model

4.3. Predictors of Successes

As a response to the treatment with doxapram, we defined avoided intubation, reduction of apnoea amount and reduced FiO2.

Twenty-one (52.5%) of all children treated with doxapram showed success with doxapram therapy (Table 7). These infants had a higher median birth weight 655.2 g (IQR 300-960 g) compared to patients with therapy failure 588 g (IQR 350-770 g).

Prenatal conditions of children with response to doxapram differ significantly from non-responders by higher numbers of complete cycle of antenatal corticosteroids 57.1% (12) (p=0.045) and antenatal complications were predominantly associated with inflammation process such as AIS or increased laboratory infectious parameters (p=0.04). The respiratory management significantly varied between responders and non-responders. Fifteen (23.8%) infants who responded to doxapram therapy were exclusively non-invasive ventilated compared to none in non-responder group. Infants with successful therapy had fewer intubations (n=4, 19% & n=15, 78.9%, p<0.01), shorter duration of invasive ventilation (3 (IQR 0-26) & 24 (IQR 5-73) days), shorter duration of O2-Therapy (p= 0.02). Despite antenatal history with infectious complications, postnatal responders frequently did not develop any sepsis (n=5, 23.8% & n=2, 10,5%; p<0.02). Furthermore, patients with therapy failure had significantly higher incidence of recurrent sepsis (n=3, 15% & n=8, 42.1%; p=0.02). Patients who did not respond to doxapram treatment were more likely to experience neurological complications during their NICU stay such as IVH II-III 26.3% (n=5) and hydrocephalus 10.5% (n=2). We did not find these parameters to be statistically significant. Patients with successful therapy had higher median postnatal age of initiation of first cycle (median 20 days, IQR 4-37 days vs. median 15 (IQR 7-60 days), fewer cycles of doxapram (mean 1,3 vs 2.2 per patient), and lower average cumulative dosage per kg (mean 203.6mg/kg, IQR 23.2-802.8mg/kg vs. 247.7 15.4-820.5mg/kg) compared to infants whose therapy was unsuccessful. However, the incidence of children without any registered mental retardation in follow-up was significantly higher in responder's group (n=8, 50% & n=5, 38,5% p=0.03).

We investigated the causes for doxapram therapy and reduction of apnea, then divided them into respiratory conditions and sepsis. Further statistical analysis did not find any difference between the groups. However, descriptive analysis showed ther-

apy success in 76.2% (n=16) with more respiratory impairment, but only in 23.8% with sepsis.

		Patients responded to Doxapram n=21	Patients non-responded to Doxapram n=19	p value*
Birth weight, g		655,2 (300-960)	588 (350-770)	
Mortality		4,8 (1)	11,8 (2)	0,4
Antenatal complications	Complete ANS	57,1 (12)	31,6 (6)	0,04
	Infection	52,4 (11)	15,8 (3)	0,045
	Placental minder perfusion	28,6 (6)	52,6 (10)	0,045
	Infection with placental minder perfusion	19 (4)	31,6 (6)	0,045
Respiratory management	Exclusive NIV	23,8 (5)	0	0,004
	Number of intubations more than 3	19 (4)	78,9 (15)	0,004
	Mean duration of invasive ventilation, d	3 (0-26)	24 (5-73)	
	O2 supply at 32 GW	45 (9)	82,4 (14)	0,02
	O2 supply at discharge	10 (2)	41,2 (7)	0,03
	Mean duration of O2-Therapy, d	58,4 (29-131)	74 (5-127)	
BPD	none	5 (1)	5,3 (1)	0,09
	light	70 (14)	31,6 (6)	0,09
	moderate	10 (2)	15,8 (3)	0,09
	severe	15 (3)	47,4 (9)	0,09
Postnatal infection	none	23,8 (5)	10,5 (2)	0,02
	Recurrent sepsis	14,3 (3)	42,1 (8)	0,02
Discharge	Mean head circumference, P	12,25 (1-81)	2,75 (1-11)	
	Mean duration of hospital stayed, d	114,8 (82-155)	165,2 (97-476)	
	Discharged with home monitor	30 (6)	52,9 (9)	0,14
Follow up		80 (16)	76,5 (13)	
	CP	12,5 (2)	38,5 (5)	0,11

		Patients responded to Doxapram n=21	Patients non-responded to Doxapram n=19	p value*
Neurodevelopmental delay	no	50 (8)	38,5 (5)	0,03
	light	12,5 (2)	38,5 (5)	0,03
	moderate	37,5 (6)	7,7 (1)	0,03
	severe	0	15,4 (2)	0,03
Doxapram therapy	Average postnatal age at initiation of first cycle, d	20 (4-37)	15 (7-60)	
	Average number of application pro infant	1,3 (1-3),	2,2 (1-.4),	
	Average total cumulative dosage per kg, mg/kg	203,6 (23,2-802,8)	247,7 (15,4-820,5)	
	Average total duration of therapy, days	5,6 (1-25)	4 (1-20)	
	Average dosage per hour, mg/kg/h	1,44 (0,5-2,07)	1,97 (1-2,34)	

Table 6: Patient characteristics classified by therapy success or therapy failure

We used binary logistic regression to analyse possible predictors of success in therapy with doxapram (Table 8). We investigated the following variables: gender, birth weight, antenatal glucocorticoids, prenatal complications (preeclampsia, pathological Doppler and CTG, antepartum bleeding, rupture of membranes), AIS, surfactant application, intubation on the first day of life, various models of respiratory support, number of intubations, duration of oxygen supply, and PDA. In our cohort, only higher birth weight (OR 1.0 95% CI 0.99), sign of moderate BPD (OR 9,0 95% CI 1,5, p=0,014), days of invasive ventilation (p= 0,014, OR 0,9, 95%CI 0,82) and fewer intubations (p=0.04 OR 137,2%, 95%CI 1,218) could be considered predictors of success in our center.

Multivariable logistic regression analysis was used. It showed no significant violations or associations.

		p value	odd ratio	95% CI
Birth Weight		0,371	1,0	0,994-1,01
Antenatal Corticosteroids	no	0,195	-	-
	not complete	0,813	0,62	0,013-30,35
	complete	0,091	0,025	0,0-1,8
Intubation in delivery room		0,309	5,592	0,2- 5590
Respiratory management	Exclusive NIV	0,238	-	-
	Invasive from 1.day	0,294	5,15	0,24-110.4
	Number of intubations more than 3	0,041	137,2	1,21-15473
BPD	none	0,091	-	-
	mild	0,457	3,3	0,14-77.2
	moderate	0,014	9,0	1,57-51,1
	severe	0,526	2,0	0,22-19,0
Reparatory support, days		0,014	0,92	0,86-0,98

Table 7: Patient characteristics. Univariable logistic regression analyses with success of doxapram therapy as predictor of success variable.

4.4. Outcomes and Potential Doxapram Therapy Improvements

4.4.1. BPD

We conduct further statistical analysis via contingency tables (crosstabs) using chi-squared test ($\chi 2$ test), to identify possible associations or correlations between severity of BPD and other variables (Table 9). The incidence and severity of BPD was significantly correlated with the frequency of various prenatal complications ($p< 0.01$). We found a statistical correlation ($p<0.001$) between the presence and quantity of postnatal infection and the severity of BPD. With an increase of BPD severity, the incidence of recurrent sepsis increased (41.2% with severe BPD). Respiratory man-

agement had an impact on the severity and rate of BPD in the observed cohort. More than three intubations during the stay in NICU was linked with increased severity of BPD (3.2% without BPD, 17.9% with mild BPD, 50% with moderate BPD, 70.6% with severe BPD). Almost half of the cohort with moderate or severe BPD had significantly higher numbers of infants with surgically corrected PDA (25% and 23.5%, respectively, p=0,02), which is almost seven times higher than in groups with no or mild BPD.70.6% of infants with severe BPD were treated with doxapram and only 25% of infants responded to doxapram therapy. With moderate BPD the rate of successful therapy was 40%. Furthermore, the group with only mild BPD were treated in 35.7%, mostly due to respiratory problems without infection; 70% responded to the therapy (p=0,023). The correlation between cerebral palsy, neurodevelopmental impairment, and severity of BPD was significant ($p< 0.01$).

BPD n=116						
		no n=31	mild n=56	moder-ate n=12	severe n=17	p val-ue
Antenatal complica-tions	none	32,3 (10)	5,4 (3)	8,3 (1)	0	0,001
	Infectious	35,5 (11)	46,4 (26)	25 (3)	29,4 (5)	
	Placental minder perfusion	25,8 (8)	30,4 (17)	58,3 (7)	29,4 (5)	
	Infection+ placental minder perfusion	6,5 (2)	17,9 (10)	8,3 (1)	41,2 (7)	
Postnatal infections	none	54,8 (17)	28,6 (16)	41,7 (5)	29,4 (5)	0,001
	Recurrent sepsis	7,1 (0)	7,1 (4)	8,3 (1)	41,2 (7)	
Respiratory support:	Without invasive venti-lation	64,5 (20)	32,1 (18)	16,7 (2)	5,9 (1)	0,024
	Number of intubations more than 3	3,2 (1)	17,9 (10)	50 (6)	70,6 (12)	
PDA surgically treated		3,2 (1)	7,1 (4)	25 (3)	23,5 (4)	0,024
Doxapram application	yes	6,5 (2)	35,7 (20)	41,7 (5)	70,6 (12)	0,075
	applied due to sepsis	100 (2)	30 (6)	40 (2)	33,3 (4)	0,26
	applied due to respiratory insufficiency	0	70(14)	60 (3)	66,7 (8)	0,26
	responded	50 (1)	70 (14)	40 (2)	25 (3)	0,023
Cerebral palsy		0	10,8 (4)	0	41,7 (5)	0,003
Neurological abnormality		13,3 (2)	48,6 (18)	44,4 (4)	75 (9)	0,014

Table 8: Long-term outcome characteristics (BPD) of the patients received doxapram contingency tables (crosstabs) using chi-squared test (χ2 test)

4.4.2. Neurodevelopmental follow-up

These data were available for 73 (50.6%) of the surviving children, 47 (64.4%) non-treated infants and 29 (78.4%) infants received doxapram (Table 10). In further cross-tabulation analysis, we investigated the possible influence of various factors (antenatal corticoids, prenatal complications, infections, respiratory management, incidence of BPD and doxapram) on long-term neurodevelopmental outcomes. Due to the lower numbers, we summarised neurodevelopmental delay, microcephaly, hear-

ing loss, and cerebral palsy in one group: neurological impairment. We also separately investigated cerebral palsy as a long-term outcome. Further analysis via cross-tabulation showed that almost a half of children from these two cohorts, without any neurodevelopmental impairment or CP, had no postnatal infection (n=22, 55% p=0,003 & n=30, 44,8%; p=0.01 respectively). Moreover, there was no child with recurrent sepsis among these without any neurological impairment (p=0,003), and only six children (9%, p=0.01) in the group with CP, while its incidence among children diagnosed in follow up with neurological impairment or CP was significantly high (24,2% p=0,003 & 22,2% p=0,01, respectively). Similar results were found for postnatal CMV-infection (24,2% p=0,001 & 44,4% p=0,005, respectively).

Respiratory management during the neonatal period and severity of BPD identified as significantly relevant factors for development of neurological impairment or CP. Compared to children with neurological impairment, infants without neurological issue in follow-up experienced significantly less invasive ventilation (45% vs. 18.2% p =0,002, respectively), multiple intubations (48,5% vs. 10% p=0,002, respectively) and rarely suffered under severe BPD (27.3% vs. 7,5% p= 0,003). Six infants with CP (66,7% p= 0,03) were intubated multiple times and five infants with CP (55,6% p= 0,03) had severe BPD. The incidence of treatment with doxapram was significantly higher among the patients with neurological impairment (57.6% vs. 25% p=0.005) and with CP (77.8% vs. 32.8% without, p=0.013). However, the rate of response to the therapy with doxapram was significantly greater in children without any neurodevelopmental deviation (70% vs. 47.4% p=0.01) as well as for infants without CP (63,6% vs. 28,6% p=0.003).

		Neurological impairment in follow up			CP in follow up		
		no n=40	**with n=33**	**p value**	**no n=67**	**with n=9**	**p val-ue**
ACS	none	7,5 (3)	15,2 (5)	0,023	11,9 (8)	0	0,3
	not complete	27,5 (11)	48,5 (16)		35,8 (24)	55,6 (5)	
	complete	65 (26)	36,4 (12)		52,5 (35)	44,4 (4)	
Antenatal complications	none	15 (6)	9,1 (3)	0,05	13,4 (9)	0	0,08
	Infectious	50 (20)	36,4 (12)		43,3 (29)	44,4 (4)	
	Placental minder per-fusion	25 (10)	27,3 (9)		29,9 (20)	11,1 (1)	
	Infection+ placental minder per-fusion	10 (4)	27,3 (9)		13,4 (9)	44,4 (4)	
Postnatal infection	none	55 (22)	21,2 (7)	0,003	44,8 (30)	11,1 (1)	0,01
	Recurrent sepsis	0	24,2 (8)		9 (6)	22,2 (2)	0,2
	CMV	0	24,2 (8)	0,001	6 (4)	44,4 (4)	0,005
Respiratory management	Without in-vasive venti-lation	45 (18)	18,2 (6)	0,002	35,8 (24)	22,2 (2)	0,03
	Number of intubations more than 3	10 (4)	48,5 (16)		20,9 (14)	66,7 (6)	
BPD	none	32,5 (13)	6,1 (2)	0,003	26,9 (18)	0	0,003
	mild	47,5 (19)	54,5 (18)		49.3 (33)	44,4 (4)	
	moderate	12,5 (5)	12,1 (4)		13,4 (9)	0	
	severe	7,5 (3)	27,3 (9)		10,4 (7)	55,6 (5)	
Doxapram application	yes	25 (10)	57,6 (19)	0,005	32,8 (22)	77,8 (7)	0,013
	applied due to sepsis	7,5 (3)	12,1 (4)	0,016	7,5 (5)	22,2 (2)	0,03

		Neurological impairment in follow up			CP in follow up		
		no n=40	**with n=33**	**p value**	**no n=67**	**with n=9**	**p val-ue**
	applied due to respirato-ry insuffi-ciency	17,5 (7)	45,4 (15)	0,016	25,4 (17)	55,6 (5)	*
	respond	70 (7)	47,4 (9)	0,01	63,6 (14)	28,6 (2)	0,003

Table 9: Long-term outcome characteristics (Neurodevelopmental follow-up and cerebral palsy) of the patients received doxapram. contingency tables (crosstabs) using chi-squared test (χ2 test)

5. Study limitations

This study has several limitations that must be addressed. First, this research is retrospective and non-blinded. Second, it is a single-centre study, which might cause the expected bias. Third, the number of included infants was restricted, limiting statistical performance. However, further multivariate analysis was impossible to perform due to lower numbers in our subgroups. Because of this, we focused on descriptive analysis and logistic analysis. Fourth, during the study period, respiratory management in the delivery room and the intensive care unit became increasingly non-invasive over time; potential bias cannot be ruled out. Finally, we did not correct the logistic regression analyses for the variables CP and neurodevelopmental delay. This may have affected the results of our study, although the impact of this is probably limited because the incidence of CP was extremely small and similar in both groups. This may have confounded our results.

6. Discussion

The aim of this study was to analyse the predictors of the successful use of doxapram for treatment of AOP in extremely preterm neonates and its possible impact on neurocognitive outcomes. We decided to define the criteria of inclusion to focus on extreme preterm neonates because, especially for this cohort, there is a remarkable lack of data and investigations. To our knowledge, this is the first study on this issue for extreme preterm patients in Germany, which also includes neurodevelopmental follow-up. While, over the last few decades, the number of surviving extreme preterm infants has increased, the vulnerability of these patients at the NICU demands the further enhancement of protocols and therapy. Every decision made in this situation has consequences, often as serious as neurodevelopmental impairment. Therefore, the main challenge for clinicians is to prevent lung injury and simultaneously provide effective, sufficient management of apnoea treatment. Doxapram could be the key to maintaining the less-invasive approach in neonatology, when other well-known and approved techniques such as caffeine, CPAP, and other treatments failed. The effectiveness of doxapram for extreme preterm patients has been demonstrated in many studies, although its impact on neurodevelopment has remained a point of discussion until recently. Hence, we analysed the possible predictors of success in preventing the unnecessary application of doxapram and investigated the neurocognitive outcomes depends on response to the treatment with doxapram.

Doxapram is an off-label analeptic and second-line treatment for severe apnoea of prematurity, however, understanding of its pharmacokinetics and metabolism is not fully understood. Only recently have the exact effects of doxapram stimulation become known. New studies have discovered that doxapram enhances the respiratory drive through the activation of both central respiratory regulation in the brainstem and peripheral chemoreceptors in the carotid and aorta. Stimulation of peripheral chemoreceptors occurs in the carotid body through the same physiological mechanisms as severe arterial hypoxemia; through the direct inhibition of the TASK-1 and TASK-3 channels, doxapram drives oxygen sensation of peripheral chemoreceptors and contributes to afferent activation (Cotten et al. 2006; Yamamoto et al. 2002; Han et al. 2003; Talley et al. 2000; O'Donohoe et al. 2018). Moreover, doxapram's stimulation of the respiratory activity involves TASK channels located in the PreBötC, spin-

al cord, medulla oblongata, and even more intense XII motor output. Theirs inhibition, increasing central inspiratory and expiratory neuronal activity, leads to the improving drive potential of respiratory neurons and possibly a stronger central CO2 response. Furthermore, the ability of doxapram to increase the drive potentials to respond and generate the respiratory rhythm in the brainstem is decreased under hypoxic conditions (Kruszynski et al. 2019). This means that, once an infant becomes chronically hypoxic, no further benefit can be expected from doxapram treatment. Moreover, we confirmed this phenomenon during therapy failure in severe sepsis or respiratory exhaustion cases. Possibly, doxapram was applied too late and, as a result, was not effective.

6.1. Characteristics of the Typical Patient Treated with Doxapram

We implemented further statistical analysis via generalised linear models to describe a typical preterm infant who might receive doxapram at our unit. This could serve us as a mirror to show which indications were used by neonatologist at our hospital. The model was built in GenLin SPSS (generalised linear model). Therefore, analysis of the typical patient treated with doxapram demonstrated higher incidence of twin pregnancy, none or incomplete cycle of ACS, various antenatal complications, especially in combination of chronic intrauterine hypoxia and inflammation. It is to expect that patients treated with doxapram will have a lower birth weight and lower gestational age. These patients frequently developed various infections such as AIS, ureaplasma, EOS and LOS. The application of catecholamine therapy was required frequently. Respiratory management for these infants was challenging, including multiple intubations, high risk of weaning failure and earlier the signs of new BPD. Also, episodes of hyperglycaemia were frequently recorded being a sign of sepsis, extreme metabolic prematurity, instability, stress, and low energy reserves. On the other hand, the decreased mortality in this cohort can be explained by the supportive effect of doxapram in terms of some stabilisation of hypoxic episodes and still reducing number of extubation failures. Probably, without doxapram, these preterm infants would have had less chances for surviving.

We found that characteristics of infants in our cohort treated with doxapram are similar to those from the data from Ten Hove et al. and Poppe (2020), which also have included a lower birth weight, lower gestational age, higher incidence of postnatal

steroids, longer duration of ventilation before initiation of therapy, increased numbers of BPD, prolonged stay at the hospital, and decreased mortality in the group treated with doxapram (Ten Hove et al. 2016; Prins et al. 2013; Poppe et al. 2020; Yamazaki et al. 2001). Furthermore, the relevance of PMA at the initiation of therapy and its duration, which was previously reported by Ten Hove, was observed in our study. This typical pattern resonates with the pathophysiology and development of AOP. Presumably, extreme immaturity in conjunction with lower birth weight, which manifests in the immature respiratory centre and nervous system, leads to early profound AOP resistance to common treatment protocols. The higher incidence of postnatal steroids administration (25.6 %, N=10), moderate and severe BPD, longer duration of invasive ventilation, multiple weaning failure, hemodynamic significant PDA, and later discharge also reflects an initially fragile, damaged respiratory system. Therefore, we can assume that the deeply impaired respiratory system and initially challenging physical condition may be a result of antenatal predisposition with the important role of the epigenetic prenatal programming.

Nevertheless, children from our unit have even lower birth weights and gestational ages than all other investigations until now; perhaps, explaining the higher morbidity (IVH, PVL, severe BPD, NEC) in short- and long-term outcomes in our cohort than those in published studies. For instance, incidence of PVL increased up to 10% (N=4) in treated patients, which may be the result of exposure to severe intermittent hypoxia. Not only intermittent hypoxia but also infection is likely to be crucial for this cohort. Our data discovered a significant role of infectious complications, prenatal or postnatal infections; although these findings differ from the data from previous investigations (Flint et al. 2017; Ten Hove et al. 2016; Poppe 2020). Due to prenatal reduced placental perfusion, chronic hypoxia, and maternal infectious complications the innate immune system confronts with activated TLR and induced production of pro-inflammatory cytokines. An exposure to high levels of pro-inflammatory effectors causes immune or endocrine imbalance and the ’fetal inflammatory response syndrome’. This is, in particular, dangerous for extreme preterm, resulting in abnormal angiogenesis, impairing development of formation of lung issues, gastrointestinal tract and an immature nervous system, resulting in health complications after birth with long-lasting consequences (Saigal et al. 2008; Gotsch et al. 2007).

6.2. Response and predictors of success

The data on the pharmacokinetics and effectiveness of doxapram in preterm infants indicates large variability between individuals, especially such factors as gender and post-menstrual age (Greze et al. 2016; Flint et al. 2021). Analysing the predictors of success in our study, we also observed that as preterm patients with successful therapy had higher median PCA at first doxapram application 20 (4-37) vs. 15 (7-60) day), however, there was no difference in response according to gender. Flint et al. in his retrospective study analysing predictors of success showed that one of the important factors was postnatal age of 20 days at the start of doxapram therapy. The PCA obviously reflects the stage of maturation and relates to undergoing crucial modification of the respiratory centres. This suggestion was supported by several studies in animal models investigating the PreBötC, showing clear functional differences within the first two weeks postnatal. In our cohort, we found that preterm infants who did not respond to doxapram therapy had lower median birth weights 588 (350-770) g and mean percentile 24,3 (1-70) P compared to patients who had successful therapy 655.2 (300-960) g and mean percentage 30.6 (1-70) P, even so it was not statistically significant. From animal studies, it is known that carotid chemoreceptors exhibit low sensitivity at birth and become more sensitive with age (Flint et al. 2017). Moreover, in our data, the cohort that responded to doxapram had a higher average birth weight and the incidence of SGA in non-responsive group was significantly higher at 31,6% compared to 9,5% in the responsive group. Similar results were represented by Poppe and Flint: infants who do not respond to doxapram therapy were characterised by significantly lower PMA and birth weight with higher numbers of SGA (Flint et al 2017; Poppe et al 2020). Furthermore, the incidence of hyperglycaemia treated with insulin was twice as high in the non-responsive cohort (52.6% vs. 23.8%). We interpret this difference as a sign of deeply immature metabolism. At the same time, SGA are characterised by impaired maturation of both the renal and respiratory centres. Therefore, immature renal excretion and its enzymes, reduced glomerular filtration rate, and/or the number of nephrons might affect the metabolism and pharmacodynamics of doxapram. In other words, prenatal growth must be considered before administration of doxapram (Allegaert et al. 2007). Other predictors of success which we could identify were completed cycle of ACS and an absence of prenatal issues related to placental insufficient perfusion combined with

inflammation, showing a role of prenatal programming and an importance of inflammatory activation antenatal. Moreover, infants who did not respond to doxapram more frequently had catecholamine therapy (52.6% vs. 23.8%), and FIP (26.3% vs. 4.8%) as well.

In this study, among the non-responders in our cohort respiratory management could stand out as especially challenging and complicated from their first hours. This includes instances of extremely increased duration of invasive ventilation, significantly longer weaning or even weaning failure and excessive time of oxygen supply, which were additionally affected by higher incidence of surgically corrected PDA (15.8% vs. 9.5%) as well. Significantly higher numbers of intubation per infant and invasive ventilation before the start of therapy with doxapram were associated with a lower therapy success rate. These results suggest that clinical characteristics of patient's physical condition and severe respiratory insufficiency can be crucial in prediction of success. Therefore, profound, prolonged hypoxic episodes alongside causing reduced physical capacity to overcome apnoea and the declined sensitivity of receptors to doxapram should be the warning signs for therapy failure (Kruszynski et al. 2019). In particular, for preterm infants with severe AOP in a poorer clinical condition, on the basis of our observation intubation should be considered instead of doxapram treatment (Flint et al. 2017). In other words, signs of respiratory exhaustion and severe pulmonary impairment can be used as predictors of non-responsiveness to doxapram therapy according to the data about the decreased ability of doxapram in the presence of prolonged hypoxic conditions and respiratory exhaustion (Kruszynski et al. 2019). Furthermore, according to the study from de Waal's (de Waal et al. 2019), which investigated diaphragmatic activity in extreme preterm infants, comparing groups in which therapy with doxapram was successful or unsuccessful; showed no difference in terms of diaphragmatic response. This study could add new important knowledge that this beneficial effect is probably not mediated through an increase in diaphragmatic activity, but the primary effect of doxapram is to regulate respiratory drive, not respiratory muscle activity. Our results support the suggestion from Flint (Flint at al. 2017) of the possibility of variable responses to doxapram in neonates, which might reflect the development and immaturity of the respiratory system.

Flint and Pope focused on the effectiveness of doxapram, predictors of success/ failure, and patient response and provided the data about their recent investigation on predictors of success for doxapram therapy, using different vital sign alarms, monitor data, oxygen requirements, and oxygen saturation (SpO2) (Flint et al. 2017; Poppe et al. 2019). In their case study, Poppe et al. analysed patient response and predictors of success with therapy according to collected data. The most significantly relevant indicators of the effects of the therapy have been parameters such as the SpO2/FiO2 ratio and hypoxia (SpO2<89%). They made the following conclusions: the first response to doxapram immediately after drug application can provide highly relevant information to clinicians and can be used as a predictor of success, as well as increasing the SpO2/FiO2 ratio after therapy and decreasing the number and duration of hypoxic episodes (Poppe et al. 2020). Pirns and Flint in their recent studies showed that doxapram prevents endotracheal intubation by stabilisation of oxygen saturation (Flint et al. 2017; Prins et al. 2013). Both studies confirmed the effectiveness of therapy with doxapram, avoiding the need for intubation and found a correlation between lower FiO2 and higher probability of preventing intubation. Prins et al. represented doxapram effectiveness in 63% of cases, preventing intubation (Prins et al. 2013). In our cohort the therapy with doxapram was considered as successful in 52,5% of children and even it was not the included in analysed features we also could observe the rapid response as a predictor of success, as well as reduction of FiO2.

Another our main finding is a significantly higher incidence of recurring sepsis among non-responders. This has an awakening interest in particular immune conditions of infants in our cohort, which can also reflect some predisposition and programming for severe BPD through cytokines and chronic inflammation. However, it is difficult to differ if this feature is only specific for our centre. This point of view must be investigated precisely; larger studies with a focus on extremely preterm ELBW are needed. Identifying predictors of non-responsiveness to doxapram therapy can prevent unnecessary doxapram treatment and, thereby, suboptimal therapy and possible harm (Poppe et al. 2020).

6.3. Doxapram, neurodevelopment and bias

Over the last decade most clinicians and neonatologists were concerned about doxapram therapy in extreme preterm infants due to studies that suggested that doxapram impacts long term neurodevelopment. Admittedly, our first finding identified a significantly higher incidence of PVL, cerebral palsy, and neurodevelopmental delay in the doxapram group, however, other factors such as antenatal corticosteroids, prenatal condition, infection, respiratory management, and number of intubations were significantly increased (relevant) in the group with CP and neurodevelopmental delay. Also, we could confirm a strong significant correlation between the severity of BPD and an impaired neurodevelopmental outcome, which was also provided by Doyle (Doyle et al. 2006). In fact, an interpretation of long-term neurodevelopmental delay/cerebral palsy (CP) and impact of doxapram is restricted without the possibility of multivariate regression analysis/high risk of bias. Some studies (Sreenan et al. 200; Lando et al.2005) reported an association between prolonged doxapram therapy and isolated mental developmental delay in low-birthweight infants, although the data was not corrected for the severity of apnoea, potential confounders, BPD, and duration of mechanical ventilation. Lando et al. (2005) used developmental information assessed via a structured telephone interview (Ten Hove et al. 2016). It is important to consider that these case-control and case-series studies provided a relatively small number of patients treated with doxapram without assisting an appropriate correction for possible comorbidity such as BPD and duration of ventilation (Sreenan et al. 2001; Lando et al. 2005; Oommen et al. 2001; Ten Hove et al. 2016). However, recent cohort-studies did not find a difference in long-term neurodevelopmental outcomes but reported that doxapram significantly reduced the risk of mental developmental impairment at the age of 24 months (Roignot et al. 2014; Zayek et al. 2008; Vliegenthart et al. 2017: Ten Hove et al. 2016). The largest current cohort-study on long-term neurodevelopmental outcomes performed by Ten Hove analysing characteristics of doxapram-treated and non-treated infants, especially those with BPD: patent ductus arteriosus and ventilation days for GA. Ten Hove conducted correction for possible confounders by using multiple regression analysis: BPD, patent ductus arteriosus and ventilation days for GA. There was no higher incidence of NEC or spontaneous intestinal perforation due to treatment with doxapram (Ten Hove et al. 2016; Yamazaki et al. 2001; Prins et al.2013). Ten Hove found no

increased short-term gastrointestinal complications. Furthermore, this study was the first which evaluated a significant correlation between doxapram therapy and improved neurodevelopmental outcomes at the age of 2 years.

6.4. Hypoxia, inflammation, and neurodevelopment

One of the main findings is the significant association between doxapram administration and neurological impairment in follow-up in a cohort of very sick and vulnerable preterms. We conducted a further analysis to reveal a role of doxapram depending on success of the therapy. In fact, a precise analysis identifies the relevance of the response to doxapram therapy for these variables. In our cross-tabulation analysis, we were able to represent that the declined rate of neurological abnormalities and cerebral palsy in the follow-up correlated with successful doxapram treatment. While the incidence of cerebral palsy was 4.3% (p= 0,0013) in the group without doxapram and 38.5% (p=0,11) in the group with therapy failure, infants who respond to doxapram had CP only in 12.5%, which is still lower than those in statistical and epidemiological reports for ELBW. Moreover, among children without neurodevelopmental delay but treated with doxapram (N=10) were 70% responders to doxapram (70% vs 47,4 % p <0.05). The same pattern was observed in the cohort without CP with exposure to doxapram (N=22), 63,8% of them responded to the therapy (63,8% vs 28,6 % p <0.11). Furthermore, 45.5% (N=15, p=0.016) of all children with neurodevelopmental delay and 55.5% (N=7, p=0.03) of those with cerebral palsy have been initially treated with doxapram due to progressive respiratory insufficiency, compared to the group without neurodevelopmental delay with only 17,5% (N=7) and 25,4% among infants without CP (N=17). In this way, children who were diagnosed with any impaired neurodevelopment in follow-up experienced significantly often prolonged hypoxia due to increased rate of BPD (p=0,003), weaning difficulties and, as a result, experienced frequently administration of doxapram. At the same time, perinatal and postnatal period of these children was accompanied by significantly declined rate of completed course of ACS, as well as by an increased number of perinatal and postnatal complications, especially infection or recurrent sepsis. We assume that these characteristics, being associated with immaturity and fragility, lead to the intermittent hypoxemic exposure, which may—as demonstrated in various studies—disrupt the maturation of the CNS at a critical time of development, resulting in neurodevelopmental impairment (Martin et al. 2015). According to the recent Co-

chrane Systematic Reviews, in genesis of cerebral palsy there are different pathways to consider, the main causes are a brain injury in the neonatal period, perinatal hypoxia, prematurity, respiratory disorders, associated prolonged ventilation (such as for respiratory distress syndrome or bronchopulmonary dysplasia), sepsis with hypotension, factors before conception, prenatal complication with insufficient placental perfusion, and infections (Shepherd et al. 2018, Oskoui et al. 2013, Himpens et al 2008) . Hence, during the phase of extensive brain growth (synaptogenesis, myelination, and cytological maturation) the critical factors for neurodevelopmental impairment are intermittent hypoxia and inflammation (Jarjour et al. 2015). Moreover, various epidemiological studies indicate a strong correlation between BPD and severe neurodevelopmental delay. After all, presence of impaired lung development intensifies further damage of brain due to exposure to intermittent hypoxia.

Therefore, according to results from our study mentioned above, we assume that due reduction of hypoxic episodes, doxapram is able to improve the neurodevelopmental outcomes for infants of higher risks and with a predisposition to negative long-term outcomes. This assumption was provided in the report from Henderson-Smart (2010) about a reduction in cerebral palsy and major disability at 18 to 21 months' corrected age with prophylactic methylxanthines (caffeine). Christine H. Ten Hove et al. (2016) also suggested that doxapram might have a positive effect on neurodevelopmental outcomes. On the other hand, Dani conducted research on the brain hemodynamics under doxapram in preterm infants and demonstrated an increased cerebral oxygen consumption and a contemporary decrease in oxygen delivery, which might be mediated by a decrease in cerebral blood flow (Dani et al. 2006). Additionally, electrophysiological data from treatment with doxapram identified doxapram’s influence in amplitude-integrated electroencephalography of preterm infants (Czaba-Hnizdo et al. 2014). This feature of doxapram can be detrimental for extreme preterm during unstable conditions and profound examination of cerebral circulation should be conducted before and during the therapy with doxapram.

6.5. BPD and respiratory system

We conducted further statistical analysis via crosstabs to identify possible associations or correlations between incidence severity of BPD, and other variables such as antenatal corticosteroids, prenatal complications, infections, respiratory support,

PDA, doxapram, successful doxapram therapy, and neurological impairment. Therefore, the incidence of various prenatal complications and failure of doxapram therapy were significantly correlated with the severity of BPD ($p<0.01$ and $p=0,0001$). The rate of response among children with mild BPD was 70%, being even higher than in all previous reports, whereas having severe BPD the therapy was successful only in 25% ($p<0.05$). Moreover, in terms of respiratory management only multiply intubations and exclusively non-invasive ventilation have significant correlation with severity of BPD. Almost seventy percent of patients with severe BPD were intubated more than three times. These findings assume that successful doxapram therapy might depend on predisposition and pulmonary function. Furthermore, PDA surgery has been associated with higher risk of BPD, though a possible confounding of indication as factor is still debated that it is due to ($p=0,024$).

By gestation age of extreme preterm, the lungs are in their canalicular stage, which lasts from 17 to 26 weeks of gestation. Then, the lungs enter the terminal sac phase (26-36 weeks). During the canalicular stage, peripheral airways enlarge to form respiratory bronchioli and sac-shaped alveolar ducts, the epithelium thins, and the extracellular matrix undergoes physiological remodelling while active angiogenesis forms the new intraacinar capillaries beneath the epithelium (Hislop et al. 2005). In the terminal sac phase from 26 to 36 weeks, the acini are refined. Due to the formation of smaller saccule and alveoli areas, the interstitial distance for diffusion decreases and capillary invasion of the alveolar-blood barrier surface area increases. Additionally, gas exchange becomes available. Further development of the surfactant system also begins at this time, although it increases at about 30 weeks (Hislop et al. 2005). A delicate balance of growth factors plays a role in vascular integrity and the remodeling of the extracellular matrix at this stage, including TGF, VEGR, HIE, platelet-derived growth factor, FGF, and VEGF. At this stage, there is also a growing network of neurons within the lung, which improves branching morphogenesis. On the other hand, lung development is also accompanied by growth of neuronal networks within the lungs which enhance branching morphogenesis. The network consists of extrinsic neurons (located outside the respiratory system), intrinsic neurons (residing in the trachea), major bronchi, and a cluster of form ganglia that enable direct connection of the pulmonary tract to the CNS. Extrinsic neurons are located in dorsal and ventral respiratory nuclei in the medulla oblongata, the jugular, and the nodose

ganglia. This neural network controls breathing, smooth muscle tone, mucous secretion, and the trigger of reflexes such as cough (Aven et al. 2013). The intrinsic neurons mainly provide parasympathetic innervation to the trachea and main bronchi in addition to functioning locally. Additionally, NEBs may play a role in O2 sensing as well as regeneration of the distal pulmonary epithelium. Collectively, a large body of evidence indicates that the lung is innervated predominantly by extrinsic neurons.

Extreme prematurity and lungs in the late canalicular or very early saccular stage of development underlie the pathophysiology of new BPD, which includes signs of disrupted and impaired lung development despite less airway injury. Every step of pulmonary care, such as surfactant and oxygen application, lung inflation, or mechanical ventilation can cause damage at the cellular level due to oxygen toxicity and volutrauma. Barotrauma and volutrauma during the terminal sac phase lead to declined surface area, enlarged diffusion distance, parenchyma stretching, and, ultimately, lung emphysema (Goldsmith et al. six edition 2011; Corrin et al. 2011), Exposure to hyperoxia, ventilation even for a short period of time and inflammation at an early stage of development impair the subsequent program of alveolarisation and vascularisation, which decrease lung cell proliferation, reduce the number of endothelial progenitor cells and cause an alteration of growth factor expression. This leads to alveolar simplification, increased apoptosis, dysmorphic capillaries, enhanced endothelial cells, enhanced smooth muscle cells, pathological remodelling of the extracellular matrix (ECM) components (e.g., elastin and collagen), and increased interstitial fluid. Many of the pulmonary responses to hyperoxia including oxidative stress are developmentally regulated, hence, induce apoptotic and proliferative patterns. Additionally, during the canalicular or saccular stages, lungs are especially sensitive to pulmonary inflammation and steroids, which can induce re-programming in lung development and restrict progress of alveolarisation. Inflammation due to structural remodeling of the lung, including altered organisation of ECM components, and impaired alveolarisation and angiogenesis increases detrimentally the risk of bronchopulmonary dysplasia. At the same time, a combination of various prenatal and postnatal factors, such as hypoxia, maternal infection (including chorioamnionitis), postnatal infection, genetic susceptibility, mechanical ventilation, oxygen injury, and poor nutrition stimulate neovascularisation, proliferation of the pulmonary capillary network, and extended diffusion distance, which leads to progression of BPD and development of pul-

monary hypertension. Understanding the mechanisms that regulate normal lung growth and the pathways that mediate lung repair after injury is the key to primary prevention of BPD. Moreover, according to Ambalavanan N et al. study and other recent research, there is a genetic susceptibility to BPD—including several genes that affect BPD-associated biological pathways (Ambalavanan et al. 2015). This finding relates with our suggestions about antenatal priming—especially placental dysfunction and various adverse intrauterine environments such as inflammation—as a main cause of epigenetic alteration involved in lung development. Additionally, such an understanding can help define the abnormal trajectory of a premature lung evolving to BPD.

Subsequent studies identified three patterns in the first 2 weeks of life based on the capacity of recover/ effective healing, resistance to injury, and oxygen sensitivity. Some extreme preterm infants with initially normal pulmonal function cannot tolerate modest initial oxygen or ventilation and progress to early respiratory death or severe BPD. Others demonstrate early severe respiratory failure and respond poorly to surfactant treatment and require aggressive ventilation, although lung function returns to normal over several weeks (Laughon et al. 2009). These differences in injury and repair patterns occur in multiple lung dysmaturity phenotypes and distinctive abilities of connective tissues. Probably, preterm with a higher predisposition to severe BPD often do not respond to doxapram therapy because of respiratory decompensation and pulmonary deterioration, where apnoea can reflect the low functional residual capacity as well as abnormalities of lung function rather than immaturity of the respiratory centres in brainstem

Analysing how response to doxapram therapy negatively correlates with severity of BPD more interest is risen to its conjunction, however, the role of doxapram in the development of BPD is still unclear and more studies on short-term are needed. Additionally, the strongest known predictors of severe BPD, such as SGA, the combination of antenatal inflammation and placental dysfunction, immaturity, postnatal sepsis, and ventilation in the first 4 days of life—which had a significantly higher incidence in our cohort with moderate and severe BPD—are also the significant predictors of therapy failure and must be considered carefully (Torchin et al. 2016; Laughon et al. 2009).

6.6. Perspectives of inflammation in triangle intestine - lungs - brain

We found a statistical correlation between the severe BPD, poor neurodevelopment and CP and an incidence of postnatal multiple or recurrent sepsis in cohort of non-responders and it is remarkable (41,2%, p= 0.001, 24,2, p= 0,0003; 22,2%, p=0,01, respectively). We suggest that recurrent infections might be considered also as a predictor of the failure of doxapram therapy (42,1% non-responded infants suffered from recurring sepsis). This is the first time when a conjunction between inflammation and possible impaired response to doxapram is reported, although the pathophysiology of this pattern remains unclear. Admittedly, due to the unique immune response of extreme preterm infants, inflammation plays a crucial role in the pathobiology of main issues in neonatology: BPD, NEC and adverse neurocognitive outcome. As it was demonstrated in various experimental and clinical studies, an exposure to antenatal and perinatal endotoxin from the mother induces expression of toll-like receptor 4 (TLR4) by macrophages and neutrophils increasing production of pro-inflammatory cytokines and other injurious factors (platelet-activating factor, TNF, Matrix metalloproteinases (MMP) in the lung parenchyma, brain and intestine tissues, and programming type of immune response. After birth, hypoxia-ischemia, biotrauma of respiratory system with impaired bronchial microbiota, immaturity of gut with disbalance of microbiome modulate further activation of innate immune system. Consequently, altering premyelinating oligodendrocyte, this inflammatory cascade and hypoxia crucially disrupt development of the vulnerable preterm brain. Moreover, as microbiome can decrease gut barrier and blood- brain barrier functioning, affect brain plasticity by impaired synaptogenesis and production of neurotransmitters, dysbiosis has also a critical influence on developmental programming of long-lasting brain function (Heijtz et al. 2016). In particular, various experimental studies could demonstrate how changes in microbiome results in predominance of anxiety-like behaviour with increased adreno-cortisone levels in response to mild stress, including an activation of vagal ascending pathways (via the vagal nerve system) (Lyte 2014; Gonzalez-Santana et al. 2020, Heijtz et al. 2016). Interestingly, vagal nerve is an important pathway for communication between lung, intestinal microbiota, gut, and brain. In this way, the lung-gut-brain axis is a communication system that integrates neural, hormonal, and immunological signalling between the gut, the lung, and the brain, and provides the intestinal and broncho pulmonal microbiota with a potential route to in-

fluence brain function and development. Interestingly, apnoea being a sign of immature brainstem and peripheral chemoreceptor, as an inadequate or absent respiratory response to hypoxia, can also be a breathing pattern impaired by inflammation, or lung parenchymas. In this way apnoea also belongs to important clinical signs of dysfunction in the lung-gut-brain axis. In presence of inflammation apnoea is caused by an activation of IL1 receptors leading to the synthesis and release of prostaglandin E2 into the brainstem; this disrupts ventilatory rhythm (Hofstetter et al. 2008; Jensen et al. 2014; Balany et al. 2015). However, it remains unclear, which impact these conditions have on function of respiratory neurons in brainstem in terms of sensitivity to CO2 and ability to respond to doxapram. An understanding of lung-gut-brain axis interaction is critical for the development of new preventative and treatment strategies including such medicaments as doxapram.

7. Conclusion

The profound, prolonged hypoxic episodes alongside causing reduced physical capacity to overcome apnoea and a declined sensitivity of receptors to doxapram should be the warning signs for therapy failure. For preterm infants with severe AOP in a poorer clinical condition, based on our observation intubation should be considered instead of doxapram treatment. We suggest that, while administration of doxapram during severe refractive apnoea and intermittent hypoxic episodes (these children are already at risk) should be critically considered, there is a positive effect of treatment with doxapram on mental and motor development for responded infants.

Doxapram has the potential to improve respiratory outcomes and reduce the incidence of neurodevelopmental delay in special groups. Identification of these factors, which can assist in the prediction of possible doxapram therapy failure, might be both essential and useful for early prognostication of success and groups of risks. Administration should be carefully considered in cases with hemodynamic instability or conditions with decreased cerebral blood flow. Additionally, doxapram should be administered with caution among children with severe lung injury or with respiratory decompensation. More studies needed to develop tools for an improvement of the personalisation of therapy and/or define an objective, relevant parameter for AOP. Developing the regulation and specific recommendations of dosage adjusted for GA, PNA, and SGA could minimise risks of overdoses and adverse effects.

Abstract

Background: When despite treatment with caffeine and CPAP ELBW infants are afflicted by chronic ischemic episodes and persistent apnea, the invasive ventilation is necessary. For such severe AOP doxapram as an off-label analeptic can be considered, which stimulates activation of respiratory regulation in the brainstem and peripheral chemoreceptors. However, its impact on neurodevelopmental outcome remains unclear.

Methods: We performed this retrospective cohort study of preterm infants born from 2013 through 2018 at the Altona Perinatal Center, Hamburg. For statistical analysis of clinical characteristics and neurodevelopmental outcome were used chi-squared test, univariable and multivariable logistic regression analyses. The definition of treatment success was an avoiding endotracheal intubation, reduction of AOP and successful weaning.

Results: The cohort included all ELBW infants born with a gestational age < 27 weeks and a birth weight (BW) < 1000 g (N=124 infants). The mean GA of children treated with doxapram (N=40) was 24.5GW with the mean BW 623.7g, which is lower than non-treated cohort (N=84, 25.5 weeks, 707.3 g). The therapy success was observed in 52.5% of treated children, they had a higher median BW (655.2g & 588g), a higher median PNA of initiation of first cycle (20 days & 15 days) and significantly lower incidence of recurrent sepsis (N=3, 15% & N=8, 42.1%; p=0.02) compared to non-responders. Patients with therapy failure had significantly longer duration of invasive ventilation (p=0,014) and more intubations (p=0.04). The incidence of children without any mental retardation in follow-up was significantly higher in responder's group (N=8, 50% & N=5, 38,5% p=0.03).

Discussion: Immaturity, lower PMA, PNA and inflammation processes might be negative predictors of therapy success with doxapram, simultaneously it might reflect the complexity and severity of AOP pathophysiology among ELBW. In our cohort responders represented improved short- and long-term outcomes compared to non-responders.

Conclusion: Although administration of doxapram during severe AOP should be critically considered, there might be a positive effect on neurodevelopment for responding infants. Further prospective randomised studies are required for recommendations and advanced protocols.

Zusammenfassung

Hintergrund: Wenn extreme Frühgeborene trotz Behandlung mit Coffein und CPAP von chronischen ischämischen Episoden und persistierender Apnoe betroffen sind, ist die invasive Beatmung notwendig. Aufgrund solcher schwerer AOP kommt Doxapram als Off-Label-Analeptikum in Betracht, das die Aktivierung der Atemregulation im Hirnstamm und periphere Chemorezeptoren stimuliert. Seine Auswirkung auf die neurologische Entwicklung bleibt jedoch unklar. **Methoden**: Wir haben diese retrospektive Kohortenstudie mit Frühgeborenen(FG) vom 2013 bis 2018 im Perinatalzentrum Altona, Hamburg, durchgeführt. Für die statistische Analyse der klinischen Charakteristik und der neurologischen Entwicklungsergebnisse wurden univariable und multivariable logistische Regressionsanalysen verwendet. Die Definition des Behandlungserfolgs war die Vermeidung einer endotrachealen Intubation, die Reduktion des AOP und die erfolgreiche Weaning. **Ergebnisse:** Die Kohorte umfasste alle ELBW-FG, die mit einem Gestationsalter (GA)< 27 Wochen und einem Geburtsgewicht (GG) < 1000 g (N=124 Säuglinge) geboren wurden. Die mittlere GA der mit Doxapram behandelten Kinder (N = 40) betrug 24,5 Wochen mit einem mittleren GG von 623,7 g, was niedriger ist als bei der nicht behandelten Kohorte (N = 84, 25,5 SSW, 707,3 g). Der Therapieerfolg wurde bei 52,5 % der behandelten FG beobachtet, sie hatten ein höheres medianes GG (655,2 g & 588 g), eine höhere mediane PNA zu Beginn des ersten Zyklus (20 Tage & 15 Tage) und eine signifikant geringere Inzidenz rezidivierender Sepsis (15 % & 42,1 %; p=0,02) im Vergleich zu Non-Respondern. FG mit Therapieversagen hatten eine signifikant längere Dauer der invasiven Beatmung (p=0,014) und mehr Intubationen (p=0,04). Die Anzahl von FG ohne geistige Behinderung im Follow-up war signifikant höher in der Responder-Gruppe (50 % & 38,5 %, p = 0,03). **Diskussion:** Die Unreife, niedrigere PMA, PNA und Entzündungsprozesse könnten negative Prädiktoren für den Therapieerfolg mit Doxapram sein, gleichzeitig könnten sie die Komplexität und Schwere der AOP-Pathophysiologie bei ELBW widerspiegeln. In unserer Kohorte zeigten Responder verbesserte kurz- und langfristige Ergebnisse im Vergleich zu Non-Respondern.
Schlussfolgerung: Obwohl die Gabe von Doxapram während schwerer AOP kritisch betrachtet werden sollte, könnte es einen positiven Effekt auf die neurologische Entwicklung von FG geben, die darauf ansprechen. Weitere prospektive randomisierte Studien sind für Empfehlungen und erweiterte Protokolle erforderlich.

Appendix

			Patients not treated with Doxapram (n = 84)	Patients treated with Doxapram (n = 40)	p value*
Gestational age, weeks			25.5 (23.5-26.6)	24.5 (23.3-26.3)	
Birth weight, g			707,3 (295- 990)	623,7 (300-960)	
Birth weight, P			33,6 (1-75)	27,7 (1-70)	
Female gender			52,5 (44)	42,5 (17)	0,2
Multiple birth (yes)			21,4 (18)	27,5 (11)	0,5
Mortality			13,1 (11)	7,7 (3)	0,29
Prenatal	ACS	no	7,1 (6)	12,5 (5)	0,32
		not complete	34,5 (29)	42,5 (17)	0,32
		complete	58,3 (49)	45 (18)	0,32
	No antenatal complications		17,9 (15)	0	0,015
	Prenatal infection		40,5 (34)	35 (14)	0,015
	pPROM and high CRP		47,6 (40)	57,5 (23)	0,2
	AIS		44 (37)	47,5 (19)	0,43
	Placental minder perfusion		27,4 (23)	40 (16)	0,015
	Pathological Doppler		11,9 (10)	20,5 (8)	0,16
	Pathological CTG		17,9 (15)	35 (14)	0,03
	Preeclampsia		11,9(10)	5 (2)	0,18
	Antepartum bleeding		14,3 (12)	27,5 (11)	0,06
	Infection associated with placental minder perfusion		14,3 (12)	25 (10)	0,015
	Premature labor		61,9 (52)	57,5 (23)	0,39
Delivery room	Birth Modus: Sectio		84,5 (71)	100 (40)	0,005
	CPR in delivery room		21,4 (18)	22,5 (9)	0,53
	LISA in delivery room		72,6 (61)	62,5 (25)	0,17
Respiratory Management	Exclusive NIV		43,4 (36)	12,5 (5)	0,05
	LISA, after intubated		24,1 (20)	22,5 (9)	0,05
	Invasive ventilation from 1.day		20,5 (17)	17,5 (7)	0,05
	Number of intubations more than 3		11,9 (10)	47,5 (19)	0,05
	Mean duration of invasive ventilation, d		9,6 (1-56)	18,3 (1-73)	

		Patients not treated with Doxapram (n = 84)	Patients treated with Doxapram (n = 40)	p value*
	Mean duration of non-invasive ventilation (CPAP), d	42,8 (0-90)	45,2 (19-79)	
	Mean duration O2-Therapy, d	41,4 (0-143)	65 (5-131)	
	O2 supply at 32 GW	43,5 (31)	62,2 (23)	0,04
	O2 supply at 36 GW	18,1 (13)	40,5 (15)	0,01
	O2 supply at Discharge	8 (6)	24,3 (9)	0,02
Ureaplasma		32,9 (27)	30 (12)	0,4
Hydrocortisone therapy		11,1 (9)	25,6 (10)	0,04
Diuretic therapy		44,4 (36)	80 (32)	*
BPD	none	37,7 (29)	6,5 (2)	*
	light	46,8 (36)	51,3 (20)	*
	moderate	9,1 (7)	12,8 (5)	*
	severe	6,5 (5)	30,8 (12)	*
Postnatal infection	no sepsis	45,1 (37)	17,5 (7)	0,02
	EOS	23,2 (19)	17,5 (7)	0,14
	LOS	25,6 (21)	45 (18)	*
	EOS+LOS	4,9 (4)	7,5 (3)	*
	Recurrent Sepsis	1,2 (1)	27,5 (11)	*
hsPDA	no	62,7 (52)	57,5 (23)	0,74
	drug-treated	28,9 (24)	30 (12)	0,74
	surgical treated	8,4 (7)	12,5 (5)	0,74
FIP		15,7 (13)	15 (6)	0,57
NEC		8,5 (7)	12,5 (5)	0,34
Hyperglycaemia		9,8 (11)	37,5 (15)	*
Administration of catecholamine		37,5 (15)	25,6 (21)	0,2
Neurological complications during hospital stay	IVH 2-3	13,3 (11)	17,5 (7)	0,35
	PVL	0 (0)	10 (4)	0,01
	Seizures	3,6 (3)	10 (4)	0,15
	Hydrocephalus	3,6 (3)	5 (2)	0,52
	CMV postnatal	3,6 (3)	15 (6)	0,03
	Overall	19,3 (16)	37,5 (15)	0,02
Discharge	Weight, P	50 (1-90)	18 (6-30)	
	Head Circumference, P	8 (1-98)	2 (1-81)	

			Patients not treated with Doxapram (n = 84)	Patients treated with Doxapram (n = 40)	p value*
	Discharge before 36.GW		9,6 (7)	2,6 (12)	0,17
	Discharge after EDD		31.5 (23)	73,7 (28)	0,17
	Mean duration of hospital stayed, d		93 (52-166)	128 (82-476)	
	Home monitor		11,5 (9)	40,5 (15)	0,001
Follow up:			64,4 (47)	78,4 (29)	0,09
	CP		4,3 (2)	24,1 (7)	0,01
	Deaf		2,1 (1)	6,9 (2)	0,32
	Mental Retardation	no	76,6 (36)	44,8 (13)	0,03
		light	14,9 (7)	24,1 (7)	0,03
		moderate	6,4 (3)	24,1 (7)	0,03
		severe	2,1 (1)	6,9 (2)	0,03
	Microcephalic		8,5 (4)	37,5 (12)	0,002
	Neurological Abnormality		31,8 (14)	65,5 (19)	0,008

Table 10: Chapter 4.2. Characteristic of cohorts with children non-treated and treated with Doxapram. p value from the Fisher's exact test and Pearson Chi-Square and χ^2 test. Values are presented as means ± SD, % (n) or median (IQR) (min–max). *cells have expected count less than 5

		Patients responded to Doxapram n=21	Patients non-responded to Doxapram n=19	p value*
Birth weight, g		655,2 (300-960)	588 (350- 770)	
Birth weight, Percentile		30,6 (1-70)	24,3 (1-70)	
Mortality		4,8 (1)	11,8 (2)	0,4
Gender female		42,9 (9)	42,9 (9)	1,0
Antenatal complications:	no ACS	14,3 (3)	10,5 (2)	0,04
	not complete ACS	28,6 (6)	57,9 (11)	0,04
	complete ACS	57,1 (12)	31,6 (6)	0,04
	Infection	52,4 (11)	15,8 (3)	0,04
	pPROM and high CRP	66,7 (14)	47,4 (9)	0,18
	AIS	57,1 (12)	36,8 (7)	0,16
	Placental minder perfusion	28,6 (6)	52,6 (10)	0,04

		Patients responded to Doxapram n=21	Patients non-responded to Doxapram n=19	p value*
	Pathological Doppler	9,5 (2)	33,3 (6)	0,11
	Pathological CTG	23,8 (5)	47,4 (9)	0,11
	Preeclampsia	4,8 (1)	5,3 (1)	0,73
	Antepartum bleeding	23,8 (1)	31,6 (6)	0,43
	Infection with placental minder perfusion	19 (4)	31,6 (6)	0,04
Respiratory management	LISA in Delivery room	71,4 (15)	52,6 (10)	0,18
	Exclusive non-invasive ventilation	23,8 (5)	0	0,004
	Invasive from 1.day	5,3 (1)	28,6 (6)	0,28
	Number of intubations more than 3	19 (4)	78,9 (15)	0,004
	Mean duration of invasive ventilation, d	3 (0-26)	24 (5-73)	
	Mean duration Non-invasive ventilation	43 (21-79)	49 (19-67)	
	O2 supply at 32 GW	45 (9)	82,4 (14)	0,02
	O2 supply at discharge	10 (2)	41,2 (7)	0,03
	Mean duration of O2-Therapy, d	58,4 (29-131)	74 (5-127)	
	Hydrocortisone therapy	15 (3)	36,8 (7)	0,11
	Diuretics therapy	75 (15)	89,5 (17)	0,22
BPD	none	5 (1)	5,3 (1)	0,09
	light	70 (14)	31,6 (6)	
	moderate	10 (2)	15,8 (3)	
	severe	15 (3)	47,4 (9)	
Infection	none	23,8 (5)	10,5 (2)	0,02
	EOS	4,8 (1)	0	0,2
	LOS	52,4 (11)	36,8 (7)	0,2
	EOS+LOS	4,8 (1)	10,5 (2)	0,2
	Recurrent sepsis	14,3 (3)	42,1 (8)	0,2
hsPDA	no	57,1 (12)	57,9 (11)	0,8
	drug- treated	33,3 (7)	26,3 (5)	
	Surgical treated	9,5 (2)	15,8 (3)	
Administration of catecholamine		23,8 (52)	52,6 (10)	0,06
FIP		4,8 (1)	26,3 (5)	0,07
NEC		9,5 (2)	15,8 (3)	0,45

		Patients responded to Doxapram n=21	Patients non-responded to Doxapram n=19	p value*
Hyperglycaemia		23,8 (5)	52,6 (10)	0,06
Discharge:	Mean head circumference, P	12,25 (1-81)	2,75 (1-11)	
	Mean duration of hospital stayed, d	114,8 (82-155)	165,2 (97-476)	
	Discharged with home monitor	30 (6)	52,9 (9)	0,14
Follow up:		80 (16)	76,5 (13)	
	CP	12,5 (2)	38,5 (5)	0,11
Neurodevelopmental delay	no	50 (8)	38,5 (5)	0,03
	light	12,5 (2)	38,5 (5)	
	moderate	37,5 (6)	7,7 (1)	
	severe	0	15,4 (2)	
Cause of severe apnoea	Respiratory insufficiency	76,2 (16)	52,6 (10)	0,1
	Sepsis	23,8 (5)	47,4 (9)	
Doxapram therapy	Average postnatal age at initiation of first cycle, d	20 (4-37)	15 (7-60)	
	Average number of application pro infant	1,3 (1-3)	2,2 (1-.4)	
	Average total cumulative dosage per kg, mg/kg	203,6 (23,2-802,8)	247,7(15,4-820,5)	
	Average total duration of therapy, days	5,6 (1-25)	4 (1-20)	
	Average dosage per hour, mg/kg/h	1,44 (0,5-2,07)	1,97 (1-2,34)	

Table 11: Chapter 7. 2. Patient characteristics classified by therapy success or therapy failure, p value from the Fisher's exact test and Pearson Chi-Square and χ^2 test. Values are presented as % (n) or median (IQR) (min–max).

BPD n=116						
		no n=31	mild n=56	moderate n=12	severe n=17	p val-ue*
ACS	no	6,5 (2)	8,9 (5)	16,7 (2)	5,9 (1)	0,056
	not complete	25,8 (8)	35,7 (20)	41,7 (5)	52,9 (9)	
	complete	67,7 (21)	55,4 (31)	41,7 (5)	41,2 (7)	
Prenatal	no complication	32,3 (10)	5,4 (3)	8,3 (1)	0	0,001
	Infection	35,5 (11)	46,4 (26)	25 (3)	29,4 (5)	
	Placental minder Per-fusion	25,8 (8)	30,4 (17)	58,3 (7)	29,4 (5)	
	Infection+ Placental minder Perfusion	6,5 (2)	17,9 (10)	8,3 (1)	41,2 (7)	
Postnatal infection	no	54,8 (17)	28,6 (16)	41,7 (5)	29,4 (5)	0,001
	EOS	19,4 (6)	19,6 (11)	8,3 (1)	0	
	LOS	22,6 (7)	35,7 (20)	41,7 (5)	29,4 (5)	
	EOS+LOS	3,2 (1)	8,9 (5)	0	0	
	Recurrent sepsis	7,1 (0)	7,1 (4)	8,3 (1)	41,2 (7)	
Respirato-ry man-agement	Exclusive NIV	64,5 (20)	32,1 (18)	16,7 (2)	5,9 (1)	*
	LISA, after intubated	22,6 (7)	25 (14)	33,3 (4)	11,8 (2)	
	Invasive from 1.day	9,7 (3)	25 (14)	0	11,8 (2)	
	Number of intubations more than 3	3,2 (1)	17,9 (10)	50 (6)	70,6 12)	
hsPDA	no	77,4 (24)	57,1 (32)	66,7(8)	52,9 (9)	0,024
	drug-treated	19,4 (6)	35,7 (20)	8,3 (1)	23,5 (4)	
	surgically treated	3,2 (1)	7,1 (4)	25 (3)	23,5 (4)	
Doxapram application		6,5 (2)	35,7 (20)	41,7 (5)	70,6 (12)	0,075
	during sepsis	100 (2)	30 (6)	40 (2)	33,3 (4)	0,26
	during respiratory insufficiency	0	70(14)	60 (3)	66,7 (8)	
	Responded	50 (1)	70 (14)	40 (2)	25 (3)	0,023
Doxapram from all	Without doxapram	93,5 (29)	64,3 (36)	58,3 (7)	29,4 (5)	0,001
	Not responded	3,2 (1)	10,7 (6)	25 (3)	52,9 (9)	
Neurode-velopmen-tal out-come	Cerebral palsy	0	10,8 (4)	0	41,7 (5)	0,003
	Neurological abnormality	13,3 (2)	48,6 (18)	44,4 (4)	75 (9)	0,014

Table 12: Chapter 9.1. Long-term outcome characteristics (BPD) of the patients received doxapram contingency tables (crosstabs) using chi-squared test (χ2 test) and Spearman correlation. * The minimum expected count is less than 5 (1,97)

		Neurological abnormalities in follow up			CP in follow up		
		no n=40	with n=33	P value *	no n=67	with n=9	P value *
ACS	no	7,5 (3)	15,2 (5)	0,023	11,9 (8)	0	0,3
	not complete	27,5 (11)	48,5 (16)		35,8 (24)	55,6 (5)	
	complete	65 (26)	36,4 (12)		52,5 (35)	44,4 (4)	
Prenatal	no complication	15 (6)	9,1 (3)	0,05	13,4 (9)	0	0,08
	Infection	50 (20)	36,4 (12)		43,3 (29)	44,4 (4)	
	Placental minder perfusion	25 (10)	27,3 (9)		29,9 (20)	11,1 (1)	
	Infection+ Placental minder perfusion	10 (4)	27,3 (9)		13,4 (9)	44,4 (4)	
Postnatal infection	no	55 (22)	21,2 (7)	0,003	44,8 (30)	11,1 (1)	0,01
	EOS	12,5 (5)	12,1 (4)		13,4 (9)	11,1 (1)	
	LOS	30 (12)	36,4 (12)		31,1 (21)	33,3 (3)	
	EOS+LOS	2,5 (1)	6,1 (2)		1,5 (1)	22,2 (2)	
	Recurrent sepsis	0	24,2 (8)		9 (6)	22,2 (2)	
	CMV	0	24,2 (8)	0,001	6 (4)	44,4 (4)	0,005
Respiratory management	Exclusive NIV	45 (18)	18,2 (6)	0,002	35,8 (24)	22,2 (2)	0,03
	after LISA intubated	32,5 (13)	24,2 (8)		31,3 (21)	11,1 (1)	
	Invasive ventilated from 1.day	12,5 (5)	9,1 (3)		11,9 (8)	0	
	Number of intubations more than 3	10 (4)	48,5 (16)		20,9 (14)	66,7 (6)	
BPD	none	32,5 (13)	6,1 (2)	0,003	26,9 (18)	0	0,003
	mild	47,5 (19)	54,5 (18)		49.3 (33)	44,4 (4)	
	moderate	12,5 (5)	12,1 (4)		13,4 (9)	0	
	severe	7,5 (3)	27,3 (9)		10,4 (7)	55,6 (5)	
Application of doxapram	yes	25 (10)	57,6 (19)	0,005	32,8 (22)	77,8 (7)	0,013
	during sepsis	7,5 (3)	12,1 (4)	0,016	7,5 (5)	22,2 (2)	0,03
	during respiratory insufficiency	17,5 (7)	45,4 (15)	0,016	25,4 (17)	55,6 (5)	
Response to Doxapram	no	30 (3)	52,6 (10)	0,01	36,4 (8)	71,4 (5)	0,11
	yes	70 (7)	47,4 (9)		63,6 (14)	28,6 (2)	

Table 13: Chapter 10.1. Long-term outcome characteristics (Neurodevelopmental follow-up and cerebral palsy) of the patients received doxapram crosstabs using chi-squared test

List of abbreviations

ACS antenatal corticosteroids

AIS amniotic infection syndrome

AOP Apnea of prematurity

BPD bronchopulmonary dysplasia

BSID-II Bayley Scales of Infant development

CI confidence interval

cm H2O centimeter of water

CMV Cytomegalovirus

CNS central nervous system

CO2 carbon dioxide

CP Cerebral palsy

CPAP continuous positive airway pressure

CRIB The Clinical Risk Index for Babies

EPICE Effective Perinatal Intensive Care in Europe

CRP C-reactive protein

CTG Cardiotocography

CVN cardiac vagal neurons

CYP3A4 Cytochrome P450 3A4

CYP3A5 Cytochrome P450 3A5

ECM extracellular matrix

EEG Electroencephalography

ELBW extreme low birth weight

EOS early onset sepsis

EXPRESS Extremely Preterm Infants in Sweden Study

FSIQ Full-Scale Intelligence Quotient

HR hazard ratio

IRR incidence rate ratio

NDI neurodevelopmental impairment

PDA patent ductus arteriosus

PEEP positive end expiratory pressure

PMA postmenstrual age,

PNA postnatal age

RR risk ratios
SDS standard deviation score
FGF fibroblast growth factor
FiO2 Fraction of inspired oxygen
GA gestational age
GABA gamma aminobutyric acid
GW Gestational weeks
HFNC High-flow nasal cannula
HIE Hypoxic-ischemic encephalopathy
hsPDA hemodynamically significant Patent Ductus Arteriosus
IL1 Interleukin 1
IL6 Interleukin 6
IQR interquartile ranges
IUGR Intrauterine growth restriction
IVH intraventricular haemorrhage
LISA less invasive surfactant application
LOS late onset sepsis
MAP median airway pressure
MDI Mental Development Index
MGA median gestational age
mmHg millimeters of mercury
MMP Matrix metalloproteinases
MRI Magnetic resonance imaging
NAVA neurally adjusted ventilation assistance
NEBs
NEC necrotizing enterocolitis
nHFO noninvasive high frequency oscillation
NICU neonatal intensive care unit:
NIPPV Nasal intermittent positive pressure ventilation
NIV non invasive ventilation
OR odds ratio
PaO2 partial pressure of oxygen in arterial blood
PCA postconceptional age
PDI Psychomotor Development Index scores

PNA postnatal age

pPROM preterm premature rupture of membranes

preBötzC Pre-Bötzinger complex

PVL periventricular leukomalacia

RCT randomized controlled trial

ROP retinopathy of preterm

SD standard deviation

SGA small for gestational age

SLN superior laryngeal nerve

SpO2 Oxygen saturation

TASK TWIK-related Acid Sensitive K channel

TGF Transforming growth factor

TLR Toll-like receptors

TNF-α Tumor necrosis factor

TWIK tandem pore domains in weak rectifying K^+ channel

VEGF vascular endothelial growth factor

VEGR receptors for vascular endothelial growth factor

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Acknowledgements

At first, I want to mention my main supervisor, Dominique Singer. Thank you for your support, for taking me in, believing in me and giving me valuable input. For you being kind, patient and encouraging me. Thank you for the opportunity to work with you. I have learned so much in the interaction and discussions with you.

My chief at the Neonatal department at the Altona Children's Hospital, Axel von der Wense. Thank you so much for all these years of your guidance and wisdom, I have learned a lot and grew as a clinician and as a person. Thank you for the opportunity to do this project.

My wife, Kristina for always being there, for your immense support and believe in me. I could not have done this without you.

To my dear friends Mahsun, Judith, Stephanie, Zara, Stephan, Johanna, Debbie who have supported me relentlessly during this period.

Finally, my extended family for all the support and my beloved grandfather Waldemar for being the best grandfather.

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