Veno-Arterial Extracorporeal Membrane Oxygenation in Relation to the Brain

Oxygenation, hemodynamics and risk factors for the brain

Amerik Cornelis de Mol
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The studies as presented in this thesis were performed at the Department of Neonatology, Radboud University Nijmegen Medical Centre, Nijmegen, The Netherlands.

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Veno-Arterial Extracorporeal Membrane Oxygenation in Relation to the Brain

Oxygenation, hemodynamics and risk factors for the brain

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door jou,

dat komt gewoon

door jou.
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Chapter 1

Introduction

1.1 Abbreviations
1.2 Background – risk factors for the brain
1.3 Outline of the thesis
1.4 Introduction in near infrared spectrophotometry
### 1.1 Abbreviations

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<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>AaDO\textsubscript{2}</td>
<td>alveolar-arterial difference in partial pressure of oxygen</td>
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<tr>
<td>ACT</td>
<td>activated clotting time</td>
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<tr>
<td>AR</td>
<td>autoregulation</td>
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<tr>
<td>CBF</td>
<td>cerebral blood flow</td>
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<td>CBFV</td>
<td>cerebral blood flow velocity</td>
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<tr>
<td>CBV</td>
<td>cerebral blood volume</td>
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<td>CDH</td>
<td>congenital diaphragmatic hernia</td>
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<tr>
<td>cHHb</td>
<td>concentration of deoxyhemoglobin</td>
</tr>
<tr>
<td>CI</td>
<td>confidence interval</td>
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<tr>
<td>cO\textsubscript{2}Hb</td>
<td>concentration of oxyhemoglobin</td>
</tr>
<tr>
<td>CPR</td>
<td>cardiopulmonary resuscitation</td>
</tr>
<tr>
<td>CT</td>
<td>computed tomography</td>
</tr>
<tr>
<td>cHb</td>
<td>concentration of total hemoglobin</td>
</tr>
<tr>
<td>CV</td>
<td>coefficient of variation</td>
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<tr>
<td>DIC</td>
<td>disseminated intravascular coagulation</td>
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<tr>
<td>ECLS</td>
<td>extracorporeal life support</td>
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<tr>
<td>ECMO</td>
<td>extracorporeal membrane oxygenation</td>
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<tr>
<td>EDV</td>
<td>end diastolic flow velocity</td>
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<tr>
<td>ELSO</td>
<td>extracorporeal life support organisation</td>
</tr>
<tr>
<td>Fi\textsubscript{O\textsubscript{2}}</td>
<td>fraction of inspired oxygen</td>
</tr>
<tr>
<td>HbD</td>
<td>hemoglobin oxygenation index</td>
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<td>HFO</td>
<td>high frequency oscillation</td>
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<tr>
<td>HR</td>
<td>heart rate</td>
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<tr>
<td>ICH</td>
<td>intracranial hemorrhage</td>
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<tr>
<td>iNO</td>
<td>inhaled nitric oxide</td>
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<td>IVA</td>
<td>intravascular volume administration</td>
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<td>MAP</td>
<td>mean arterial pressure</td>
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<td>MAS</td>
<td>meconium aspiration syndrome</td>
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<tr>
<td>MRI</td>
<td>magnetic resonance imaging</td>
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<td>NIRS</td>
<td>near infrared spectrophotometry</td>
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<tr>
<td>NS</td>
<td>not significant</td>
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<tr>
<td>OI</td>
<td>oxygenation index</td>
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<tr>
<td>OR</td>
<td>odds ratio</td>
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<tr>
<td>paCO\textsubscript{2}</td>
<td>arterial partial pressure of carbon dioxide</td>
</tr>
<tr>
<td>paO\textsubscript{2}</td>
<td>arterial partial pressure of oxygen</td>
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<tr>
<td>patm</td>
<td>atmospheric pressure</td>
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<tr>
<td>pH\textsubscript{2}O</td>
<td>vapour pressure</td>
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<tr>
<td>PEEP</td>
<td>positive end expiratory pressure</td>
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<tr>
<td>PI</td>
<td>pulsatility index</td>
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<tr>
<td>PIP</td>
<td>peak inspiratory pressure</td>
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<tr>
<td>PPHN</td>
<td>persistent pulmonary hypertension of the newborn</td>
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<tr>
<td>PSV</td>
<td>peak systolic flow velocity</td>
</tr>
<tr>
<td>Qcar</td>
<td>blood flow in the left carotic artery</td>
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<tr>
<td>RDS</td>
<td>respiratory distress syndrome</td>
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<tr>
<td>RIJV</td>
<td>right internal jugular vein</td>
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<tr>
<td>saO\textsubscript{2}</td>
<td>arterial oxygen saturation</td>
</tr>
<tr>
<td>SD</td>
<td>standard deviation</td>
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<tr>
<td>TAM</td>
<td>time averaged mean flow velocity</td>
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<tr>
<td>va-ECMO</td>
<td>veno-arterial extracorporeal membrane oxygenation</td>
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<tr>
<td>vv-ECMO</td>
<td>veno-venous extracorporeal membrane oxygenation</td>
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1.2 Background – risk factors for the brain

*Historical perspectives*

From a historical point of view, extracorporeal membrane oxygenation (ECMO) is not even such an old treatment modality for neonates suffering from severe respiratory failure. Although several forms of extra corporeal life support (ECLS) are possible, like temporary cardiac support in infants with poor cardiac output after cardiac surgery, this thesis will concentrate on ECLS in newborns with severe, but potentially reversible, respiratory failure, often complicated by pulmonary hypertension. Right from its introduction in neonatology, ECMO became a rescue, life-saving, therapy in newborns who were considered to have a high mortality risk when treated with conventional methods only. It was in such a manner, that Bartlett and colleagues treated the first newborn, called Esperanze (hope) by the nursing staff, with ECMO for severe respiratory failure due to meconium aspiration syndrome (MAS) in 1976 (1). Several years later, in 1982, Bartlett presented the results of 45 newborns treated with ECMO (2). In these newborns, ECMO was used as a rescue therapy after maximal conventional therapy had failed in severely ill neonates with an estimated mortality risk of 90%. Treatment with ECMO resulted in an over 50% survival. Studies with a more randomized character were indicated to determine the usefulness of ECMO. Again, Bartlett et al, presented a study in which ECMO and conventional therapy were compared by using the so called “randomized play the winner” method. In this method the chance of randomly assigning an infant to one treatment or the other is influenced by the outcome of treatment of each patient in the study. If one treatment is more successful, more patients are assigned to that treatment (3). Finally 1 patient was treated by conventional therapy and died, 11 consecutive patients were treated with ECMO and survived. Results were considered as supportive for using ECMO. In another study of O’Rourke et al (4), neonates were prospectively randomized to ECMO or conventional ventilator management, but with an adapted design, in which randomisation continued until 4 deaths occurred in either treatment group. At that point, randomization would cease and all subsequent patients would be enrolled in the group with less than four deaths. Enrollment would continue until the fourth death occurred in that group or the number of survivors was significantly larger than the number of survivors in the arm that had been discontinued first. The survival in the ECMO group was 97% compared to 60% in the conventional group. Although there were concerns about the quality, especially the randomization methods, of the studies so far, in the following period, interest in ECMO was growing, resulting in the institution of the Extracorporeal Life Support Organization (ELSO) and a National Institute of Health consensus to continue the use of ECMO. The ELSO counts 109 members from 17 countries nowadays. From ELSO data a survival rate of 83% in 3500 newborns suffering from respiratory failure (estimated mortality 80%) was demonstrated (5). An important additive study came from the UK collaborative ECMO group in 1996. Till today this has been the only large randomized trial on survival of newborns treated with ECMO (6). Newborns with severe respiratory failure were randomised to either ECMO treatment in one of the five ECMO centres or conservative treatment at the partici-
pating centres. The conclusion was that survival until discharge in all diagnoses groups of newborns with severe respiratory failure was better in the ECMO group, compared to the conventional treatment group. Cost-effectiveness was also evaluated and it was concluded that ECMO was likely to be as cost-effective as other life-extending technologies (7). In 2008 a Cochrane Review, an update of an earlier review in 2003, was performed (8), including the studies mentioned before and one more study by Bifano et al (9). The Cochrane group concluded that the use of ECMO in newborns with severe, but potentially reversible, respiratory insufficiency resulted in significantly improved survival, without an increased risk of severe disability in survivors. Although the risk of death was significantly reduced, the effect of ECMO was less in newborns suffering from congenital diaphragmatic hernia (CDH). That this group of infants might be a special group is confirmed by the fact that in terms of death or disability, the advantage of ECMO treatment over conventional treatment in CDH patients had disappeared at 4 years of follow-up in the UK Trial (10). The authors of the Cochrane review group advised further studies to optimize optimal time of the initiation of ECMO and to determine which infants will benefit most. In general, the results of follow-up studies might play a role in the decision to use ECMO. Further on these long-term outcomes will be discussed. As mentioned before, ECMO has always been considered to be a rescue therapy in newborns. This persisted while other techniques were introduced to treat neonates with severe respiratory failure, complicated by pulmonary hypertension, like surfactant, high frequency oscillation (HFO) ventilation and inhaled nitric oxide (INO).

Neonatal ECMO criteria in severe respiratory failure
In table 1 selection criteria and (relative) contra-indications for the initiation of ECMO are given as used at the department of Neonatology of the Radboud University Nijmegen Medical Centre, Nijmegen, The Netherlands. Criteria fit closely to those described by the ELSO (11). These criteria concern newborn patients with severe respiratory failure, often complicated by pulmonary hypertension. Criteria for ECMO in patients with cardiac failure are not further described here. Historically, developed inclusion criteria are aimed to initiate ECMO in newborns with an estimated mortality of 80% with maximal conservative therapy. Exclusion criteria are mainly based on the fact that, when present, they increase the risk for ECMO related complications, especially (intracranial) hemorrhage. However with modern developments in intensive care treatment, like surfactant, HFO ventilation and the use of iNO, predicted mortality without ECMO is probably less than 80%. With the criteria used in the UK trial mentioned before (6), the control group had, depending on underlying disease a mortality rate of 43% (MAS) to 100% (CDH). On the other hand, to avoid a delay in the use of ECMO because of an inadequate response to prior iNO treatment and other techniques like HFO ventilation, it has been suggested to adapt the current ECMO criteria and to lower the threshold for the initiation of ECMO in patients suffering from severe hypoxic respiratory failure, who are already treated with iNO and HFO ventilation (12,13).
**Table 1.** Inclusion criteria for neonatal ECMO at the department of Neonatology of the Radboud University Nijmegen Medical Centre

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<tr>
<td>1</td>
<td>AaDO$_2$ &gt; 600 mmHg (80 kPa) for more than 8 hours</td>
</tr>
<tr>
<td>2</td>
<td>AaDO$_2$ &gt; 605 mmHg (80.6 kPa) for more than 4 hours with peak inspiratory pressure ≥ 38 mbar or mean airway pressure ≥ 22 mbar</td>
</tr>
<tr>
<td>3</td>
<td>Acute deterioration for at least 2 hours with pH &lt; 7.15 and paO$_2$ &lt; 40 mmHg (5.3 kPa)</td>
</tr>
<tr>
<td>4</td>
<td>No clinical improvement on maximal conventional therapy for 3 hours with paO$_2$ &lt; 40 mmHg (5.3 kPa)</td>
</tr>
<tr>
<td>5</td>
<td>Signs of barotrauma (at least 4): lung emphysema, pneumothorax or pneumomediastinum, pneumopericardium, pneumoperitoneum, subcutaneous emphysema, air leak &gt; 24 hours, mean airway pressure &gt; 15 mbar</td>
</tr>
<tr>
<td>6</td>
<td>OI &gt; 40 for 3 to 5 hours</td>
</tr>
<tr>
<td>7</td>
<td>In patients with CDH, at least once a documented paO$_2$ &gt; 80 mmHg (10.6 kPa)</td>
</tr>
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(Relative) contra-indications

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<tr>
<td>1</td>
<td>Gestational age &lt; 34 weeks or birth weight &lt; 2000 grams</td>
</tr>
<tr>
<td>2</td>
<td>Underlying lung disease or cause of respiratory insufficiency, that is not expected to be reversible within 10-14 days</td>
</tr>
<tr>
<td>3</td>
<td>Mechanical ventilation &gt; 10 days</td>
</tr>
<tr>
<td>4</td>
<td>Chromosomal or other congenital or acquired abnormalities incompatible with life, including severe asphyxia</td>
</tr>
<tr>
<td>5</td>
<td>Intraventricular or cerebral parenchymal haemorrhage ≥ grade II</td>
</tr>
<tr>
<td>6</td>
<td>Coagulopathy or bleeding complication for which heparin administration is contra-indicated</td>
</tr>
</tbody>
</table>

AaDO$_2$ = Alveolar-arterial difference in partial oxygen pressure, AaDO$_2$ = (patm - pH$_2$O) x FiO$_2$ - (paO$_2$ + paCO$_2$), where patm = atmospheric pressure (mmHg), pH$_2$O = vapour pressure (being 47 mmHg at 37°C), paO$_2$ = arterial partial pressure of oxygen (mmHg), paCO$_2$ = arterial partial pressure of carbon dioxide (mmHg), FiO$_2$ = fraction of inspired oxygen, OI = Oxygenation Index = (MAP x FiO$_2$ x 100)/paO$_2$, where MAP = mean airway pressure in mbar.
One study of Schumacher et al showed a shorter length of hospitalization and better cost-effectiveness when infants were randomized to lower threshold criteria (Oxygenation Index >25 but <40 instead of an oxygenation index > 40). Outcome at 1 year of age seemed to be better in the group with the lower criteria (14). A more recent study of Radhakrishnan et al suggested to use “relaxed” entry criteria for ECMO in newborns suffering from meconium aspiration syndrome (15). From the ELSO database we know that newborns with MAS have the best prognosis compared to other neonates treated with ECMO.

**ECMO and technical aspect**

Neonatal ECMO is a form of extracorporeal life support (ECLS) in which lung function of the newborn with severe, but potentially reversible, respiratory failure is temporary supported by an external circuit with an artificial oxygenator. As ECMO is considered to be a rescue therapy, it is only initiated in those newborns with high mortality rates despite treatment with maximal con-

**Figure 1.** overview of the veno-arterial ECMO circuit.

![ECMO Circuit Diagram](printed with permission from CNMC ECMO Training Manual, Short BL, Mikesell G, and Muir R, (eds), 2004)

ventional therapy. Figure 1. shows the va-ECMO circuit. During ECMO venous blood is passively drained from the right atrium through the venous cannula of the ECMO circuit, which is inserted into the right internal jugular vein (RIJV). Blood then passes the bladderbox, a device that will control the venous drainage from the right atrium. When the venous drainage from the right
atrium is inadequate, the bladderbox will be less filled by blood and some collapse of the bladderbox will occur, resulting in release of a switch which will slow down and finally interrupt the ECMO pump. After the bladderbox, the blood is actively pumped by the ECMO pump at a regulated variable ECMO flow rate through a membrane oxygenator. In the membrane oxygenator, a gas mixture of air, oxygen and carbon dioxide passes in the opposite direction to the blood flow. Here the blood is oxygenated and the partial carbon dioxide pressure is regulated. Before the oxygenated blood is returned to the patient, it is rewarmed by a heat exchanger. In case of veno-arterial ECMO, the blood will be returned to the aortic arch through an arterial cannula, inserted in the right common carotid artery. If veno-venous ECMO is used, the blood will return through the same venous cannula (in this case a double lumen cannula) into the right atrium, as is shown in figure 2. As the artificial external oxygenator will take care of the lung function, the lungs will be able to “rest and recover” at low intensity of artificial ventilation. Since the ECMO system is made of artificial materials that will have continuous contact with the patient’s blood, clot formation has to be avoided by heparinization of the patient. The (partial) bypass is continued until lung function recovers, after which the ECMO flow rate of the pump is decreased and finally stopped. At the end of the ECMO treatment, after decanunulation, mechanical ventilation is still required, but at much lower intensity and with less oxygen than before the initiation of the ECMO treatment. ECMO in general therefore has two positive effects. Firstly, it prevents newborns from dying due to persistent severe hypoxia. Secondly, it prevents the lungs from being damaged due to intense artificial ventilation and high inspiratory oxygen fraction.

Figure 2. The double lumen venous cannula used in veno-venous ECMO.

Neonatal ECMO and outcome.
Cerebral injury, intracranial hemorrhage (ICH) as well as ischemia belong to the most devastating complications of ECMO. Imaging studies revealed hemorrhagic or ischemic intracranial abnormalities in 10% up to as high as 52% of the neonates (16-18). Although ECMO has increased survival rates, the occurrence of hemorrhagic and ischemic cerebral lesions, resulting in future neurological and neurodevelopmental dysfunction are of major concern (19,20). Next to this, most of non-survivors of ECMO treatment do not die from respiratory problems, but as a result of ICH (ELSO data). An important study, describing the relation between severity of neonatal brain injury as detected by ultrasound and CT-scan related to ECMO and neuropsychological and neurobehavioral outcome at age 5 years was performed by Glass et al in 1997 (21). Disability was found from 10% in children with no radiologic brain abnormalities up to 57% in those with severe radiologic brain abnormalities. In the control group, newborns without ECMO and without hospital admission, disability was detected in 2% of cases. Although limited, some studies concern the more short-term outcome of newborns treated with ECMO, like feeding problems, growth and persistent pulmonary problems (22-24). For the purpose of this thesis, however, this introduction will concentrate on long-term neurodevelopmental and neuropsychological outcome. Most long-term follow-up studies show mild to major neurological impairment in 15 up to 25% of ECMO survivors. Impairment involves different kinds of neurological development, cognitive development, IQ and neuromotor development. More subtle signs of neurological involvement occur at higher rates in ECMO survivors. Learning problems at school age appear to be as high as 50% and ECMO parents reported behavioral problems more often compared to normal controls (10,20-23,25-28). A Dutch follow-up study found the veno-arterial ECMO population to be highly at risk for developmental problems, most prominently in the motor domain as compared to normal reference populations (29). Assessment in 149 survivors over a 7 years period (1993-2000) showed normal development in all fields in 49%, severe disability in 13% and impaired motor development combined with cognitive and/or behavioral problems in 9%. Outcome differed between primary diagnosis with CDH showing lowest normal outcome in all domains, 37.5% versus 52.6% in the MAS group.

In all long-term follow-up studies the selection of the control group is very important, especially as neonates treated with ECMO are generally severely ill and suffered from episodes of hypoxia and/or hemodynamic instability. Although several authors use near-ECMO patients as a control group, the most reliable control group can probably be found in the follow-up studies of the UK collaborative randomized trial of neonatal extracorporeal membrane oxygenation (6,30-32). In their studies, the UK ECMO trial group found an improved survival at 1, 4 and 7 years of age in ECMO treated newborns compared to those treated with conventional therapy. Even though 1 in every 4 patients treated with ECMO showed signs of impairment, there seemed to be a favorable outcome at 4 and 7 years of age in those newborns treated with ECMO. Finally it might be important to mention that in an additional study, ECMO showed to be cost-effective compared to conventional treatment based on the 7 years results in the UK (33).
Neonatal ECMO and risk factors for the brain

The studies described above make clear that ECMO is an effective therapy to increase survival in newborns with severe respiratory failure, but that the morbidity like intracranial injury, ICH as well as ischemia, are very important complications that determine long term outcome. From this point of view a continuous effort for recognizing the risk factors for intracranial injury in an attempt to further improve outcome is absolutely justified. The origin of cerebral injury in ECMO treated newborns is probably a combination of factors, including pre-ECMO, patient and disease related factors as well as factors related to the ECMO treatment itself and a combination of both (19,34). Literature concerning these risk factors can be divided in two categories. The first category contains those studies looking for risk factors, like primary diagnosis, in patients who did develop cerebral lesions, with or without a control group. Studies of the second category focus more indirectly on possible risk factors in patients and on technical aspects and consequences of ECMO, like the changes in pulsatility of the cerebral blood flow pattern. Several of the studies of the first category are important to mention; Hardart et al (35,36) determined gestational age, postconceptional age, acidosis, sepsis, coagulopathy and treatment with epinephrine as independent pre-ECMO factors associated with ICH in neonates who were treated with ECMO. Dela Cruz et al (37) demonstrated that an elevated activated clotting time (ACT) and low platelet counts requiring transfusion were statistically associated with an increase in the development of ICH. Especially the relation between ACT and ICH might reflect the role of anticoagulation, which is necessary to perform during ECMO. Hirthler et al (38) reviewed that instability of ACT and platelet count are early predictors of ICH. In general the equilibrium between anticoagulation and clotting complications is difficult to manage. Clotting complications and especially disseminated intravascular coagulation (DIC) might result in embolization of (micro) clots to different organs. Finally lactate before ECMO treatment was found to be a useful marker for the development of ICH during ECMO (39). Major ischemic lesions seem to be related to longer ECMO treatment and more conservative use of heparin (40,41). Since randomized controlled studies cannot be performed due to ethical considerations, the second category of studies contains mainly observational aspects and concerns in patients treated with ECMO. One interesting aspect both influenced by patient factors like severity of illness and hypoxia as well as by the ECMO treatment itself is the disturbance of cerebral autoregulation (AR). In normal, healthy situations cerebral AR maintains the cerebral blood flow over a wide range of cerebral perfusion pressures with a contra regulation response occurring with a delay of 2 seconds after sudden changes in blood pressure. It has been shown, however, that AR is disturbed in severely ill term infants (42-45). Additional studies in newborn lambs showed that prolonged hypoxia and/or the ECMO treatment itself will significantly disturb the cerebral AR (46-49). As a result of this disturbed AR, the capillary vasculature of the brain might be at increased risk in case of systemic blood pressure changes. This could be an additional risk for ischemic complications due to hypoperfusion as well as hemorrhagic complications due to hyperperfusion.

Right from the start of ECMO treatment, cannulation of the right common carotid artery and in-
ternal jugular vein became a concern. Some authors describe predominance of cerebral lesions in especially the right hemisphere of the brain, while in other studies this predominance, possibly related to ligation of the right common carotid artery and right internal jugular vein, could not be confirmed (49-56). Theoretically, in the presence of an intact circle of Willis, ischemia of the right cerebral hemisphere will never occur. However, incompleteness of the circle of Willis has been found in 0.6% of normal brains, which is a possible risk factor for ischemic lesions of the right hemisphere (57). Reassuring data concerning the cannulation came from two studies. First Short et al showed that 4 hours of hypoxia in lambs before cannulation caused a 100% increase in CBF, with maintenance of oxygen transport to the brain and cerebral oxygen metabolism. These parameters were not altered by cannulation of the right common carotid artery and/or the right internal jugular vein (58). A second study, by Klein et al. found no statistically significant or persistent differences on MRI performed directly after ligation between rats in which either the jugular vein and/or the carotid artery where ligated compared to control rats (59). Less reassuring studies, considering the cannulation procedure occurred as well. Liem et al found alterations in cerebral oxygenation after carotid artery ligation in human newborns, possibly reflecting an increased O2 extraction due to increased transit time of the blood resulting from the diversion of arterial blood supply from the left common carotid artery to the right hemisphere. After jugular vein ligation, no changes were found. At 60 minutes after the initiation of ECMO there was a significant increase in cerebral blood flow velocity and cerebral blood volume (60). In a consecutive study van Heijst et al confirmed these data. They measured changes in the right and left cerebral hemisphere separately, but found no right – left differences (61). These findings combine well with the results of the studies of Raju et al and Matsumoto et al, who both demonstrated a temporary decrease in CBFV after ligation of the right common carotid artery (62,63). Finally Hunter et al. performed a study in which the CBF of lambs was measured by laser Doppler flowmetry during va- as well as vv-ECMO. Ligation of the right common carotid artery resulted in temporary (1 minute) decrease in CBF to the right cerebral cortex (64). It can at least be concluded that there are some concerns about possible negative effects for the brain related to the ligation of the right common carotid artery. Besides this, there is a lack of knowledge about life-long consequences of permanent ligation of the right common carotid artery. All these concerns mentioned, stimulated the development of vv-ECMO, using a double-lumen cannula, inserted in the right atrium of the patient. Even though, for the purpose of this thesis, attention is directed mainly on va-ECMO it has to be mentioned that vv-ECMO might have its own risks for the brain. There is especially fear for venous congestion due to obstruction caused by the thicker double lumen venous cannula. This is supported by three, somewhat older, studies. Two of them described a high frequency of ICH in the posterior fossa of the brain (65,66). The third study found a high incidence of subarachnoid space enlargement at the interhemispheric fissure and frontal, temporal and parietal convexity (68%) and ventricular enlargement (35%) in patients treated with vv-ECMO. In this same study ICH occurred in 7 out of 31 (22%) patient, all neonates (67). However, a possible positive effect of vv-ECMO compared to va-ECMO might be the maintenance of pulsatile arterial
flow to the brain. Once va-ECMO is initiated, Taylor et al found the systolic phase broadened and diastolic flow velocities markedly increased, as measured by Doppler imaging of the pericallosal portion of an anterior cerebral artery (68). This resulted in a decrease of the pulsatility index [PI, in which PI = (systolic CBF velocity – diastolic CBF velocity) / systolic CBF velocity] most clearly at higher ECMO flow rates. There was also an increase in true CBF expressed by the area under the velocity curve. Although clinical relevance and importance of these findings remain unclear, as is the fact for many observational studies, this loss of arterial pulsatility might be additionally harmful for the vulnerable brain (19). One of the underlying mechanisms might be the increased disturbance of cerebral AR in va-ECMO compared to vv-ECMO in studies by Ingyinn et al. Vascular reactivity to acetylcholine was measured in middle cerebral arteries from lambs exposed to ECMO (69,70). There are still conflicting data about the influence of non-pulsatile flow on cerebral perfusion (71,72). However, in recent literature there is some evidence for more benefit of pulsatile circulation on cerebral blood flow and increased vital organ flow (73,74). A concern for va-ECMO as well as for vv-ECMO could be the extensive contact of blood with the plasticizers containing tubings of the ECMO system, which might result in exposure of the brain to potential toxic agents (75,76).

Other ECMO related factors might further influence the occurrence of complications in the vulnerable brain. A good example is the use of the veno-arterial bridge (va-bridge) between the arterial and venous side of the ECMO system. Historically this va-bridge was filled with blood and opened and closed several times per hour to avoid clotting in it. Studies of Liem et al and van Heijst et al showed significant changes in cerebral oxygenation and hemodynamics related to the use of the va-bridge that can be avoided if the va-bridge does not have to be opened intermittently by filling it with normal saline instead of blood and connecting it with stop cocks to the venous and arterial cannula (77,78). Other aspects of the ECMO treatment, like bladderbox alarms and different kinds of pumps seem all to be interesting to be further studied in their possible relation to cerebral complications.

Conclusion
ECMO has proven to be an effective and life-saving therapy with increased survival and possibly a favorable outcome compared to newborns with severe but reversible respiratory failure treated conventionally. However, cerebral hemorrhage and ischemia are important complications resulting in long-term neurological and neurodevelopmental sequelae. More research is necessary to gain more knowledge of pathophysiological mechanisms underlying these complications. When this mechanism has been recognized, improved ECMO treatment strategies can be developed in order to reduce these cerebral complications. This thesis will try to contribute to further improvement of this knowledge.
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1.3 Outline of the thesis

This thesis describes the results of several studies, all concerning possible risk factors for cerebral hemorrhage and/or ischemia during va-ECMO. In general these factors can be patient as well as ECMO therapy specifically related. The studies presented can be considered to be part of a continuous search to improve the ECMO outcome.

Besides an introduction focusing on the relation between ECMO and outcome and cerebral complications, **chapter 1** gives a short introduction in near infrared spectrophotometry. This technique might be less known by several readers and was used in two of the studies presented.

In **chapter 2** the possible relation between prior iNO use and the occurrence of (cerebral) hemorrhagic complications, disseminated intravascular coagulation and clotting complications during consecutive ECMO treatment were studied.

**Chapter 3** describes disturbances of the cerebral circulation caused by repetitively opening and closure of the veno-arterial bridge, a connecting tube between the venous and arterial side of the ECMO system. CBFV measurements were performed by echo-Doppler in the pericallosal artery and the relation between the observed changes in CBFV and ECMO flow rate was studied.

**Chapter 4** contains three studies focusing on a possible relation between bladderbox alarms and consecutive intravascular volume administration and the effects on cerebral oxygenation and hemodynamics and the occurrence of cerebral complications. In the first study, the relation between the frequency and amount of intravascular volume administration and the occurrence of ICH during va-ECMO is described. The second, consecutive study in lambs, was developed to determine the changes in cerebral oxygenation and hemodynamics caused by simulated bladderbox alarms during va-ECMO. The third study describes the effects of intravascular volume administration on cerebral oxygenation and hemodynamics in human newborns treated with va-ECMO.

In **chapter 5** the main findings of the studies of this thesis are described in a general discussion and thoughts about future perspectives and proposals for studies and the place of ECMO in the treatment of severe neonatal respiratory failure, mostly complicated by persistent pulmonary hypertension, are shared with the readers.

Finally a summary, together with a Dutch version, is presented in **chapter 6**.
1.4 Introduction in near infrared spectrophotometry

In two of the studies presented in this thesis, near infrared spectrophotometry (NIRS) is used as a tool for measurement of changes in cerebral oxygenation and hemodynamics in events related to ECMO. Although this technique is widely used in medical research it might be less known compared to several other techniques used. For this reason a short introduction is presented in this section.

Since Jöbsis was the first to use NIRS in humans in 1977 (1), it became a frequently used noninvasive and safe technique to study tissue oxygenation and hemodynamics. In our research group, NIRS has been used extensively to study changes in cerebral oxygenation and hemodynamics in term as well as preterm neonates. These studies concern ECMO-related topics as well as a variety of other situations (2,3,4).

Background of NIRS

NIRS is based on the principle of oxygenation-dependent changes in the absorption by chromophores of near infrared light passing through relatively transparant tissue like the brain. Although not the only chromophore, the most important oxygen-dependent chromophore in the brain is hemoglobin. Total hemoglobin (tHb) in the brain consists of oxyhemoglobin ($O_2$Hb) and deoxyhemoglobin (HHb). Both forms of Hb have different absorption spectra in the near infrared region (1,5,6). In homogenous and non-scattering tissue, the concentration of a chromophore can be determined from light absorption at specific wavelengths using the Lambert-Beer law:

$$OD(\lambda) = \varepsilon(\lambda) \cdot c \cdot d$$

In this formula OD is the light absorption in optical densities, $\lambda$ is the used wavelength, $\varepsilon$ is the extinction coefficient of the chromophore (1 mmol$^{-1}$ cm$^{-1}$) for the used wavelength, $c$ is the concentration of the chromophore (mmol l$^{-1}$) and $d$ is the optical pathlength (cm), representing the distance between the point where near infrared light enters and leaves the tissue, usually the distance between the transmitting and receiving optode. In this thesis, Hb is the only chromophore used. The extinction coefficients of $O_2$Hb and HHb are obtained in vitro with hemoglobin solutions (5,6).

There are two important restrictions for the use of the Lambert-Beer law in brain tissue. Since brain tissue is non-homogenous, anisotropic and also containing other chromophores, photon loss will occur due to scattering and absorption by oxygen-independent chromophores in the brain, skull or skin. Besides this, scattering will lengthen the pathlengths of photons. However, with some modification, the Lambert-Beer law can be used;
Chapter 1

\[ \text{OD}(\lambda) = \varepsilon(\lambda) \cdot c \cdot d \cdot B + \text{OD}(\lambda)_R \]

In this modified formula, \( B \) is the multiplication factor for the increase in optical path length caused by scattering and considered to be constant and wavelength independent (7,8). \( \text{OD}(\lambda)_R \) reflects the loss of photons caused by scattering and absorption by oxygen-independent chromophores. Although the absolute value of \( \text{OD}(\lambda)_R \) is unknown, it is assumed to be a constant parameter in the same patient as long as positions of the transmitter and receiver are not changed. By measuring OD changes at three different wavelengths, changes in concentration of \( O_2\text{Hb} \) (\( \Delta cO_2\text{Hb} \)) and \( HHb \) (\( \Delta cHHb \)) can be obtained. These changes can be expressed in \( \mu \text{mol}.100\text{g}^{-1} \) brain tissue.

Application of NIRS in this thesis.

We studied changes in \( cO_2\text{Hb} \) and \( cHHb \) by using continuous wave NIRS (Oxymon, Artinis, the Netherlands). Three wavelengths of near infrared light (905, 850 and 767 nm) were transmitted through the brain with a three-branch fiberoptic bundle and the transmitter placed in the midline of the skull. Two separate receiving optodes were placed on the left and right parietotemporal region of the skull. This enabled us to study \( \Delta cO_2\text{Hb} \) and \( \Delta cHHb \) separately for the right and left hemisphere of the brain, which might be especially interesting in studies concerning extracorporeal membrane oxygenation as the right carotid artery and/or jugular vene are cannulated. Although several important NIRS variables can be derived using continuous wave NIRS, we used changes in hemoglobin oxygenation index (\( \Delta HbD \)) and changes in cerebral blood volume (\( \Delta CBV \)) to study changes in cerebral oxygenation and hemodynamics.

Changes in hemoglobin oxygenation index (\( \Delta HbD \))

\( \Delta HbD \) is defined as the difference between \( \Delta cO_2\text{Hb} \) and \( \Delta cHHb \) (\( \Delta HbD = \Delta cO_2\text{Hb} - \Delta cHHb \)) and expressed in \( \mu \text{mol}.100\text{g}^{-1} \). It represents changes in global cerebral oxygenation and its use is preferable above isolated changes in \( cHHb \) and \( cO_2\text{Hb} \) as \( \Delta HbD \) is not influenced by simultaneous changes in \( \Delta cO_2\text{Hb} \) and \( \Delta cHHb \) caused by changes in CBV. It provides a continuous and direct measure for global cerebral oxygenation. Changes in Hbd can be explained by changes in cerebral oxygen consumption or by changes in cerebral oxygen delivery. The latter is mainly determined by cerebral blood flow (CBF), arterial oxygen saturation (saO.) and blood hemoglobin concentration (cHb). In general, \( \Delta HbD \) like used in our studies, is strongly correlated with CBF over a wide range of intracranial pressures (9,10). CBF is determined by cerebral perfusion pressure (CPP) and cerebral vascular resistance (CVR). CPP is the difference between mean arterial blood pressure (MAP) and cerebral venous pressure, which can be replaced by the intracranial pressure (ICP). The most important limitation of \( \Delta HbD \) is the lack of reference values below which cerebral oxygenation or CBF is compromised and potentially harmful to the brain. Reference values might also depend on the clinical situation. Baseline variability of Hbd has been reported to be from -0.12 to +0.13 \( \mu \text{mol}.100\text{g}^{-1} \) brain (11). In the same study a decrease in Hbd of > 0.3
\( \mu \text{mol.}100\text{g}^{-1} \) reflects the effect of reducing \( \text{saO}_2 \) by approximately 12%. Another comparison can be found in relation to other physiologic parameters. During hypercapnia in piglets on ECMO after hypoxia, a mean \( \Delta \text{cO}_2 \text{Hb} \) of 0.16 \( \mu \text{mol.}100\text{g}^{-1} \cdot \text{kPa}^{-1} \) occurred (12). \( \Delta \text{cO}_2 \text{Hb} \) is one of the main factors for calculating \( \Delta \text{HbD} \).

Changes in cerebral blood volume (\( \Delta \text{CBV} \))

If blood hemoglobin concentration (cHb) is stable, \( \Delta \text{CBV} \), expressed in ml.100g\(^{-1}\), can be calculated using the following formula:

\[
\Delta \text{CBV} = (4 \cdot \Delta \text{ctHb}) \cdot (R \cdot \text{cHb})^{-1}
\]

In this formula \( \Delta \text{ctHb} \) is the sum of \( \Delta \text{cHHb} \) and \( \Delta \text{cO}_2 \text{Hb} \) (expressed in \( \mu \text{mol.}100\text{g}^{-1} \)), \( R \) is the cerebral arterial hematocrit ratio which is stated to be 0.69 (13), and 4 is a correction factor, since \( \text{ctHb} \) is expressed in mmol.100g\(^{-1}\) as a tetraheme molecule. cHb is expressed in mmol.l\(^{-1}\) as monoheme molecule.

Cerebral vasodilatation or vasoconstriction as well as changes in CBF might result in changes in CBV. The most important limitation in using \( \Delta \text{CBV} \) is the lack of reference values below or above which \( \Delta \text{CBV} \) might cause or reflect harm to the brain. Some reference might be found in two studies by Wyatt et al. In the first study, changes in CBV, calculated in relation to changes in \( \text{paCO}_2 \) in term infants, were 0.51 mL.100g\(^{-1}\)·kPa\(^{-1}\) (13). In a second study mean CBV was demonstrated to be 2.2 ± 0.40 mL.100g\(^{-1}\) in normal infants and 3.00 ± 1.04 mL.100g\(^{-1}\) in infants with brain injury (15). Additional data come from animal studies in which changes in CBV have been compared with usual physiological parameters like changes in \( \text{paCO}_2 \) and mean blood pressure. During hypercapnia in normoxemic piglets on ECMO, a mean increase of CBV of 0.03 mL.100g\(^{-1}\)·kPa\(^{-1}\) occurred. In piglets after hypoxemia this increase was 0.10 mL.100g\(^{-1}\)·kPa\(^{-1}\) (12). In another study an increase in CBV of 0.07 mL.100g\(^{-1}\)·mmHg\(^{-1}\) has been shown in relation to changes in mean blood pressure in newborn lambs (16). Finally the value of CBV in lambs has been found to be 2.5 mL.100g\(^{-1}\) of brain tissue (17).

Conclusion

Despite its limitation, NIRS is a valuable tool for investigation of changes in cerebral hemodynamics in newborns during ECMO treatment. This can improve our knowledge about the cerebral hemodynamic response to many events during ECMO.
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Chapter 2

iNO before the start of ECMO and complications of the brain

2.1 Abnormalities of coagulation related to the use of inhaled nitric oxide before extracorporeal membrane oxygenation

2.2 The effect of inhaled nitric oxide on the course of extracorporeal membrane oxygenation and the occurrence of hemorrhagic complications
Chapter 2.1

Abnormalities of coagulation related to the use of iNO prior to extracorporeal membrane oxygenation

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Abstract

Objective. Evaluation of the influence of previous inhaled nitric oxide (iNO) treatment on the occurrence of clotting complications and disseminated intravascular coagulation during extra corporeal membrane oxygenation (ECMO).

Design: Retrospective study in newborns treated with veno-arterial ECMO during a 5-yr period.

Setting: Neonatal intensive care unit of a university medical center

Patients: A total of 59 Newborns with severe respiratory insufficiency treated with veno-arterial ECMO.

Interventions: Patients received iNO before ECMO (iNO group) or not (control group).

Measurements and main results: There were no differences between the groups for patient characteristics and medication use before ECMO, except for norepinephrine. After correction for diagnosis and duration of ECMO, significantly more clotting complications and DIC as individual variables were seen in the iNO group. For the combination of clotting complications and disseminated intravascular coagulation there was a significantly higher prevalence in the iNO group.

Conclusions: In our population we found a remarkable relationship between clotting complications or a disseminated intravascular coagulation and iNO use prior to ECMO treatment, which needs further prospective research before conclusions can be drawn.
Introduction

Veno-arterial extracorporeal membrane oxygenation (ECMO) is a rescue therapy for neonates with severe respiratory failure (1). One of the major causes for complications of ECMO is the disturbance in normal coagulation, resulting in an increased risk of disseminated intravascular coagulation (DIC) and clotting complications in the ECMO system (2, 3). Because persistent pulmonary hypertension is one of the most important indications for ECMO in newborns, inhaled nitric oxide (iNO) is usually part of the treatment before the start of ECMO (4). Although used for its specificity as a pulmonary vasodilator, iNO has systemic effects as well (5). There are suggestions in literature that iNO might be involved in the initiation of the coagulation cascade (6-13). The aim of this study is to evaluate the influence of previous iNO treatment on the occurrence of clotting complications and DIC during the ECMO treatment.

Materials and methods

In a retrospective study of a 5-yr period, 85 patients treated with ECMO in our hospital were analyzed. The project was approved by the institutional review board. Patients with congenital diaphragmatic hernia were excluded (n=26) because in our center iNO is not part of the standard treatment protocol in congenital diaphragmatic hernia (14). In addition, patients with congenital diaphragmatic hernia on ECMO receive tranexamic acid, an anti-fibrinolytic drug, during the operation. Finally 59 patients were included, of which 25 patients received iNO treatment before ECMO (the iNO-group) and 34 patients started with ECMO without previous iNO treatment (control group). The control group consists of patients from the time prior to the introduction of iNO at our institution and from the time that there was limited availability of iNO. No more patients were included from the time when iNO was fully available. Through the study period selection criteria to start iNO were based on an oxygenation index of ≥ 25 while on assisted ventilation. Patients were treated with 20 ppm NO. The iNO group and the control group were compared for gestational age, birth weight, lowest arterial oxygen saturation (saO₂), lowest arterial partial pressure of oxygen (pao₂), maximum alveolo-arterial difference in partial pressure of oxygen (AaDO₂) before ECMO, ventilator settings, postnatal age at the initiation of ECMO, duration of ECMO, worst pH before ECMO and Apgar scores at 1 and 5 minutes using a student t-test. Primary diagnoses were compared using Fisher exact test. The independence between sex and iNO in this study was analyzed using a chi-square test.

All patients received a heparin-loading dose of 150 units/kg at the initiation of ECMO. After this, heparinization was checked every hour by measuring the activated clotting time aiming for values between 200 and 220 secs (Hemochron, ITC Europe, Italy). iNO was stopped immediately after the initiation of ECMO. We studied differences in the occurrence of clotting complications or DIC between the iNO group and the control group. Clotting complications were defined as cerebral infarction on ultrasound, computed tomographic scan or magnetic resonance image or clot formation in the ECMO-system by visual inspection. The diagnostic of DIC was based on laboratory results performed on a daily basis with D-dimer concentration of > 10,000 units/L in combination with decreasing fibrinogen levels to values of < 1500 mg/L. We performed a linear logistic regression analysis (Statistical Analysis
System, SAS Institute, Cary, NC) to look for significant relations with iNO treatment before ECMO. For clotting complications and DIC, iNO treatment and primary diagnosis were included in the regression models. Other factors included were gestational age, birth weight, postnatal age at start of ECMO and pre-ECMO medication. Patient characteristics are presented as mean ± SD. Odds ratios for complications are presented with a 95% confidence interval; p values of <.05 were considered significant.

## Results

### Table 1. Characteristics of inhaled nitric oxide and control group

<table>
<thead>
<tr>
<th>Pre-ECMO Parameter</th>
<th>iNO group (n=25)</th>
<th>Control group (n=34)</th>
<th>p-values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre-ECMO Parameter</td>
<td>Mean SD</td>
<td>Mean SD</td>
<td>p</td>
</tr>
<tr>
<td>Gestational age (weeks)</td>
<td>39 ± 2</td>
<td>39 ± 3</td>
<td>0.69</td>
</tr>
<tr>
<td>Birth weight (gram)</td>
<td>3422 ± 586</td>
<td>3392 ± 626</td>
<td>0.86</td>
</tr>
<tr>
<td>Lowest saO2 (%)</td>
<td>64 ± 22</td>
<td>63 ± 21</td>
<td>0.85</td>
</tr>
<tr>
<td>Lowest paO2 (mmHg)</td>
<td>42 ± 17</td>
<td>35 ± 11</td>
<td>0.11</td>
</tr>
<tr>
<td>Maximum AaDO2 (mmHg)</td>
<td>636 ± 21</td>
<td>644 ± 14</td>
<td>0.12</td>
</tr>
<tr>
<td>PIP (cm H2O)</td>
<td>36.8 ± 1.8</td>
<td>37.5 ± 2.6</td>
<td>0.45</td>
</tr>
<tr>
<td>MAP (cm H2O)</td>
<td>19.6 ± 2.1</td>
<td>19. ± 2.5</td>
<td>0.74</td>
</tr>
<tr>
<td>PEEP (cm H2O)</td>
<td>5.0 ± 1.2</td>
<td>4.6 ± 1.2</td>
<td>0.25</td>
</tr>
<tr>
<td>Duration of ECMO (hours)</td>
<td>173 ± 52</td>
<td>155 ± 74</td>
<td>0.27</td>
</tr>
<tr>
<td>Postnatal age at start ECMO (hours)</td>
<td>59 ± 68</td>
<td>53 ± 63</td>
<td>0.70</td>
</tr>
<tr>
<td>Worst pH</td>
<td>7.31 ± 0.16</td>
<td>7.26 ± 0.16</td>
<td>0.29</td>
</tr>
<tr>
<td>Apgar 1 minute</td>
<td>5.4 ± 3.1</td>
<td>5.8 ± 2.8</td>
<td>0.63</td>
</tr>
<tr>
<td>Apgar 5 minutes</td>
<td>6.3 ± 2.4</td>
<td>6.9 ± 2.2</td>
<td>0.30</td>
</tr>
<tr>
<td>Gender (male/female)</td>
<td>13/12</td>
<td>25/9</td>
<td>0.05</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Primary diagnosis</th>
<th>Number (%)</th>
<th>Number (%)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>MAS</td>
<td>19 (76)</td>
<td>19 (56)</td>
<td>0.17</td>
</tr>
<tr>
<td>Sepsis</td>
<td>4 (16)</td>
<td>8 (24)</td>
<td>0.53</td>
</tr>
<tr>
<td>Pneumonia</td>
<td>2 (8)</td>
<td>7 (21)</td>
<td>0.28</td>
</tr>
<tr>
<td>RDS</td>
<td>2 (8)</td>
<td>2 (6)</td>
<td>1.00</td>
</tr>
<tr>
<td>Primary PPHN</td>
<td>2 (8)</td>
<td>5 (15)</td>
<td>0.69</td>
</tr>
<tr>
<td>Total number of primary diagnosis</td>
<td>29</td>
<td>41</td>
<td></td>
</tr>
</tbody>
</table>

*a Several patients had more than one primary diagnosis. iNO group = group treated with inhaled nitric oxide, saO2 = arterial oxygen saturation, paO2 = arterial partial pressure of oxygen, AaDO2 = alveolo-arterial difference in partial pressure of oxygen, PIP = positive inspiratory pressure before ECMO, MAP = mean airway pressure before ECMO, PEEP = positive end-expiratory pressure before ECMO. ECMO = extracorporeal membrane oxygenation, MAS = meconium aspiration syndrome, RDS = respiratory distress syndrome. PPHN = persistent pulmonary hypertension of the newborn.*
No significant differences were found for pre-ECMO characteristics between groups (table 1). More than one primary diagnosis was found in several patients. In the iNO group, two patients were diagnosed with the combination of sepsis and pneumonia, one patient with meconium aspiration syndrome and sepsis, and one patient with respiratory distress syndrome and sepsis. There was no significant difference in pre-ECMO medication between the two groups except for the use of norepinephrine (four patients in the iNO group vs. no patient in the control group [Fisher's exact test, p=0.03]). Mean OI at or near the time iNO was initiated was 38 ± 9.

Table 2. Occurrence of clotting complications and DIC during extra corporeal membrane oxygenation in the inhaled nitric oxide group and control group

<table>
<thead>
<tr>
<th>Complication</th>
<th>INO group (n=25)</th>
<th>Control group (n=34)</th>
<th>Regression analysis</th>
<th>Corrected</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.(%)</td>
<td>No.(%)</td>
<td>OR (95% CI)</td>
<td>p</td>
</tr>
<tr>
<td>Clotting</td>
<td>10 (40)</td>
<td>6 (18)</td>
<td>3.1 (1.0-10.8)</td>
<td>0.056</td>
</tr>
<tr>
<td>DIC</td>
<td>10 (40)</td>
<td>6 (18)</td>
<td>3.1 (1.0-10.8)</td>
<td>0.056</td>
</tr>
<tr>
<td>Clotting and/or DIC</td>
<td>17 (68)</td>
<td>9 (26)</td>
<td>5.9 (2.0-19.3)</td>
<td>0.001</td>
</tr>
</tbody>
</table>

Odd’s ratios (OR) are presented with a 95% confidence interval (CI) for each complication before and after correction for diagnosis and diagnosis as well as duration of extracorporeal membrane oxygenation (ECMO). iNO group = group treated with inhaled nitric oxide, DIC = disseminated intravascular coagulation. * Statistically significant (p<0.05) after correction for diagnosis or after correction for diagnosis and ECMO run-time.

The occurrence of clotting complications or DIC during ECMO is shown in table 2. A p value just above the defined level of significance was observed for the relationship between previous iNO use and clotting complications and DIC. This became statistically significant (odds ratio, 3.4 [95% confidence interval, 2.1-22.6] and 4.5 [1.3-19.6]) after correction for diagnosis and duration of ECMO. For the combination of clotting complications or DIC a statistically significant relationship with previous iNO was found, which persisted after correction for diagnosis and duration of ECMO (odds ratio, 5.6 [95% confidence interval, 1.7-20.4]).

Discussion
iNO effectively reduces the number of ECMO treatments, but does not reduce mortality in term infants with hypoxic respiratory failure (15). Clotting complications and DIC are major problems
for patients receiving ECMO. Because of the potential effects on the coagulation cascade, the use of iNO in this population has been of our concern (6-13).

In this retrospective study we found a remarkable relationship between the occurrence of clotting complications or DIC and the use of iNO prior to ECMO. After correction for diagnosis and duration of ECMO, both clotting complications and DIC were significantly more present in the iNO group than in the control group. When combined, clotting complications or DIC occurred significantly more often in the iNO group than in the control group. The fact that the difference in clotting complications and DIC between the iNO group and control group increased after correction for diagnosis might be explained by the fact that there were more patients with sepsis in the control group. Because sepsis, even without ECMO, is related to the occurrence of clotting complications and DIC, these complications might already be present in these patients.

Pre-treatment with iNO of newborns in need of ECMO therapy seems to have an effect on the coagulation cascade in this study. It is hard to explain this from the known effects of iNO. Based on literature hypothetical explanations might be found in vascular and tissue damage, apoptosis, an increase in free radicals and increased expression of factors initiating the coagulation cascade, like tissue factor. In term infants prolonged, 24 to 48 hours, exposure to iNO is associated with an increase in markers of oxidative injury in serum samples (6). Kobayashi et al. found a relationship between long-term inhalation of NO and activation of the clotting system by increasing the lung expression of tissue factor (7). It is known that free radicals and apoptosis are likely to play a role in the expression of tissue factor and increased thrombin generation on the surface of monocytes, macrophages and endothelial cells (8-11). Finally NO can influence coagulation parameters in vitro via nitrosylation of thiol groups (12,13). The clinical relevance of these mechanisms remains unclear. It is speculative whether there might be a cumulative effect in the initiation of the coagulation cascade by the combination of iNO and a consecutive ECMO treatment.

We recognize the limitations of a retrospective study design in which patients are not randomized and findings could be influenced by many factors. However, we are not aware of any study focusing on the possible negative effects of iNO treatment before ECMO. Because iNO has been accepted as standard therapy for newborns with severe respiratory insufficiency with pulmonary hypertension, prospective studies are hard to perform because of medical as well as ethical considerations. Due to the limited capacity of iNO treatment in our hospital during the study period, the start of iNO depended on its availability. If more patients with an iNO indication were presented at the same time with limited availability of iNO the choice to use iNO in which patient was at the discretion of the treating physician and bias related to myriad factors cannot be ruled out. Although the two groups were not completely homogeneous for diagnosis, especially sepsis and MAS, this was not statistically significant. To correct for the possible influence of primary diagnosis on complications we did however perform a logistic regression analysis. Treatment in both groups was equal except for the use of norepinephrine, which was significantly more used in the iNO group. It is unclear whether this was related to the use of iNO. Finally the control group had a higher percentage of males than the iNO group. Although this was just above of the level
of significance, any influence on the findings cannot be excluded. If our results would be confirmed by prospective studies it is of great interest to further investigate the possible mechanisms of iNO on the coagulation system. Major clot formation in the circuit can be detected by circuit inspection, but small clots can escape into the circulation of the patient and cause (small) infarctions. Disturbance in other organ functions can also be involved in DIC. We should be aware of this potential risk of using iNO in newborns with respiratory failure qualifying for ECMO treatment.

Conclusions
In this retrospective study we found a remarkable relationship between clotting complications and/or DIC and iNO use prior to ECMO treatment. A prospective, randomized study is indicated, which should also investigate the mechanisms of possible adverse effects of iNO on the coagulation system before and during ECMO, before strong conclusions can be made.
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Chapter 2.2

The effect of inhaled nitric oxide on the course of extracorporeal membrane oxygenation and the occurrence of hemorrhagic complications

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Abstract
This study evaluated the relation between prior inhaled nitric oxide (iNO) and the time to initiation and duration of treatment with veno-arterial extracorporeal membrane oxygenation (ECMO) and the occurrence of hemorrhagic complications.
A retrospective study was conducted in 59 human newborns treated for respiratory insufficiency with ECMO over a 5-years period. Patients received iNO before ECMO (iNO group) or not (control group). Both groups were compared for patient characteristics, postnatal age at the initiation of ECMO, duration of ECMO treatment and hemorrhagic complications.
There were no significant differences between the iNO group and the control group for patient characteristics and medication use before the ECMO treatment, except for norepinephrine. There was no significant difference for postnatal age at the initiation of ECMO and mean duration of ECMO treatment. We found no statistically significant difference in hemorrhagic complications between both groups. This persisted after correction for diagnosis and ECMO run-time in linear logistic regression analysis.
Inhaled nitric oxide before ECMO did not result in a significant delay in the initiation of ECMO or longer duration of the ECMO treatment. There was no significant relationship between the use of prior iNO and the occurrence of hemorrhagic complications during the ECMO treatment.
Introduction

Since Bartlett et al. first used neonatal veno-arterial extracorporeal membrane oxygenation (ECMO) in 1975, ECMO became an effective rescue therapy for newborns suffering from severe, but potentially reversible respiratory insufficiency. Without ECMO there is a high mortality in this patient group. The occurrence of hemorrhagic and ischemic cerebral lesions, resulting in neurological and neurodevelopmental dysfunction are major complications of ECMO treatment. One of the causes for these devastating complications is the disturbance of normal coagulation resulting in an increased risk for clotting complications and hemorrhagic complications, the latter occurring in up to 20% of cases.

Because persistent pulmonary hypertension is one of the most important indications for ECMO treatment in newborns, the application of inhaled nitric oxide (iNO) is usually part of the conventional treatment before the start of ECMO. The use of iNO to treat pulmonary hypertension might result in a delayed initiation of ECMO since a certain time is required to evaluate the effect of iNO. Due to a possible longer pre-ECMO treatment period, lung recovery might be prolonged, resulting in an increased duration of ECMO treatment, which consequently might result in more complications. Although used for its specificity as a pulmonary vasodilator, iNO has systemic, extra pulmonary effects as well. NO is known to have an effect on platelet function in vitro. The relevance in vivo is unclear, especially as NO in the blood is reported to have a short half life time and will bind to proteins like hemoglobin and albumin. However, iNO in the systemic circulation might diffuse to circulating platelets and inhibit adhesion and aggregation. Next to this, elevated levels of NO metabolites, nitrite and nitrate, may result in prolonged reactivation into bioactive NO, especially in case of acidosis and hypoxemia, conditions occurring often in pre ECMO patients. Furthermore, the production of toxic free radicals by iNO with consecutive vascular and tissue damage may play a role. It is unknown whether this might result in an increased vulnerability for hemorrhagic complications when iNO treatment is followed by ECMO for which the patient needs to be heparinized. In an earlier publication of our group in the same population, we found a remarkable and statistically significant relation between the use of iNO before veno-arterial ECMO and clotting complications or disseminated intravascular coagulation (DIC). The aim of this study is to evaluate the relation between the use of iNO before ECMO and the time to initiation and duration of treatment with ECMO. Furthermore, the occurrence of hemorrhagic complications in relation to iNO treatment was studied.

Materials and methods

In a retrospective study over a 5-year period, all newborn patients treated with veno-arterial ECMO for severe, but potentially reversible, pulmonary insufficiency and pulmonary hypertension in our hospital (n=85) were analyzed. The study was approved by the institutional review board. Patients with congenital diaphragmatic hernia were excluded (n=26) because, in our center, iNO is not part of the standard treatment protocol in congenital diaphragmatic hernia. In addition, patients with congenital diaphragmatic hernia treated with ECMO receive tranexamic...
acid, an anti-fibrinolytic drug, during and 24 hours after surgery. Finally, 59 patients were included in our study, of which 25 patients received iNO treatment before ECMO (iNO-group) and 34 patients started with ECMO without prior iNO treatment (control group). The control group consists of patients from the time before the introduction of iNO at our institution and from the time that there was limited availability of iNO. No more patients were included from the time when iNO was fully available. During the study period, selection criteria to start iNO were based on an oxygenation index of ≥ 25 although on assisted ventilation. Patients in the iNO group were treated with 20 ppm NO. All patients received a heparin-loading dose of 150 U/kg at the initiation of ECMO. After this, heparinisation was checked every hour by measuring the activated clotting time aiming for values between 200 and 220 seconds (Hemochron, ITC Europe, Italy). Inhaled nitric oxide was stopped shortly after the initiation of ECMO. During the course of ECMO, platelet transfusions were given to maintain a platelet count greater than 80,000/mm³. Erythrocyte transfusions were given to maintain a hemoglobin level > 8.0 mmol/l and a hematocrit value > 0.40 L/L. Hemoglobin level and platelet count were determined every 4 hours. The iNO group and the control group were compared for gestational age, birth weight, lowest arterial oxygen saturation (saO₂), lowest arterial partial pressure of oxygen (paO₂), maximum alveolo-arterial difference in partial pressure of oxygen (AaDO₂) before ECMO, ventilator settings before ECMO (peak inspiratory pressure, mean airway pressure, positive end-expiratory pressure), worst pH before ECMO, postnatal age at the start of ECMO, duration of ECMO and Apgar scores at 1 and 5 minutes using student t-tests. Primary diagnoses were compared using Fisher's exact test. The independency between gender and iNO and different medications and iNO in this study was analyzed using a Chi-square test. We studied differences in the occurrence of hemorrhagic complications between the iNO- and control group, which were defined as intracranial hemorrhage as assessed by daily ultrasound during the ECMO treatment or computed tomographic scan or magnetic resonance image directly after the ECMO treatment, cannulation site hemorrhage, gastrointestinal hemorrhage or pulmonary hemorrhage. We performed linear logistic-regression analysis (Statistical Analysis System, SAS Institute, Cary, NC) to study the relation between iNO treatment before veno-arterial ECMO and hemorrhagic complications. Inhaled nitric oxide treatment or not, duration of ECMO and diagnosis were included in the regression models. Other factors included were gestational age, birth weight, postnatal age at start of ECMO and pre-ECMO medication. Patient characteristics are presented as mean ± SD. Odds ratios for hemorrhagic complications are presented with a 95% confidence interval; p values of <0.05 were considered significant.
Results

Table 1. Characteristics of inhaled nitric oxide and control group

<table>
<thead>
<tr>
<th>Pre-ECMO Parameter</th>
<th>iNO group (n=25)</th>
<th>Control group (n=34)</th>
<th>p-values</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>SD</td>
<td>Mean</td>
</tr>
<tr>
<td>Gestational age (weeks)</td>
<td>39</td>
<td>2</td>
<td>39</td>
</tr>
<tr>
<td>Birth weight (gram)</td>
<td>3422</td>
<td>586</td>
<td>3392</td>
</tr>
<tr>
<td>Lowest saO₂ (%)</td>
<td>64</td>
<td>22</td>
<td>63</td>
</tr>
<tr>
<td>Lowest paO₂ (mmHg)</td>
<td>42</td>
<td>17</td>
<td>35</td>
</tr>
<tr>
<td>Maximum AaDO₂ (mmHg)</td>
<td>636</td>
<td>21</td>
<td>644</td>
</tr>
<tr>
<td>PIP (cm H₂O)</td>
<td>36.8</td>
<td>1.8</td>
<td>37.5</td>
</tr>
<tr>
<td>MAP (cm H₂O)</td>
<td>19.6</td>
<td>2.1</td>
<td>19.5</td>
</tr>
<tr>
<td>PEEP (cm H₂O)</td>
<td>5.0</td>
<td>1.2</td>
<td>4.6</td>
</tr>
<tr>
<td>Duration of ECMO (hours)</td>
<td>173</td>
<td>52</td>
<td>155</td>
</tr>
<tr>
<td>Postnatal age at start ECMO (hours)</td>
<td>59</td>
<td>68</td>
<td>53</td>
</tr>
<tr>
<td>Worst pH</td>
<td>7.31</td>
<td>0.16</td>
<td>7.26</td>
</tr>
<tr>
<td>Apgar 1 minute</td>
<td>5.4</td>
<td>3.1</td>
<td>5.8</td>
</tr>
<tr>
<td>Apgar 5 minutes</td>
<td>6.3</td>
<td>2.4</td>
<td>6.9</td>
</tr>
<tr>
<td>Gender (male/female)</td>
<td>13/12</td>
<td></td>
<td>25/9</td>
</tr>
<tr>
<td>Primary diagnosis*</td>
<td>Number (%)</td>
<td>Number (%)</td>
<td></td>
</tr>
<tr>
<td>MAS</td>
<td>19 (76)</td>
<td>19 (56)</td>
<td>0.17</td>
</tr>
<tr>
<td>Sepsis</td>
<td>4 (16)</td>
<td>8 (24)</td>
<td>0.53</td>
</tr>
<tr>
<td>Pneumonia</td>
<td>2 (8)</td>
<td>7 (21)</td>
<td>0.28</td>
</tr>
<tr>
<td>RDS</td>
<td>2 (8)</td>
<td>2 (6)</td>
<td>1.00</td>
</tr>
<tr>
<td>Primary PPHN</td>
<td>2 (8)</td>
<td>5 (15)</td>
<td>0.69</td>
</tr>
<tr>
<td>Total number of primary diagnosis</td>
<td>29</td>
<td>41</td>
<td></td>
</tr>
</tbody>
</table>

* Several patients had more than one primary diagnosis. iNO group = group treated with inhaled nitric oxide, saO₂ = arterial oxygen saturation, paO₂ = arterial partial pressure of oxygen, AaDO₂ = alveolo-arterial difference in partial pressure of oxygen, PIP = positive inspiratory pressure before ECMO, MAP = mean airway pressure before ECMO, PEEP = positive end expiratory pressure before ECMO. ECMO = extracorporeal membrane oxygenation, MAS = meconium aspiration syndrome, RDS = respiratory distress syndrome, PPHN = persistent pulmonary hypertension of the newborn.

No significant differences were found for pre-ECMO characteristics between the iNO group and the control group (Table 1). More than one primary diagnosis was found in several patients. In the iNO group there were two patients diagnosed with the combination of sepsis and pneumo-
nia, one patient with meconium aspiration syndrome and sepsis and one patient with respiratory distress syndrome and sepsis. Mean oxygenation index at or near the start of iNO treatment was $38 \pm 9$. There was no statistically significant difference in pre-ECMO medication between the iNO group and the control group except for the use of norepinephrine (4 patients in the iNO group versus no patient in the control group, $p = 0.03$). The postnatal age at the start of ECMO in the iNO group was $59 \pm 68$ hours versus $53 \pm 63$ hours in the control group ($p = 0.70$). Mean duration of ECMO in the iNO group was $173 \pm 52$ hours versus $155 \pm 74$ hours in the control group ($p = 0.27$). Regression analysis showed a statistically significant relationship between the postnatal age at the start of ECMO and the duration of ECMO ($p = 0.003$). There was an average 0.41 hour increase in duration of ECMO for every hour of postnatal age at the initiation of ECMO. In our study, however, the difference in postnatal age between the iNO group and control group at the start of ECMO was not related to a clinically relevant prolonged mean total duration of ECMO (2.9 hours).

Table 2. Occurrence of hemorrhagic complications during extra corporeal membrane oxygenation (ECMO) in the inhaled nitric oxide (iNO) group and control group

<table>
<thead>
<tr>
<th>Complication</th>
<th>iNO group (n=25)</th>
<th>Control group (n=34)</th>
<th>Regression analysis</th>
<th>Corrected for diagnosis</th>
<th>Corrected for diagnosis and duration of ECMO</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. (%)</td>
<td>No. (%)</td>
<td>OR (95% CI)</td>
<td>P</td>
<td>OR (95% CI)</td>
</tr>
<tr>
<td>Hemorrhage</td>
<td>5 (20)</td>
<td>6 (18)</td>
<td>1.2 (0.3-4.4)</td>
<td>0.819</td>
<td>1.7 (0.4-7.7)</td>
</tr>
</tbody>
</table>

Odd's ratios (OR) are presented with a 95% confidence interval (CI) before and after correction for diagnosis and for diagnosis and duration of ECMO

The occurrence of hemorrhagic complications during ECMO is shown in Table 2. There was no statistically significant difference in hemorrhagic complications between the iNO group and the control group ($p = 0.82$). This did not change after correction for diagnosis and duration of ECMO. Hemorrhagic complications in the iNO group consisted of four patients with cerebral hemorrhage and one patient with pulmonary hemorrhage. In the control group, there were three patients with a cerebral hemorrhage, two with a gastro-intestinal hemorrhage and one with a pulmonary hemorrhage.

Discussion

Inhaled nitric oxide is effective in reducing the number of ECMO treatments, but does not reduce mortality in term infants with hypoxic respiratory failure who do not have a diaphragmatic hernia. ECMO related complications might be related to later initiation of ECMO and a prolonged duration of the ECMO treatment. To avoid a delay in the use of ECMO because of an inadequate response to prior iNO treatment and other techniques like high frequency oscilla-
tion (HFO), it has been suggested to adapt the current ECMO criteria and to lower the threshold for the initiation of ECMO in patients suffering from severe hypoxic respiratory failure, who are already treated with iNO and HFO.\textsuperscript{3,24} Although there is a statistically significant relationship between the postnatal age at the start of ECMO and the duration of ECMO, in this study, the use of iNO did not result in a significant increase in the postnatal age at the start of ECMO nor in the duration of ECMO.

In earlier research, using a similar study in this population, we found a remarkable relation between iNO use and clotting complications or DIC.\textsuperscript{19} In this current study we focused on hemorrhagic complications, but did not find a statistically significant difference in the occurrence of hemorrhagic complications between the iNO group and control group. Because of the effect iNO is supposed to have on platelet aggregation and bleeding time,\textsuperscript{12,14,25,26,27} this is an important finding. This is especially true in its relation to the use of ECMO in which bleeding is one of the most devastating complications. The effect of NO, added to the sweep gas, on the coagulation system has also been used in ECMO research to increase biocompatibility of the extracorporeal circulation system and to reduce clotting formation on the oxygenator surface.\textsuperscript{28,29} Although not in combination with ECMO as a possible additive risk factor, a Cochrane review did not find evidence of an effect of iNO on intraventricular hemorrhage in preterm infants.\textsuperscript{30} A recent study, however, showed a significant association between increased nitrates and nitrites with hypoxic ischemic encephalopathy and possibly increased blood-brain barrier.\textsuperscript{18} Finally there is one study in piglets, that does not support the hypothesis that iNO increases the risk of bleeding in humans.\textsuperscript{31} Again this was not in combination with ECMO treatment.

Of all hemorrhagic complications, there were four cases of intracranial hemorrhage in the iNO group and three in the control group. As these intracranial hemorrhages were not predominantly located at one site of the brain, numbers are by far too small to make any conclusion about a possible relation between cannula position, of which depth of location was always checked by x-ray, and the occurrence of intracranial hemorrhage. Whether other treatment strategies like maintaining lower ACT levels would result in a decrease of hemorrhagic complications cannot be concluded from this study.

We recognize the limitations of a retrospective study design in which patients are not randomized and findings could be influenced by many factors. However, to our knowledge, this is the first study concerning the relation between prior iNO treatment and the occurrence of hemorrhagic complications, one of the most important complications of ECMO. Because iNO has been accepted as standard therapy for newborns with severe respiratory insufficiency with pulmonary hypertension, prospective studies are nowadays hard to perform because of medical and ethical considerations. Retrospective studies like ours might indicate whether there is a need for a prospective study or not. Due to the limited capacity of iNO treatment in our hospital during the study period, the start of iNO depended on its availability. If more patients with an iNO indication were presented at the same time with limited availability of iNO the choice to use iNO in which patient was at the discretion of the attending physician and bias related to myriad factors
cannot be ruled out. Even though primary diagnosis was not significantly different between the two groups we performed a logistic-regression analysis to correct for any possible influence of primary diagnosis on the outcome. Treatment in both groups was equal, except for the use of norepinephrine. It is unclear whether this was related to the use of iNO.

**Conclusion**

Based on this retrospective study, we demonstrated that the use of iNO in severe respiratory insufficiency and pulmonary hypertension does not necessarily result in a delay in the initiation of ECMO or in an increase in the duration of ECMO treatment in our population. Additionally, we conclude that the use of iNO prior to veno-arterial ECMO treatment does not result in an increase of hemorrhagic complications, one of the most devastating complications.
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Chapter 3

Disturbed cerebral circulation during opening of the veno-arterial bypass bridge in extracorporeal membrane oxygenation
Chapter 3

Disturbed cerebral circulation during opening of the veno-arterial bypass bridge in extracorporeal membrane oxygenation

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Abstract

**Purpose:** To describe the effects on cerebral blood flow velocity (CBFV) of intermittent opening of the venoarterial bridge (VA bridge) during venoarterial extracorporeal membrane oxygenation (VA-ECMO).

**Study design:** Prospective study in 22 newborns during VA-ECMO. CBFV was measured in the pericallosal artery by Doppler-ultrasound. Changes in peak systolic flow velocity (PSV), end diastolic flow velocity (EDV) and time averaged mean flow velocity (TAM) on day 1, 2, 3, and 5 and at low ECMO-flow (50-150 ml/min) were analyzed (mean percentage ± standard deviation (t-tests, p<0.05)). Changes >25% were considered relevant. The relationship between changes in CBFV and ECMO-flow rate (Pearson correlation, p<0.01) was studied.

**Results:** Opening of the VA bridge resulted in statistically significant and relevant decreases in PSV (35 ± 18%), EDV (93 ± 15%) and TAM (68 ± 13%), persisting during the consecutive days of treatment. Smaller changes in CBFV at low ECMO flow were statistically significant and mostly relevant, PSV (15 ± 7%), EDV (76 ± 21%) and TAM (40 ± 12%). Changes in CBFV were positively correlated to the ECMO-flow.

**Conclusion:** Use of the VA bridge results in significant and relevant, ECMO flow dependent, changes in CBFV, persisting during the treatment. The VA bridge should be used in such a way as to allow regular unclamping to be omitted.
Introduction

The first newborn was successfully treated with veno-arterial extracorporeal membrane oxygenation (VA-ECMO) by Bartlett and colleagues in 1976 (1). Since then, ECMO has become a rescue therapy for more than 22,000 neonates with severe respiratory failure, of which about 15,000 patients were treated with VA-ECMO, with an overall survival rate of 80% (2). Although ECMO has increased survival rates, the occurrence of hemorrhagic and ischemic cerebral lesions, resulting in future neurological and neurodevelopmental dysfunction are major complications (3,4). Imaging studies revealed hemorrhagic or ischemic intracranial abnormalities in 10% up to as high as 52% of the patients (5,6,7).

The occurrence of complications might be determined by pre-ECMO, as well as ECMO-related events or conditions (3,8). Pre-ECMO conditions like hypoxia, asphyxia, acidosis, hypercapnia, and cardiovascular instability, including cardio-pulmonary resuscitation are considered to increase the risk for cerebral injury by direct damage to the brain and potential disturbance of cerebral autoregulation. Once on ECMO, heparinization, microthrombi and disturbances of cerebral blood flow (patterns) and autoregulation are supposed to be other risk factors. Understanding of physiologic processes in the patient as a response to the ECMO-treatment is crucial to avoid potential harmful events. Part of the va-ECMO system is “a bridge” between the arterial and venous cannula (VA bridge). In the event of emergencies the arterial and venous cannula can be clamped and the va-bridge opened to allow continuation of circulation through the ECMO system, while the patient is taken off from ECMO. Under normal conditions the VA bridge is clamped. To prevent clotting in the VA bridge because of blood stasis, it has been recommended to release the clamp on the VA bridge every 15 to 60 minutes during the course of ECMO (9-11). There is, however, a limited number of studies concerning the impact of this procedure. Inqyinn et al. found a decrease in blood pressure and renal blood flow during opening of the VA bridge (12). Our group previously reported on the disturbance of cerebral oxygenation and hemodynamics following opening of the VA bridge during the course of VA-ECMO treatment. In piglets and newborn infants, Liem et al, using near infrared spectrophotometry (NIRS) found that opening of the VA bridge resulted in a decrease in cerebral blood volume and cerebral oxygen supply. After this initial decrease there is a compensatory increase in cerebral oxygen extraction and vasodilatation (13). In a consecutive study in lambs, van Heijst et al described that opening of the VA bridge during VA-ECMO resulted in a massive shunt of blood from the arterial side of the circuit to the venous side, causing significant changes in blood pressure, cerebral oxygenation and hemodynamics in lambs(14). These changes were found to be present at different ECMO flow rates and opening times of the VA bridge. As there is an ongoing discussion about how to use the VA bridge, additive studies, other than the NIRS studies discussed above may further demonstrate the potential harm of intermittent opening of the VA bridge.

The aim of this prospective study was to describe the effect of opening and closure of the VA bridge on cerebral blood flow velocity (CBFV) in a terminal artery to the brain, as measured by Doppler-ultrasound, in human newborn infants. In addition, the relationship between the changes in CBFV and the duration of ECMO treatment and ECMO flow rate were also studied.
Methods
In a prospective study over a one-year period, 22 consecutive patients treated with VA-ECMO in our university medical center were enrolled to study the effects of opening and closure of the VA bridge of the VA-ECMO system on CBFV. Approval was obtained from the Institutional Review Board and Ethics Committee. CBFV in the pericallosal artery, a terminal branch of the anterior cerebral artery, was measured by Doppler-ultrasound (HDI-3000, ATL ultrasound, Inc., Bothwell, WA, USA) through the anterior fontanel (15,16). Measurements were performed just before opening (unclamping), during opening and after closure (clamping) of the VA bridge, which was opened for 2 to 3 seconds to achieve a clear visible flow through the VA bridge in accordance with our standard clinical protocol, which was used throughout the study period. Measurements were performed in duplo in each patient, with a 15-minute interval and repeated on day 1, 2, 3, and 5 by the same investigator (AFJvH) during the consecutive days of the ECMO treatment and thus at different ECMO flow rates. Additionally measurements were also performed at a low ECMO flow defined as 50 to 150 ml/min, when this was not already achieved at day 5. The ECMO circuit itself consisted of a custom packed ¼ inch flexible polyvinylchloride tubing (Baxter, Uden, The Netherlands), with a silicone reservoir, the bladderbox (Seabrook Medical System, Værlose, Denmark), a 0.6 m² membrane oxygenator (Scimed Life Systems, Minneapolis, MN, USA), a heat exchanger (Cincinnati Sub Zero, Cincinnati, OH, USA) and a rollerpump (Polystan A/S, Denmark). To describe possible changes in CBFV we studied the peak systolic flow velocity (PSV), end diastolic flow velocity (EDV) and time averaged mean flow velocity (TAM), all in centimeters per second (cm/s). All parameters were determined as the average value measured over three consecutive heart cycles. For analysis purposes, we used the averaged value of the duplo measurements. Changes in CBFV during opening and after closure of the bridge were expressed as mean percentage of change ± standard deviation (SD) of the CBFV as compared to the value before opening. Statistical analysis was performed using paired t-tests (SPSS 12.0). A p-value < 0.05 was considered statistically significant. We defined changes in CBFV of more than 25% to be relevant. By repeating the measurements during the consecutive days of treatment, we looked at the relationship between the magnitude of changes in CBFV and the duration of ECMO treatment in days. Finally, we studied the relationship between the magnitude of changes in CBFV during opening of the bridge and the ECMO flow rate. For this purpose data of all days of ECMO and at low ECMO flow rate were combined. Changes in CBFV were analyzed in their relationship to the ECMO flow rate expressed in ml/min/kg (Pearson correlation, SPSS 12.0). Correlations were considered statistically significant at a p-value< 0.01 (2-tailed).

Results
Of the 22 newborns included in our study, 10 patients suffered from meconium aspiration syndrome. Other diagnoses were: 5 cases of congenital diaphragmatic hernia; 1 of sepsis; 1 of respiratory distress syndrome; 1 of total abnormal pulmonary venous return; and finally 4 patients had an idiopathic pulmonary hypertension. Mean birthweight (±SD) was 3474 (± 443) grams, mean gestational age (±SD) was 276 (± 19) days and mean ECMO runtime (±SD) was 152 (± 44) hours.
On day one of the ECMO treatment we found a significant decrease in PSV (35 ± 18%), EDV (93 ± 15%) and TAM (68 ± 13%) after opening of the VA bridge (Fig. 1). All changes were statistically significant (p<0.001). In 9 patients no end diastolic flow was detected while the VA bridge was opened (EDV=0). In 2 patients even a backward end diastolic flow (negative EDV) was found with a 112 and 120 percent decrease compared to the pre-opening EDV value. The effects on PSV, EDV and TAM during the consecutive days and at low flows while unclamping and clamping the va-bridge are shown in Table 1. Changes in PSV, EDV and TAM found at day 2 and 3 of the ECMO-treatment were equal to the results at day 1. Again there was no end diastolic flow over the pericallosal artery in 6 patients on both days and a backward flow with negative EDV was measured in 1 patient on both days.

Opening of the VA bridge on day 5 of the ECMO treatment still resulted in a statistically significant decrease in PSV (23 ± 23%), EDV (85 ± 23%) and TAM (61 ± 15%), which was also relevant for EDV and TAM. This effect was lower compared to the first three days. If, however, only patients are analyzed who had an ECMO flow rate of 250 ml/min or more on day 5, changes in PSV, EDV and TAM were equal to the results on day 1 through 3. Fluctuations in PSV (15 ± 7%), EDV (76 ± 21%) and TAM (40 ± 12%) measured at low ECMO flow of 50 to 150 ml/min were less pronounced than during the first 5 days of treatment. All changes, however, remained statistically significant (p<0.001) and were relevant for EDV and TAM. There were two missing measurements for values after closure of the VA bridge on day 1 and 2. Four patients could not be analyzed on day 5 as three of them were already decannulated after successful ECMO treatment and one died at day 4 of ECMO. Additional measurements at low ECMO flow were performed in 14 patients, while this was not possible in 4 patients. In some patients the measurement at low ECMO flow rate was already achieved at day 5. By combining all the data from day 1, 2, 3 and 5 and the data of additional measurements at low ECMO flow we studied the relationship between ECMO flow rate (ml/min/kg) and the magnitude of changes in CBFV. A statistically significant linear correlation between the ECMO flow rate and the magnitude of changes in CBFV was found for
Table 1. Changes in percentages in peak systolic flow velocity (ΔPSV), end diastolic flow velocity (ΔEDV) and time averaged mean flow velocity (ΔTAM).

<table>
<thead>
<tr>
<th></th>
<th>Changes in PSV, EDV, and TAM after opening of the VA-bridge compared to pre-opening values.</th>
<th>Changes in PSV, EDV, and TAM after closure of the VA-bridge compared to pre-opening values.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ΔPSV (%)</td>
<td>ΔPSV (%)</td>
</tr>
<tr>
<td></td>
<td>Mean  SD</td>
<td>Mean  SD</td>
</tr>
<tr>
<td>Day 1</td>
<td>-35  18</td>
<td>-93  15</td>
</tr>
<tr>
<td></td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Day 2</td>
<td>-34  16</td>
<td>-93  12</td>
</tr>
<tr>
<td></td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Day 3</td>
<td>-38  14</td>
<td>-91  13</td>
</tr>
<tr>
<td></td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Day 5</td>
<td>-23  23</td>
<td>-85  23</td>
</tr>
<tr>
<td></td>
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<td>&lt;0.001</td>
</tr>
<tr>
<td>LowFlow</td>
<td>-15  7</td>
<td>-76  21</td>
</tr>
<tr>
<td></td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

PSV (Pearson correlation 0.43, p<0.001), EDV (Pearson correlation 0.31, p= 0.009) and TAM (Pearson correlation 0.55, p<0.001). After dichotomization it became clear that for ECMO flow rates >100 ml/min/kg the Pearson correlations were not statistically significant in PSV (Pearson correlation 0.07, p=0.60) and TAM (Pearson correlation −0.13, p=0.37). For ECMO flow rates <100 ml/min/kg the Pearson correlation was higher than for all combined data and statistically significant for PSV (Pearson correlation 0.59, p=0.004) and TAM (Pearson correlation 0.59, p=0.009). Although the Pearson correlation for EDV for ECMO flow rates <100 ml/min/kg was higher than for ECMO flow rates >100 ml/min/kg (0.25 vs. −0.14) neither was statistically significant (p=0.31 and 0.34 respectively). Figure 2 shows the decrease in TAM related to the ECMO flow rate.

After closure of the VA bridge there is an immediate recovery of PSV, EDV and TAM. A few seconds after closure, at the end of our measurement, however, we still found some decrease in all three
parameters compared to pre-opening values. Especially changes in EDV still showed a statistically significant decrease compared to pre-opening values (Tab. 1.).

**Figure 2.**

![Decrease in TAM related to ECMO flow rate](image)

*Decrease in Time Averaged Mean flow velocity (TAM) in relation to the flow rate during veno-arterial extracorporeal membrane oxygenation (ECMO). The correlation curves are given for ECMO flow rates <100 ml/min/kg (Pearson correlation 0.59, p=0.009) and for ECMO flow rates >100 ml/min/kg (Pearson correlation –0.13, p=0.37).*

**Discussion**

Cerebral Doppler-ultrasound through the anterior fontanel as an acoustic window has been used in different clinical settings and treatments to investigate fluctuations in CBFV (17,18). Our study is the first to use this method in human newborns on consecutive days of VA-ECMO to describe the effects of opening and closure of the VA bridge, which is considered to be controversial, as mentioned above. The pericallosal artery is a terminal branch of the anterior cerebral artery and frequently used in (ECMO) literature to describe changes in cerebral blood flow patterns or velocity to a part of the brain (19,20). Changes in cerebral blood flow (CBF) in this vessel cannot be compensated by other arteries.

We found a very acute and relevant decrease in PSV, EDV and TAM after opening of the va-bridge,
which was statistically significant. After closing the VA bridge again, there was an immediate recovery of CBFV, although at the end of our measurements CBFV parameters were not completely normalized to pre-opening values.

In general, changes in CBFV are considered to be a contributing risk factor for cerebral hemorrhage and ischemia. Regarding a mean ECMO runtime of 152 hours in our study population, in which the bridge is opened four times per hour, acute changes in CBFV occurred more than 600 times during a complete ECMO treatment. The earlier study of our group by van Heijst et al (14) describes the underlying flow changes in the ECMO circuit and changes in hemodynamics and cerebral oxygenation in lambs. When unclamping the va-bridge, blood is shunting from the arterial cannula towards the venous cannula over the bridge. This results in reversed flows on either side of the VA bridge with a consecutive decrease in mean arterial blood pressure and a rise in central venous pressure. As the diameter of the cerebral arteries cannot be determined by Doppler-ultrasound we only measured the velocity of the CBF. However, we assume that the decrease in cerebral oxygenation during the opening of the VA bridge, earlier demonstrated in the NIRS studies of Liem and van Heijst, is caused by a decrease in cerebral blood flow. This results in a significant and acute decrease in blood supply to the brain as measured in arterial branches like the pericallosal artery, which is potentially harmful to brain tissue. This might be true for the fast recovery of CBFV after closure of the VA bridge as well. Measuring CBFV by Doppler-ultrasound and cerebral oxygenation by NIRS simultaneously might have been of contributing value. Due to the fixed position of the head during ECMO and limited space on the head it is difficult to combine both techniques. Next to this the NIRS signal is influenced by movements of the head possibly induced by performing Doppler ultrasound. Finally our group demonstrated changes in cerebral hemodynamics and oxygenation during the performance of cranial ultrasound itself, at least in preterm infants (21, submitted). This makes the interpretation of data difficult.

The study of Inqyinn et al (12) referred to previously showed that cerebral perfusion disturbances in lambs are substantially less severe in veno-venous ECMO (VV-ECMO), but because the design and use of the bridge was already adapted when we started VV ECMO in our center this was something we could not analyze.

In normal, healthy subjects, cerebral autoregulation (AR) maintains CBF over a wide range of cerebral perfusion pressures (22). However, the changes in CBFV demonstrated in this study are too acute and too huge to make AR possible. The autoregulatory contraregulation response after sudden blood pressure changes occurs with a delay of 2 seconds (23-25) and in this study CBF decreases beyond the normal autoregulatory range. Beside this it has been shown, that AR is disturbed in severely ill term infants (26) and also in newborn lambs placed on VA ECMO after prolonged hypoxia (27-29).

This is the first study describing a correlation between the ECMO flow rate and the magnitude of changes in CBFV during opening of the va-bridge. The statistically significant overall correlation between the changes in CBFV and the ECMO flow rate found in our study is mainly caused by flow dependent fluctuations in CBFV at lower ECMO flows, up to 100 ml/min/kg. At lower ECMO
flow rate the arterio-venous shunt across the va-bridge might be less severe. In addition, we suppose that at lower ECMO flow the contribution of the patient's own cardiac output to organ perfusion is relatively higher thereby preventing the dramatic changes that occurred at higher flow rates.

To avoid the repetitive acute changes in CBFV, we have made a construction by which regular unclamping of the VA bridge can be omitted. We use stopcocks on both sides, while the bridge is filled with normal saline. Flushing of the bridge can be avoided, while the benefit in emergency circumstances is retained. Based on our studies we seriously recommend this technique, which is becoming more common, but is not generally used, in ECMO community (11).

We conclude that although a direct relationship between opening and closure of the VA bridge and cerebral hemorrhage or ischemia has never been proven, it seems to be reasonable to use the VA bridge in such a way to be able to omit regular unclamping and prevent significant changes in CBFV, a potential threat to the brain.
References

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Chapter 4

Volume administration and bladderbox alarms and their relation to cerebral oxygenation and hemodynamic

4.1 Intravascular Volume Administration: A Contributing Risk Factor for Intracranial Hemorrhage During Extracorporeal Membrane Oxygenation?

4.2 The effect of bladderbox alarms during veno-arterial extracorporeal membrane oxygenation on cerebral oxygenation and hemodynamics in lambs.

4.3 The effect of intravascular volume administration on cerebral oxygenation and hemodynamics during veno-arterial extracorporeal membrane oxygenation in human newborns.
Chapter 4.1

Intravascular volume administration; A contributing risk factor for intracranial hemorrhage during extracorporeal membrane oxygenation?

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Abstract

Objective. The objective of this study was to determine the relationship between the frequency and total volume of intravascular volume administration and the development of intracranial hemorrhage during veno-arterial extracorporeal membrane oxygenation.

Patients and Methods. In a retrospective matched case-control study 24 newborns who developed an intracranial hemorrhage during veno-arterial extracorporeal membrane oxygenation treatment were compared with 40 control subjects. Both groups were analyzed for gestational age, gender, race, Apgar scores at 1 and 5 minutes, birth weight, cardiopulmonary resuscitation before veno-arterial extracorporeal membrane oxygenation, age at the start of treatment, duration of treatment, worst arterial blood gas sample preceding treatment, activated clotting time values, need of platelet transfusions, mean blood pressure and the use of inotropics and steroids before the treatment. For both groups, total number and volume of intravascular infusions of normal saline, pasteurized plasma protein solution, erythrocytes and platelets during the first 24 hours of treatment were determined. Variables were analyzed in their relationship to intracranial hemorrhage using univariate and multivariate conditional logistic regression.

Results. The only statistically significant difference in patient characteristics between the case patients and control subjects was arterial blood gas values. Newborns who developed intracranial hemorrhage during the treatment received both a statistically significantly higher number and a statistically higher total volume of intravascular volume administrations compared with control patients. After adjustment for pH, $\text{paCO}_2$, and $\text{paO}_2$, in the multivariate analysis, we found a significant relation between the development of intracranial hemorrhage and $>8$ infusions or $>300$ mL of volume infusion in the first 8 hours and $>10$ infusions in the first 24 hours of treatment.

Conclusion. The number and total volume of intravascular volume administration in the first 8 and 24 hours of veno-arterial extracorporeal membrane treatment are statistically significantly related to the development of intracranial hemorrhage.
Introduction

Since Bartlett and colleagues in 1976 treated the first newborn successfully with veno-arterial extracorporeal membrane oxygenation (va-ECMO), ECMO has become a rescue therapy for neonates with severe, but potentially reversible, respiratory failure when maximal conventional therapy has failed. Without ECMO, the mortality rate is high. More than 22,000 neonates have been treated with ECMO, 15,000 of whom were treated with va-ECMO, with an overall survival rate of 80%. Although ECMO has increased survival, the occurrence of hemorrhagic and ischemic cerebral lesions, resulting in future neurological and neurodevelopmental dysfunction are major complications. Imaging studies revealed intracranial hemorrhage (ICH) or ischemic abnormalities in 10 to even 52% of the patients. The cause of ICH occurring during va-ECMO treatment is probably multifactorial and might be determined by pre-ECMO- as well as ECMO-related events or conditions. Optimizing neurological outcome remains one of the priorities in ECMO research. The population at greatest risk for ICH is however not clearly defined. Hardart et al determined gestational age, postconceptional age, acidosis, sepsis, coagulopathy and treatment with epinephrine as independent factors associated with ICH in neonates who were treated with ECMO. A study of Dela Cruz et al demonstrated that an elevated activated clotting time (ACT) and low platelet counts requiring transfusion were statistically associated with an increase in the development of ICH. Hirthler et al reviewed that instability of ACT and platelet count are early predictors of ICH. Finally lactate before ECMO treatment was found to be a useful marker for the development of ICH during ECMO.

Especially during the first days of ECMO intravascular volume administration is regularly required. One reason is the occurrence of bladderbox alarms, as a sign of inadequate venous drainage from the right atrium to maintain the required flow of the ECMO system which is aimed for to realize adequate oxygenation of the patient. In a reaction, volume administration is often given when other causes of bladderbox alarms are ruled out. Next to this anemia or thrombocytopenia are reasons to add volume with blood products. From our clinical experience we had the impression that neonates, who required frequent volume administration during the first days of the va-ECMO treatment developed ICH more frequently. The objective of this study was to investigate whether there is a relationship between intravascular volume administration and the occurrence of ICH in the neonate, during treatment with va-ECMO.

Methods

From September 1989 through November 2005, 236 newborns (64% boys, 36% girls) were treated with va-ECMO at the Radboud University Nijmegen Medical Centre. Overall survival rate was 77% and ICH occurred in 25 patients (11%). All patients with ICH were analyzed to participate in a matched case-control study. One patient was excluded because of congenital abnormality of the heart, so 24 patients with ICH were included in the study. ICH was diagnosed either by cerebral ultrasound, performed daily during the ECMO treatment (n=18), by computer tomography...
Chapter 4

(CT) or magnetic resonance imaging (MRI) in the period directly after decannulation (n=6). The 24 newborns that developed an ICH related to va-ECMO treatment (case patients) were matched with 40 patients who did not develop ICH (control subjects). Each pair of 1 case patient and 1 or 2 control subjects was matched for diagnosis, gestational age (± 1 week) and birth weight (±500 g). For statistical reasons we aimed for 2 controls for each case patient. If >2 possible candidates were available, the patients whose dates of birth were closest to that of the case patient were selected as control subjects. Newborns were excluded from the study if there were congenital abnormalities other than congenital diaphragmatic hernia; a second ECMO treatment; ICH before the initiation of ECMO; or coagulation disorders as screened for by the determination of protrombine time, activated partial thromboplastine time and platelet count. Cerebral ultrasound, none of them showing ICH, was performed for all patients, case patients as well as control subjects, before the ECMO treatment. Before the initiation of ECMO informed consent from the parents was obtained and all case patients and control subjects met institutional criteria for ECMO treatment, which did not change during the study period. All patients were treated with va-ECMO. The ECMO circuit itself consisted of a custom packed ¼-in flexible polyvinylchloride tubing (Baxter, Uden, The Netherlands), with a silicone reservoir, the bladderbox (Seabrook Medical System, Værløse, Denmark), a 0.6 m² membrane oxygenator (Scimed Life Systems, Minneapolis, MN, USA), a heat exchanger (Cincinnati Sub Zero, Cincinnati, OH, USA) and a rollerpump (Polystan A/S, Copenhagen, Denmark).

A loading dose of heparin was given before cannulation to achieve an activated clotting time (ACT) of >300 seconds. After this heparinization was checked every hour by measurement of the ACT aiming for values between 200 and 220 seconds (Hemochron). During the course of ECMO platelet transfusions were given to maintain a platelet count > 80,000/mm³. Erythrocyte transfusions were given to maintain a hemoglobin level > 8.0 mmol/l and a hematocrit value > 0.40 l/l. Hemoglobin level and platelet count were determined every 4 hours. Normal saline (NaCl 0.9%) and pasteurized plasma protein solution (albumin 3.8%) were used to restore the ECMO flow after bladderbox alarms caused by insufficient blood drainage from the right atrium.

Cerebral ultrasound imaging through the anterior fontanel (Philips HDI-3000, Ultrasound, Inc Bothell, WA, USA) was performed daily to check for ICH. Images were interpreted by the neonatologist and the pediatric neurologist. After decannulation, CT or MRI was performed on all patients and analyzed by the neuroradiologists.

To check the matching procedure and possible differences between the case patients and the control subjects, both groups were analyzed for gestational age, gender, race, Apgar scores at 1 and 5 minutes, birth weight, cardiopulmonary resuscitation (CPR) before ECMO, age at the start of ECMO, duration of ECMO treatment, survival, worst arterial blood gas sample in the six hours preceding ECMO, number and volume of required thrombocyte transfusions, ACT value after the loading dose at the start of ECMO, mean of ACT and mean blood pressure values as well as the coefficient of variation for ACT and mean blood pressure during the first 8 and 24 hours of ECMO and the use of inotropics (dopamine, dobutamine, epinephrine, nor-epinephrine) and steroids before the start of ECMO.
To investigate the relationship between intravascular volume administration and the occurrence of ICH we analyzed the number and volume of intravascular infusions of normal saline, pasteurized plasma protein solution, erythrocytes and platelets during the first 8 and 24 hours of ECMO treatment. Data were collected from the international Extracorporeal Life Support Organization (ELSO) forms, patient files and patient medical checklists in which physiological parameters, laboratory results and ACT values were written down every hour and all volume administrations are registered.

Statistical analysis was performed by using SPSS 13.0 (SPSS Inc., Chicago, USA). Patient characteristics were analyzed by Student’s $t$ tests for comparison of means ($P < .05$) and $\chi^2$ tests ($P < .05$) for dichotomized characteristics. Univariate and multivariate testing of variables in their relation to ICH was performed by use of conditional logistic regression. For several variables, the cutoff points for which dichotomization of the variable leads to a statistically significant odds ratio (OR) at univariate analysis were determined by stepwise changing the segregation value for dichotomization. Steps were 0.1 for pH, 5 mmHg for $\text{paCO}_2$ and $\text{paO}_2$, 100 mL for the volume of infusions and 1 for the number of infusions. Variables that were found to have a significant relationship with the development of ICH in the univariate analysis were further used in the multivariate conditional logistic regression. ORs and 95% confidence intervals (CIs) were determined. Because CPR, gestational age and age at the start of ECMO are generally considered to be important in their relation to ICH, we performed an univariate as well as a multivariate analysis for these terms. In the latter, the outcome for volume administration was corrected for each separate parameter, CPR, gestational age and age at the start of ECMO.

Results
All 24 newborns that developed an ICH during va-ECMO treatment could be matched with one or two control patients with the same primary diagnosis. Primary diagnoses for the case patients in this study were as follows: congenital diaphragmatic hernia in 8 (33%), sepsis in 8 (33%), meconium aspiration syndrome in 5 (21%) and primary pulmonary hypertension in 3 (13%). The other matching parameters used (gestational age and birth weight) were not significantly different between the case patients and control subjects (Table 1). Maximum time span between a case patient and its control subject was 8 months. In this study we found the following types of ICH: parenchymal 10 (42%), intraventricular 9 (38%), subependymal 3 (13%) petechial 1 (4%) and 1 ICH in the posterior fossa (4%). Of these 24 cases of ICH, 18 were diagnosed by cerebral ultrasound during va-ECMO and 6 were diagnosed by either CT or MRI after decannulation. Of the 18 cases of ICH on cerebral ultrasound 8 (44%) were demonstrated within 24 hours after the start of va-ECMO and 14 (78%) within 72 hours. Also for other than the matching variables we found no statistically significant differences in patient characteristics, except for pH and survival (Table 1). Next to these characteristics we found no significant differences in ACT values, platelet requirement, blood pressures and the use of inotropics and steroids nor in race of the patients.
Table 1. Patient Characteristics of Newborns With ICH (Case Patients) and Newborns Without ICH (Control subjects) During ECMO Treatment

<table>
<thead>
<tr>
<th></th>
<th>ICH – cases (n=24)</th>
<th>Controls (n=40)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Mean</strong></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>birthweight (kg)</td>
<td>3.3 ± 0.5</td>
<td>3.3 ± 0.5</td>
<td>.79</td>
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<tr>
<td>gestational age (wks)</td>
<td>38 ± 3</td>
<td>39 ± 2</td>
<td>.64</td>
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<tr>
<td>age at start of ECMO (hours)</td>
<td>35 ± 30</td>
<td>53 ± 64</td>
<td>.20</td>
</tr>
<tr>
<td>duration of ECMO (hours)</td>
<td>155 ± 76</td>
<td>171 ± 81</td>
<td>.43</td>
</tr>
<tr>
<td>median Apgar at 1 minute</td>
<td>6.0 ± 2.6</td>
<td>6.1 ± 2.5</td>
<td>.94</td>
</tr>
<tr>
<td>median Apgar at 5 minutes</td>
<td>7.0 ± 2.0</td>
<td>7.1 ± 2.1</td>
<td>.82</td>
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<tr>
<td>pH</td>
<td>7.23 ± 0.15</td>
<td>7.33 ± 0.15</td>
<td>.01</td>
</tr>
<tr>
<td>paCO₂ (mmHg)</td>
<td>45.8 ± 13.9</td>
<td>42.7 ± 16.7</td>
<td>.46</td>
</tr>
<tr>
<td>paO₂ (mmHg)</td>
<td>48.1 ± 29.6</td>
<td>53.7 ± 19.1</td>
<td>.36</td>
</tr>
<tr>
<td>gender (male/female)</td>
<td>17/7</td>
<td>28/12</td>
<td>.93</td>
</tr>
<tr>
<td>CPR before ECMO</td>
<td>3/24</td>
<td>3/40</td>
<td>.51</td>
</tr>
<tr>
<td>Survival</td>
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<td>32/40</td>
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<tr>
<td>Number platelet T24</td>
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<td>1.6 ± 1.2</td>
<td>.64</td>
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<tr>
<td>Volume platelet T24 (ml)</td>
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<td>67 ± 48</td>
<td>.18</td>
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<tr>
<td>loading ACT</td>
<td>399 ± 136</td>
<td>397 ± 161</td>
<td>.96</td>
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<tr>
<td>ACT first 8 hours</td>
<td>276 ± 35</td>
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<td>.42</td>
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<td>ACT first 24 hours</td>
<td>258 ± 34</td>
<td>246 ± 34</td>
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<td>CoV ACT first 8 hours</td>
<td>0.17 ± 0.09</td>
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<td>.41</td>
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<td>CoV ACT first 24 hours</td>
<td>0.15 ± 0.07</td>
<td>0.15 ± 0.10</td>
<td>.75</td>
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<tr>
<td>mean MAP first 8 hours</td>
<td>53 ± 10</td>
<td>56 ± 7</td>
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<tr>
<td>Mean MAP first 24 hours</td>
<td>52 ± 8</td>
<td>53 ± 7</td>
<td>.63</td>
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<td>CoV MAP first 8 hours</td>
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<td>0.14 ± 0.06</td>
<td>.60</td>
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<td>CoV MAP first 24 hours</td>
<td>0.13 ± 0.07</td>
<td>0.15 ± 0.06</td>
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<tr>
<td>Use of steroids</td>
<td>3/24</td>
<td>5/40</td>
<td>.82</td>
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<tr>
<td>Use of inotropic</td>
<td>21/24</td>
<td>8/40</td>
<td>.31</td>
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</tbody>
</table>

Number and volume platelet T24 = number and total volumes (mL) of platelet transfusions to keep platelet counts at > 80,000/mm³ in first 24 hours of ECMO. Inotropics used were dopamine, dobutamine, epinephrine and norepinephrine. CV indicates coefficient of variation; MAP, mean arterial blood pressure

At dichotomization, we found the following statistically significant values in the univariate conditional logistic regression for the worst arterial blood gas samples in the 6 hours preceding the ECMO treatment in relation to the development of ICH: pH ≤ 7.3, paCO₂ ≥ 45 mm Hg and paO₂ ≤ 53 mm Hg.
50 mm Hg (Table 2). These parameters were further used in the multivariate conditional logistic regression.

Table 2. ORs and CIs for the Relation Between pH, paCO₂, paO₂ and the Number and Total Volume of Intravascular Infusion in the First 8 and 24 Hours of ECMO Treatment and the Development of ICH.

<table>
<thead>
<tr>
<th></th>
<th>Univariate analysis</th>
<th>Multivariate analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>OR (95% CI)</td>
<td>OR (95% CI)</td>
</tr>
<tr>
<td>pH ≤ 7.3 worst arterial gas</td>
<td>4.5 (1.4-14.0)</td>
<td></td>
</tr>
<tr>
<td>paCO₂ ≥ 45 mmHg worst arterial gas</td>
<td>3.3 (1.0-10.9)</td>
<td></td>
</tr>
<tr>
<td>paO₂ ≤ 50 mmHg worst arterial gas</td>
<td>5.3 (1.5-19.0)</td>
<td></td>
</tr>
<tr>
<td>Every 100 ml of volume first 8 hours</td>
<td>1.5 (1.1-2.2)</td>
<td>1.6 (1.0-2.6)</td>
</tr>
<tr>
<td>≥300 ml first 8 hours</td>
<td>6.3 (1.4-29.1)</td>
<td>10.0 (1.9-83.5)</td>
</tr>
<tr>
<td>Every single infusion first 8 hours</td>
<td>1.2 (1.0-1.5)</td>
<td>1.3 (1.0-1.6)</td>
</tr>
<tr>
<td>≥8 infusions first 8 hours</td>
<td>13.6 (1.7-106.3)</td>
<td>26.0 (1.6-430.2)</td>
</tr>
<tr>
<td>Every 100 ml of volume first 24 hours</td>
<td>1.4 (1.1-1.8)</td>
<td>1.6 (1.0-2.4)</td>
</tr>
<tr>
<td>≥400 ml first 24 hours</td>
<td>5.7 (1.3-25.6)</td>
<td>6.4 (0.5-77.1)</td>
</tr>
<tr>
<td>Every single infusion first 24 hours</td>
<td>1.1 (1.0-1.3)</td>
<td>1.2 (1.0-1.4)</td>
</tr>
<tr>
<td>≥10 infusions first 24 hours</td>
<td>3.6 (1.1-11.2)</td>
<td>8.5 (1.2-59.2)</td>
</tr>
</tbody>
</table>

Results are given for the univariate analysis and the multivariate analysis (adjustment for pH, paCO₂ and paO₂ mentioned) for the first 8 and 24 hours of the treatment with va-ECMO. NS indicates not statistically significant.

Single infusions of intravascular volume administration ranged from 20 to 50 mL. In Fig 1, it is shown that the newborns, who developed ICH during the treatment with va-ECMO had received both a statistically significantly higher number as well as a statistically significantly higher total volume of intravascular volume administrations during the first 8 and 24 hours of the treatment with va-ECMO, compared to the matched control patients. Statistically, there was a significant increase in the risk of ICH for every single extra infusion in the first 8 and 24 hours of treatment with va-ECMO. This was also true for every 100 ml of extra volume administration in the first 8 and 24 hours (Table 2). After dichotomization and adjustment for pH, paCO₂ and paO₂ in the multivariate conditional regression we found the following conditions to be significantly related to the development of ICH during the va-ECMO treatment; >8 infusions or >300 mL of intravascular infusion in the first 8 hours of va-ECMO and >10 infusions in the first 24 hours of va-ECMO. An overview of ORs and 95% CIs for the univariate and multivariate analysis is shown in Table 2.
significant risk factors for the development of ICH. ORs and CIs were 2.0 (0.4 – 9.0), 0.4 (0.1 – 1.4) and 1.0 (0.9 – 1.0) respectively. When these terms were used in a straight multivariate conditional regression, data found for volume administration, as shown in Table 2, were still statistically significant.

Figure 1.
Total number and volume of intravascular volume suppletions in the first 8 and 24 Hours between patients with and without ICH during the treatment with ECMO

ICH = intracranial hemorrhage, ECMO = veno-arterial extracorporeal membrane oxygenation.
# p=0.03; † p=0.005; ‡ p=0.04

Discussion
Because ICH is one of the most devastating complications of ECMO, it is of ongoing importance to further identify those patients most at risk and eliminate risk factors as much as possible. This matched case-control study is the first to describe a statistically significant relationship between the total number and volume of intravascular volume administrations and the development of ICH during the treatment with va-ECMO. We found that neonates, receiving >8 infusions or >300 ml of volume infusion during the first 8 hours or >10 infusions during the first 24 hours of va-ECMO were statistically significant more at risk to develop an ICH. We stress, that this relationship does not have to be necessarily causal. A hypothetical explanation, however, might be found in disturbances of cerebral hemodynamics caused by the infusions of intravascular volume administration and/or the often preceding bladderbox alarms with consecutive acute interruption of the rollerpump resulting in interruption of blood flow to the patient. In normal, healthy situations cerebral autoregulation maintains the cerebral blood flow over a wide range of cerebral perfusions pressures with a contraregulation response occurring with a delay of 2 seconds after sudden changes in blood pressure. It has been shown, however, that autoregulation is disturbed in severely ill term infants. Additionally studies of newborn lambs showed that prolonged hypoxia and/or the ECMO treatment itself will significantly disturb the
autoregulation\textsuperscript{20-22}; therefore, frequent interruption of the roller pump, followed by infusion of intravascular volume administration may result in frequent fluctuations in cerebral blood flow, a known risk factor for ICH. Besides this, possible disturbances in cerebral blood flow at bladderbox alarms might be too acute to be compensated for by the autoregulation system, even if not disturbed. The combination of most bladderbox alarms and intravascular volume infusion in the first 24 hours and disturbed autoregulation may be a part of the explanation of why most ICHs occur in the first few days of the ECMO treatment; however, another explanation of the relationship found in this study might be that the pathological process which leads to ICH also causes the inadequate venous drainage to the ECMO circuit, which in turn leads to the need for volume administration. Birth weight and gestational age were not significantly different between the ICH group and the group without ICH, which suggests that the matching procedure was successfully performed. The significant relationship between pH, $\text{paCO}_2$ and $\text{paO}_2$ and ICH found in this study confirms the findings of previous studies.\textsuperscript{12,13,15} Confirmation of acidosis using lactate as another variable was not possible, as this was not always routinely determined.

In contradiction to the study of Dela Cruz et al,\textsuperscript{13} ACT levels, analyzed for the first 8 and 24 hours between case patients and control subjects, were not significantly different. Instability of ACT during the ECMO treatment was described by Hirthler et al,\textsuperscript{14} but not further confirmed in this study, neither by the mean ACT nor by the coefficient of variation of the ACT. Because the exact time of ICH occurrence is not known, instability of ACT could be a risk factor as well as a consequence of the ICH. This could also be the question for possible differences in platelet counts. In all our patients, case patients and control subjects, platelet counts were kept strictly above 80,000/mm$^3$. Platelet requirement was slightly higher in the group of patients developing an ICH, which seems to fit with the previous studies described. In our study this was however not statistically significant. We did not find a statistically significant difference in the use of epinephrine as was found in the study of Hardart et al,\textsuperscript{12} or in the use of other inotropic medication or steroids before the ECMO treatment used to treat hypotension.

Although any confounding factor is not completely excluded, we think that this matched case-control study shows an important relationship between intravascular volume administration and the development of ICH. The results of this study can be translated to clinical practice. Any newborn treated with va-ECMO and requires high amounts or number of intravascular volume administration should alert the ECMO team. Because of the increased chance of ICH more frequent neurologic assessment and cerebral ultrasounds are recommended to detect any ICH in an early stage and if possible avoid extension of the hemorrhage.

Additional prospective studies, as currently initiated in our ECMO center, are required to analyze the mechanisms by which volume administration and/or the often preceding bladderbox alarms are related to the development of ICH. Knowledge of these mechanisms may be useful for the development of strategies to reduce the risk of ICH during the va-ECMO treatment, such as how to react on bladderbox alarms and whether it might be indicated to be cautious with intravascular volume administration.
Conclusion
To our knowledge, this is the first study to show a statistically significant relationship between the total amount and number of intravascular volume administration and the development of ICH during va-ECMO treatment. Additional studies are needed to investigate the pathophysiological mechanisms of this relationship.
References

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Chapter 4.2

The effect of bladderbox alarms during veno-arterial extracorporeal membrane oxygenation on cerebral oxygenation and hemodynamics in lambs

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Abstract

To determine the effects of bladderbox alarms during veno-arterial extracorporeal membrane oxygenation (va-ECMO) on cerebral oxygenation and hemodynamics, six lambs were prospectively treated with va-ECMO and bladderbox alarms were simulated. Changes in concentrations of oxyhemoglobin (ΔcO$_2$Hb), deoxyhemoglobin (ΔcHHb) and total Hb (ΔctHb) were measured using near infrared spectrophotometry. Fluctuations in hemoglobin oxygenation index (ΔHbD) and cerebral blood volume (ΔCBV) were calculated. Heart rate (HR), mean arterial pressure (MAP), blood flow in the left carotid artery (Qcar) and central venous pressure (CVP) were registered. Bladderbox alarms were simulated by increasing the ECMO flow or partially clamping the venous cannula and resolved by decreasing the ECMO flow, unclamping the cannula or intravascular volume administration. CBV, HbD, MAP and Qcar decreased significantly during bladderbox alarms, whereas HR and CVP increased. After the bladderbox alarms, CBV and HbD increased significantly to values above baseline. For HbD this increase was higher during intravascular volume administration. MAP, Qcar and CVP recovered to preexperiment values, but increased further with volume administration. HR was increased at the end of our measurements. We conclude that bladderbox alarms during va-ECMO treatment result in significant fluctuations in cerebral oxygenation and hemodynamics, a possible risk factor for intracranial lesions.
Introduction
Extracorporeal membrane oxygenation (ECMO) has become a rescue therapy for neonates with severe, but potentially reversible, respiratory failure when maximal conventional therapy has failed. Without ECMO there is a high mortality rate (1,2). Since the first newborn was successfully treated with veno-arterial extracorporeal membrane oxygenation (va-ECMO) by Bartlett et al. in 1976 (3), over 22,000 neonates have been treated with ECMO, of which 15,000 with va-ECMO, with an overall survival rate of 80% (4). Although ECMO has increased survival, the occurrence of hemorrhagic and ischemic cerebral lesions resulting in future neurologic and neurodevelopmental dysfunction are of major concern (5,6). Imaging studies revealed hemorrhagic or ischemic intracranial abnormalities in 10 up to even 52% of the patients (7-9). These complications justify a continuous search for factors related to intracranial hemorrhage and ischemia and treatment strategies aimed to improve outcome.

Part of the va-ECMO system is a bladderbox, which is situated between the venous cannula and the rollerpump of the extracorporeal system. The bladderbox controls venous drainage of blood from the right atrium of the patient. If the venous drainage becomes inadequate to maintain the established flow of the ECMO system, the bladderbox will give an alarm, slow down the pump, and finally cause an acute interruption of the rollerpump resulting in interruption of blood flow to the patient. If other causes of inadequate venous drainage from the right atrium and consecutive bladderbox alarms, like disturbed position of the cannula, are ruled out, intravascular volume administration is often given to restore the circulation through the ECMO system and the patient. Recently, our group first showed a statistically significant relation between the total number and volume of intravascular volume administration and the occurrence of intracranial hemorrhage (ICH) during va-ECMO treatment (10). We hypothesize that this relation may be explained by fluctuations in cerebral oxygenation and hemodynamics caused by either the bladderbox alarms with a consecutive interruption of the ECMO blood flow to the patient or by intravascular volume administration itself. Both might be a contributing risk factor for intracranial hemorrhage and ischemia. The aim of this study is to describe possible fluctuations in cerebral oxygenation and hemodynamics, as measured by near infrared spectrophotometry (NIRS), caused by simulated bladderbox alarms during va-ECMO in lambs.

Methods
Study population and ECMO
In a prospective interventional setting, six healthy lambs were studied for changes in cerebral oxygenation and hemodynamics caused by bladderbox alarms during va-ECMO. The Lambs were obtained from local farmers, and the Ethical Committee on Animal Research of the Radboud University of Nijmegen approved the study. The care and handling of the animals were in accordance with the guidelines issued by the National Institutes of Health. Experimental settings were mainly based on earlier research in our group (11). General anesthesia was induced by intravenous administration of midazolam 0.2 mg.kg⁻¹, ketamine 15 mg.kg⁻¹, atropine 0.01 mg.kg⁻¹ and
Muscle relaxation was obtained with 0.02 mg.kg⁻¹ pancuronium. After endotracheal intubation, mechanical ventilation was started using a Babylog 8000 (Dräger, Lübeck, Germany) to maintain normal blood gas values (pH 7.30-7.40; \(p_{O_2}\) 10-15 kPa; \(p_{CO_2}\) 4.5-5.5 kPa). During mechanical ventilation, anesthesia was maintained by infusion of midazolam (0.2 mg.kg⁻¹.h⁻¹), ketamine (10 mg.kg⁻¹.h⁻¹), sufentanyl (20 μg.kg⁻¹.h⁻¹) and pancuronium (0.02 mg.kg⁻¹.h⁻¹). Heart rate (HR) was monitored by needle electrodes on the chest wall. Central temperature was measured with a rectal probe and maintained between 38.5 and 39.5 °C. A catheter was placed in the aorta through the right femoral artery for continuous measurement of mean arterial blood pressure (MAP). The right femoral vein was used to place a 7.5-Fr catheter into the inferior caval vein to create a venous access for medication and measurement of central venous pressure (CVP). Both MAP and CVP were represented on one monitor (HP 68S, Hewlett Packard, Boeblingen, Germany). Blood flow in the left common carotid artery (Qcar) was measured using an ultrasonic flowmeter (Transonic Systems Inc, NY, USA). Finally arterial oxygen saturation (Sao₂) and end-tidal CO₂ were continuously registered.

Before veno-arterial cannulation, the ECMO circuit was primed with full fresh sheep blood. The right common carotid artery was cannulated with an arterial catheter (8-12 Fr) and the right internal jugular vein with a venous catheter 10-12 Fr (both Biomedicus, Medtronics Bio-Medicus, Eden Prairie, MN). Thereafter, va-ECMO was initiated. During the cannulation procedure a loading dose of heparin was administered (150 IU.kg⁻¹) followed by continuous i.v. infusion of heparin (100–200 IU.kg⁻¹.h⁻¹) to maintain the activated clotting time between 200 and 250 s (Hemochron, Edison, NJ). The ECMO circuit itself consisted of a custom packed ¼-inch flexible polyvinylchloride tubing (Baxter, Uden, The Netherlands), with a silicone reservoir, the bladderbox (Seabrook Medical Systems, Inc, Cincinnati, OH), a hollow fiber membrane oxygenator with integrated heat-exchanger (Medos Hiiite 2400LT, Medos, Stolberg Germany), and a rollerpump (Jostra RPM 20-320, Lung, Sweden). An ultrasonic flow meter (Transonic Systems, Ithaca, NY) was placed on the venous site of the ECMO-system continuously measuring actual flow in the circuit. Aimed flow rate of the ECMO pump was 100-150 ml.kg⁻¹.min⁻¹.

Near infrared spectrophotometry (NIRS)

Cerebral Oxygenation and hemodynamics were studied with NIRS. The NIRS equipment (OXY-MON) used was developed by the instrumentation department and the department of physiology of the Radboud University Nijmegen Medical Centre, The Netherlands (12). The technique is based on the spectrophotometric measurement of changes in the absorption properties of Hb in the near infrared region, depending on its oxygenation state, first described by Jöbsis in 1977 (13). The NIRS procedure used in our ECMO center has been described to more extend in earlier studies of our research group (14,15). From an optode, placed frontal in the midline of the skull, three wavelengths of near infrared light (905, 850 and 767 nm) were transmitted through the head and received by two other optodes placed both on the left and right parietotemporal region of the skull, to evaluate the oxygenation and hemodynamics in each hemisphere sepa-
Concentration changes of oxyhemoglobin (ΔcO₂Hb) and deoxyhemoglobin (ΔcHHb) were calculated from changes in light absorption using the modified Lambert-Beer law. We used the Keele absorption matrix and a constant path length multiplier factor of 4.27 times the distance between both optodes (16-18). Using a value of 1.05 g.mL⁻¹ for brain specific mass, concentration changes are expressed in μmol.100.g⁻¹ (19). ΔcO₂Hb and ΔcHHb reflect changes in cerebral oxygen supply, if oxygen consumption remains constant. Differences between ΔcO₂Hb and ΔcHHb were calculated and indicated as changes in the hemoglobin oxygenation index (ΔHbD, where ΔHbD = ΔcO₂Hb - ΔcHHb in μmol.100.g⁻¹). Concentration changes in total hemoglobin (ΔctHb) were calculated as the sum of ΔcO₂Hb and ΔcHHb (ΔctHb = ΔcO₂Hb + ΔcHHb). ΔHbD represents changes in cerebral blood flow (20), whereas ΔctHb reflects changes in cerebral blood volume (ΔCBV), which are calculated from the formula: DCBV = 4 x ΔctHb/0.69 x cHb, expressed in mL.100.g⁻¹. In this formula cHb is the hemoglobin concentration in circulating blood (mmol/L), 0.69 is the cerebral-arterial hematocrit ratio and 4 is a correction factor. Also because cHb is calculated from changes in light absorption using an extinction coefficient based on the tetrahaem molecule, whereas cHb determination is based on the monohaem molecule (21).

**Experimental procedures**

After the initiation of va-ECMO, we waited for a stable NIRS signal. Once this was established bladderbox alarms were simulated. During the first experiment (experiment A) the ECMO flow rate of the rollerpump was increased stepwise with 10 mL.kg⁻¹.min⁻¹ until bladderbox alarms occurred. After 10 s, the ECMO flow rate was decreased to the preexperiment value and bladderbox alarms were thereby resolved. Experiment A was repeated three times in each lamb with a minimal time-interval of 2 min to obtain a stable NIRS signal before each experiment for at least 30 s. During the second experiment (experiment B), the venous cannula of the ECMO circuit was partially occluded by a nonocclusive clamp that was developed for this experiment. Bladderbox alarms occurred as result of insufficient venous drainage and after 10 s, the clamp was removed to restore the preexperiment ECMO flow and resolve the bladderbox alarms. Again experiment B was repeated three times in each lamb with a minimal time interval of 2 min.

During the third experiment (experiment C) bladderbox alarms were simulated similar to experiment A. However, after 10 s, bladderbox alarms were resolved by starting intravascular volume administration with 50 mL of normal saline. To avoid an overload of intravascular volume administration, experiment C was repeated two times in each lamb, with a time interval long enough to obtain at least a 30 second stable NIRS signal.

Arterial blood samples were obtained before and after each experiment to check for changes in pH, paO₂, paCO₂ and cHb values.

**Data analysis**

All physiologic parameters were registered by a data acquisition system (Poly®, Inspector Research System, Amsterdam, The Netherlands) with a sampling frequency of 1 Hz and synchro-
Chapter 4

nized with the NIRS data. Baseline values (mean ± SD) of cO_2 Hb, cHHb, ctHb and physiological parameters were determined during a 15-s period during the stable NIRS signal before (t = 0) each single experiment (A, B and C). Maximal changes in cO_2 Hb, cHHb, ctHb and physiological parameters were searched by a computer analysis during (t = 1) and after resolving (t = 2) the bladder-box alarms as mean values during a 3-s period. Because of the continuous registration, related changes in physiological data could be described during changes of the NIRS signals. Changes in HbD and CBV were calculated by using the formulas mentioned earlier. All changes were described separately for the left and right hemisphere of the brain. Therefore ΔHbD is expressed as ΔHbD_{right} and ΔHbD_{left}, while ΔCBV is expressed as ΔCBV_{right} and ΔCBV_{left}. Statistical analysis was performed for maximal changes at t = 1 and t = 2 compared with the baseline values by using Wilcoxon signed ranks tests and p values < 0.05 were considered to be statistically significant.

Results

All the three experiments A, B and C were successfully performed in six lambs, weight (mean ± SD) 5.2 ± 1.1 kg. The flow rate of the ECMO pump aimed for (100-150 m.l.kg^{-1}.min^{-1}) was reached in 5 lambs. In one lamb maximal flow rate of the ECMO pump was 90 m.l.kg^{-1}.min^{-1}. As measured by arterial blood gas samples, all lambs had blood gas values within the normal range before and after each experiment. For each experiment, chb could be determined and used for calculations of ΔCBV. An overview of mean changes in important physiological parameters, HbD and CBV is given in Table 1, while Figure 1 shows the typical pattern of cO_2 Hb, cHHb, HbD and CBV during and after bladderbox alarms in one lamb.

Baseline HbD and CBV values, calculated during a 15-s period showed a baseline variability with a mean SD of 0.03 ± 0.01 μmol.100.g^{-1} and 0.02 ± 0.01 mL.100.g^{-1} respectively. Significant changes in Sao_2 and end-tidal CO_2 were not observed.

Bladderbox alarms caused a significant and acute decrease in O_2 Hb and increase in HHb. After the acute decrease, there was a slight increase in O_2 Hb during the period of bladderbox alarms, followed by an acute increase to values above baseline values when bladderbox alarms were resolved. After the acute increase, HHb did show a further very slight increase during the period of bladderbox alarms as well, followed by a more acute decrease to values lower than baseline values after the bladderbox alarms were resolved.

Bladderbox alarms caused a significant and acute decrease in HbD, CBV, MAP and Qcar. There was a significant increase in CVP. During the 10 s course of bladderbox alarms HR increased as did HbD, CBV, MAP and Qcar; however, just before resolving the bladderbox alarms, values were still lower than pre-experiment baseline values. Patterns for all parameters were not different between those bladderbox alarms simulated by increasing the ECMO flow rate and those simulated by partially clamping of the venous cannula. We did not find any significant difference between ΔHbD_{right} and ΔHbD_{left}, or between ΔCBV_{right} and ΔCBV_{left}. The decrease in HbD, when simulating bladderbox alarms, ranged from -0.05 to -0.45 μmol.100.g^{-1}, while the decrease in CBV ranged from 0 to -0.20 mL.100.g^{-1}. 
Table 1. Changes in hemoglobin oxygenation index (HbD), cerebral blood volume (CBV), mean arterial pressure (MAP), blood flow in the left carotid artery (Qcar), heart rate (HR) and central venous pressure (CVP) during and after bladderbox alarms, compared to baseline values.

<table>
<thead>
<tr>
<th>Exp</th>
<th>HbD right (µmol.100g-1)</th>
<th>Baseline (t = 0)</th>
<th>Bladderbox alarms (t = 1) Change</th>
<th>After recovery (t = 2) Change</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>NA</td>
<td>-0.20±0.10*</td>
<td>0.11±0.12f</td>
<td></td>
</tr>
<tr>
<td></td>
<td>HbD left (µmol.100g-1)</td>
<td>NA</td>
<td>-0.20±0.10*</td>
<td>0.10±0.11f</td>
</tr>
<tr>
<td></td>
<td>CBV right (ml.100g-1)</td>
<td>NA</td>
<td>-0.11±0.05*</td>
<td>0.13±0.11*</td>
</tr>
<tr>
<td></td>
<td>CBV left (ml.100g-1)</td>
<td>NA</td>
<td>-0.11±0.05*</td>
<td>0.12±0.08*</td>
</tr>
<tr>
<td></td>
<td>MAP (mmHg)</td>
<td>65±11</td>
<td>-9.8±5.8*</td>
<td>-0.4±1.2</td>
</tr>
<tr>
<td></td>
<td>Qcar (mL/min)</td>
<td>119±63</td>
<td>-19.2±13.6*</td>
<td>0.7±3.9</td>
</tr>
<tr>
<td></td>
<td>HR (beats.min-1)</td>
<td>147±36</td>
<td>5±4*</td>
<td>7±6*</td>
</tr>
<tr>
<td></td>
<td>CVP (mmHg)</td>
<td>5.2±2.7</td>
<td>0.7±0.9*</td>
<td>-0.3±0.5</td>
</tr>
<tr>
<td>B</td>
<td>HbD right (µmol.100g-1)</td>
<td>NA</td>
<td>-0.17±0.05*</td>
<td>0.14±0.07*</td>
</tr>
<tr>
<td></td>
<td>HbD left (µmol.100g-1)</td>
<td>NA</td>
<td>-0.13±0.05*</td>
<td>0.11±0.05*</td>
</tr>
<tr>
<td></td>
<td>CBV right (ml.100g-1)</td>
<td>NA</td>
<td>-0.11±0.05*</td>
<td>0.10±0.05*</td>
</tr>
<tr>
<td></td>
<td>CBV left (ml.100g-1)</td>
<td>NA</td>
<td>-0.09±0.04*</td>
<td>0.08±0.06*</td>
</tr>
<tr>
<td></td>
<td>MAP (mmHg)</td>
<td>62±10</td>
<td>-9.3±1.7*</td>
<td>0.2±1.6</td>
</tr>
<tr>
<td></td>
<td>Qcar (mL/min)</td>
<td>102±58</td>
<td>-12.1±9.0*</td>
<td>1.7±3.7</td>
</tr>
<tr>
<td></td>
<td>HR (beats.min-1)</td>
<td>158±42</td>
<td>3±3*</td>
<td>4±3*</td>
</tr>
<tr>
<td></td>
<td>CVP (mmHg)</td>
<td>5.8±2.9</td>
<td>0.9±0.6*</td>
<td>-0.6±0.4*</td>
</tr>
<tr>
<td>C</td>
<td>HbD right (µmol.100g-1)</td>
<td>NA</td>
<td>-0.21±0.11*</td>
<td>0.33±0.17*</td>
</tr>
<tr>
<td></td>
<td>HbD left (µmol.100g-1)</td>
<td>NA</td>
<td>-0.21±0.15*</td>
<td>0.33±0.24*</td>
</tr>
<tr>
<td></td>
<td>MAP (mmHg)</td>
<td>67±15</td>
<td>-8.3±3.5*</td>
<td>13.6±5.5f</td>
</tr>
<tr>
<td></td>
<td>Qcar (mL/min)</td>
<td>102±48</td>
<td>-11.2±8.5f</td>
<td>34.5±23.2f</td>
</tr>
<tr>
<td></td>
<td>HR (beats.min-1)</td>
<td>155±35</td>
<td>3±4†</td>
<td>3±3†</td>
</tr>
<tr>
<td></td>
<td>CVP (mmHg)</td>
<td>6.5±3.8</td>
<td>0.6±0.5†</td>
<td>2.6±0.8f</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SD. In Exp A and C, bladderbox alarms are simulated by increase of the ECMO flow rate. In Exp B, bladderbox alarms are simulated by partially clamping the venous cannula.

All changes are compared to baseline values.

* p < 0.001.
† p < 0.01.
‡ p < 0.05.
NA = not available.
Figure 1. Typical pattern of changes in cerebral oxyhemoglobin ($\Delta cO_2Hb$), deoxyhemoglobin ($\Delta cHHb$), hemoglobin oxygenation index ($\Delta HbD$) and cerebral blood volume ($\Delta CBV$) during and after bladderbox alarms.
When resolving the bladderbox alarms there was a fast increase in HbD to values significantly higher than baseline values before the experiment. In experiment C, when bladderbox alarms were resolved by the administration of intravascular volume, this increase in HbD (range 0.09 to 0.84 μmol.100g⁻¹) was significantly larger than in experiments A and B [range -0.06 to 0.35 μmol.100g⁻¹ (p<0.05)]. Because normal saline is used as intravascular volume administration to resolve bladderbox alarms in experiment C, this will result in hemodilution (decrease of chb). Therefore changes in CBV cannot be reliably calculated. However, in experiment A and B, CBV increased to values significantly higher than baseline values (range 0 to 0.26 ml.100g⁻¹). MAP and Qcar recovered to baseline values in experiments A and B, but increased significantly when intravascular volume was administered (experiment C). CVP tends to decrease after bladderbox alarms are resolved, but increases during intravascular volume administration. HR was still increased at the end of each experiment. Again, we did not find any significant difference between ΔHbD_right and ΔHbD_left or between ΔCBV_right and ΔCBV_left.

Discussion
Because cerebral hemorrhage and ischemia belong to the devastating complications of ECMO treatment, it is important to identify risk factors related to the use of the ECMO technique itself and eliminate these as much as possible. After describing a significant relation between intravascular volume administration during va-ECMO treatment and ICH (10), this is the first study to describe statistically significant changes in cerebral oxygenation and hemodynamics, expressed by ΔHbD and ΔCBV, in relation to bladderbox alarms. These alarms often precede the need for intravascular volume administration.

Bladderbox alarms cause a stop of the ECMO pump, which results in an acute interruption of the arterial circulation and an acute decrease in MAP, Qcar, Hbd and CBV. While bladderbox alarms persist, several mechanisms might explain observed findings of our study. First, in an attempt to restore the circulation, the body will try to increase cardiac output with an increase in HR. Especially, as lambs in our study have healthy lungs, an increase in cardiac output will result in an increased arterial blood flow, which explains the increase in MAP, Qcar, Hbd and CBV.

When the ECMO pump is interrupted, venous drainage from the right atrium will be reduced and CVP will increase, resulting in less venous drainage from the brain. This might contribute to an increase in CBV. Finally, cerebral vasodilatation might occur as compensation for decreased CBF during bladderbox alarms. When bladderbox alarms are resolved, an acute recovery of ECMO flow will occur with a consecutive increase in MAP and Qcar, occurring while the system was just adapting to reduced CBF by vasodilatation. This might, partially, explain why there is an overshoot in Hbd and CBV to values higher than baseline values. Once intravascular volume administration is used, there is a further increase of MAP, Qcar and cerebral blood flow with a consecutive further increase in Hbd.

Several authors reported that the majority of cases of ICH occurred within 72 h after the start of ECMO (22-24). This might fit with our findings, as the majority of bladderbox alarms occur
in the first days of ECMO treatment, when a relatively high ECMO flow rate is required to maintain adequate arterial oxygen saturation in a period that the patient's lung is passing a phase of “white out” and the patient is still hemodynamically stabilizing. Next to this the acute changes in HbD and CBV, related to bladderbox alarms in the first days, occur while cerebral autoregulation (AR) in the patient seems to be disturbed. In normal, healthy infants, AR maintains the cerebral blood flow over a wide range of cerebral perfusion pressures with a contra-regulation response occurring with a delay of 2 seconds after sudden changes in blood pressure. However, it has been shown, that AR is disturbed in severely ill term infants (25-28). Additional studies in newborn lambs showed that prolonged hypoxia and/or the ECMO treatment itself significantly disturbs the AR (29-32). These studies demonstrated that AR in lambs placed on ECMO was more disturbed in animals that had hypoxia before ECMO compared with animals without preceding hypoxia. Therefore, the hemodynamic changes that occur during bladderbox alarms as demonstrated in our study could even be more profound in the clinical situation of newborns with respiratory and/or circulatory distress.

Large prospective randomized studies in human newborns to investigate the relation between (simulated) bladderbox alarms and the occurrence rate of intracranial lesions during treatment with ECMO are impossible to perform. Therefore, it is important to establish observational intervention studies. The most important limitation of using NIRS, with its variables like HbD and CBV, is the lack of reference values below which global cerebral oxygenation and hemodynamics are compromised and clinically relevant for the individual patient. Findings can be used to describe trends in a group of patients and the relevance can only be suspected when compared with studies where known risk factors for the brain are related to changes in NIRS variables. Baseline variability of HbD has been described to range from -0.12 to +0.13 µmol.100g⁻¹ (33). With a variability of 0.03±0.01 this was lower in our study. In the same study a decrease in HbD of > 0.3 µmol.100g⁻¹ is likely to be clinically relevant since this was found to correspond with the effect of reducing Sao₂ by approximately 12%. Compared with these values, mean changes in HbD of 0.11 to 0.33 µmol.100.g⁻¹ and changes for individual lambs in HbD up to 0.45 µmol.100.g⁻¹ in experiments A and B and up to 0.84 µmol.100.g⁻¹ when intravascular volume administration was used could be important. Fluctuation of HbD, expressed as changes between maximal decrease in HbD and the consecutive maximal increase in HbD are even more profound. For ΔCBV, changes have been compared with usual physiologic parameters like changes in Paco₂ and MAP. During hypercapnia in normoxemic piglets on ECMO, a mean ΔCBV of 0.03 mL.100.g⁻¹.kPa⁻¹ occurred (34). In another study, ΔCBV was calculated in relation to changes in MAP in newborn lambs to be 0.07 mL.100.g⁻¹. mmHg⁻¹ (35). Compared with these values a mean change in CBV of 0.11 to 0.13 mL.100g⁻¹ and changes for individual lambs in CBV up to 0.26 mL.100.g⁻¹ as found in our study could be important. Changes between the maximal decrease in CBV and consecutive maximal increase in CBV are more profound. Finally, the value of CBV in lambs has been found to be 2.5 mL.100.gr⁻¹ (36). Changes in CBV found in our study are up to 10%. Besides the magnitude, changes in HbD and CBV occur very acute, which might be an additional risk factor for cerebral lesions by itself.
Some authors describe predominance of cerebral lesions in especially the right hemisphere of the brain, while in other studies this predominance, possibly related to ligation of the right carotid artery and right internal jugular vein, could not be confirmed (37-43). To study differences between both hemispheres we used two receiving NIRS optodes. However, changes in HbD and CBV were not different between the left and right hemisphere of the brain.

Cerebral hemorrhage and ischemia are generally considered to be caused by pre-ECMO as well as ECMO-related factors. We showed a significant relation between bladderbox alarms and changes in cerebral oxygenation and hemodynamics. This might play a role in the development of neurological sequelae. As changes in HbD and CBV are more extended when bladderbox alarms are resolved by administration of normal saline it would be interesting to further study the effects on cerebral oxygenation and hemodynamics of intravascular volume administration by itself as well. The results of our study are limited to ECMO systems with a rollerpump and a bladderbox and might support the use of centrifugal pumps, systems without a bladderbox or ECMO strategies with reduced bladderbox alarms. However, more research is needed for other systems as well to study, for example, the effects of less controlled drainage from the right atrium of the patient and cerebral oxygenation and hemodynamics. On the basis of this study we conclude that bladderbox alarms during treatment with va-ECMO result in statistically significant fluctuations in cerebral oxygenation and hemodynamics. This might be an additional risk factor for the occurrence of intracranial hemorrhage and ischemia.
References

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Chapter 4.3

The effect of intravascular volume administration on cerebral oxygenation and hemodynamics during veno-arterial extracorporeal membrane oxygenation in human newborns

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Submitted
Abstract

Objective: This study determines the possible relation between intravascular volume administration (IVA) and cerebral oxygenation and hemodynamics during veno-arterial extracorporeal membrane oxygenation (va-ECMO) in human newborns.

Patients and Methods: In a prospective observational study human newborns on the first day of va-ECMO were studied during IVAs. Administration of normal saline, thrombocytes and erythrocytes was needed due to bladderbox alarms, low platelet count or low hemoglobin level. Changes in concentrations of oxyhemoglobin and deoxyhemoglobin were measured using near infrared spectrophotometry. Changes in total Hb (ctHb) and hemoglobin oxygenation index (cHbD) were calculated. cHbD represents cerebral blood flow, ctHb reflects cerebral blood volume. Heart rate and mean arterial blood pressure were measured continuously. Of the studied variables maximal changes during and 2 minutes after fluid administration were compared to baseline values by using Student-t tests. Values are expressed as mean ± SD.

Results: 51 IVAs were observed in 10 neonates. The mean volume administered was 42 ± 11 ml, the mean administration velocity was 0.41 ± 0.25 ml.s⁻¹. IVA resulted in a significant increase of mean arterial blood pressure, ctHb and cHbD and a significant decrease of HR.

Conclusion: IVA on the first day during va-ECMO results in fluctuation of cerebral oxygenation and hemodynamics. This might be an additional risk factor for brain damage.
Introduction
Veno-arterial extracorporeal membrane oxygenation (va-ECMO) is a rescue therapy for (near) term newborns with severe but potentially reversible cardiorespiratory insufficiency unresponsive to maximal conventional therapy (1,2). It temporarily provides partial cardiopulmonary bypass by supporting the cardiac output and improving oxygen uptake via an artificial lung. The use of partial cardiopulmonary bypass will alter the cardiopulmonary hemodynamics. The ligation of the right common carotid artery and right internal jugular vein in combination with decreased arterial pulsatility due to the use of a rollerpump might also alter the cerebral hemodynamics (3). The most devastating complication of ECMO is the occurrence of cerebral vascular injury, both hemorrhagic and ischemic (4,5). The origin of cerebral injury in ECMO treated newborns is probably a combination of factors, including pre-ECMO, patient and disease related factors, as well as factors related to the ECMO treatment itself and a combination of both (5,6).

Part of the va-ECMO system is the bladderbox, which is situated between the venous cannula and the rollerpump of the ECMO system. The bladderbox controls venous drainage of blood from the right atrium of the patient. In case of inadequate drainage to maintain the established flow of the rollerpump, the bladderbox will give an alarm, slow down the pump, and finally cause an acute interruption of the rollerpump. This results in an acute interruption of blood flow from the ECMO system to the patient. If other causes of inadequate venous drainage from the right atrium, like disturbed position of the cannula, are ruled out, intravascular volume is often administered to restore the circulation through the ECMO system and the patient. Recently, our group first showed a statistically significant relationship between the total number and volume of intravascular volume administration (IVA) and the occurrence of intracranial hemorrhage (ICH) during va-ECMO treatment (7). In a consecutive study in lambs we demonstrated that bladderbox alarms are significantly related to fluctuations in cerebral oxygenation and hemodynamics (8). These fluctuations were larger if bladderbox alarms were resolved by IVA. The principle aim of this consecutive study is to further determine the relation between different kinds of IVA and changes in cerebral oxygenation and hemodynamics in daily practice in human newborns treated with va-ECMO.

Patients and Methods
Study population and setting
In a prospective observational study between November 2006 and December 2008 human newborns on va-ECMO were studied for changes in cerebral oxygenation and hemodynamics related to IVA. The study was approved by the Radboud University Nijmegen Medical Centre ethics committee and parental informed consent was obtained before measurements. The study was performed on the first day of ECMO when usually administration of normal saline, thrombocytes and erythrocytes is needed most frequently due to bladderbox alarms, low platelet count or low hemoglobin level.
Chapter 4

ECMO
All patients met institutional criteria for ECMO treatment, which did not change during the study period and were treated with va-ECMO (9). The ECMO circuit itself consisted of a custom packed 1/4" flexible polyvinylchloride tubing (Baxter, Uden, The Netherlands), with a silicone reservoir, the bladderbox (Seabrook Medical Systems, Inc, Cincinnati, Ohio, USA), an artificial lung with integrated heat-exchanger (Medos Hillite 2400LT, Medos, Stolberg Germany), and a rollerpump (Jostra RPM 20-320, Lung, Sweden). Aimed flow rate of the ECMO pump was 100-150 ml.kg⁻¹.min⁻¹. Normal saline (NaCl 0.9%) was used to restore the ECMO flow after bladderbox alarms. During the course of ECMO, thrombocyte transfusions were given at thrombocytes count < 80,000.mm⁻³. Erythrocyte transfusions were given at a hemoglobin level < 8.0 mmol.L⁻¹ and a hematocrit value < 0.40 L.L⁻¹. Hemoglobin level and thrombocyte count were determined every 4 hours. The indication for administration of NaCl 0.9%, thrombocytes or erythrocytes was made by the attending physician. The administration of these fluids was performed manually by the attending nurse.

NIRS
Cerebral oxygenation and hemodynamics were studied by using NIRS during the first 24 hours after the start of ECMO. The NIRS equipment used (OXYMON') was developed by the instrumentation department and the department of physiology of the Radboud University Nijmegen Medical Centre, The Netherlands (10). The technique is based on the spectrophotometric measurement of changes in the absorption properties of hemoglobin in the near infrared region, which is dependent on its oxygenation state, first described by Jobsis in 1977 (11). The NIRS procedure used in our ECMO centre has been described in earlier studies of our research group (12-14). From an optode, placed frontal in the midline of the skull, three wavelengths of near infrared light (908, 857 and 783 nm) were transmitted through the head and received by two other optodes placed on the left and right parietotemporal region of the skull, in order to evaluate the oxygenation and hemodynamics in each hemisphere separately. Concentration changes of oxyhemoglobin (ΔcO₂Hb) and deoxyhemoglobin (ΔcHHb) were calculated from changes in light absorption using the modified Lambert-Beer law. We used the Keele absorption matrix and a constant path length multiplier factor of 4.27 times the distance between both optodes (15-17). Using a value of 1.05 g.ml⁻¹ for brain specific mass, concentration changes are expressed in μmol.100g⁻¹ (18). ΔcO₂Hb and ΔcHHb reflect changes in cerebral oxygen supply, if oxygen consumption remains constant. Differences between ΔcO₂Hb and ΔcHHb were calculated and indicated as changes in the hemoglobin oxygenation index (ΔcHbD, where ΔcHbD = ΔcO₂Hb - ΔcHHb in μmol.100g⁻¹). ΔcHbD represents changes in cerebral blood flow (CBF) (19). Concentration changes in total hemoglobin (ΔcTbHb) were calculated as the sum of ΔcO₂Hb and ΔcHHb (ΔcTbHb = ΔcO₂Hb + ΔcHHb). ΔcTbHb reflects changes in cerebral blood volume (CBV). The NIRS variables were registered at a sample rate of 5 Hz.
Other measurements
Using a neonatal monitor (Philips Intellivue) arterial oxygen saturation (saO₂), mean arterial blood pressure (MABP) and heart rate (HR) were measured continuously. All variables were registered at a sample rate of 1 Hz and were synchronized with the NIRS registration.

Data management and analysis
We selected data in the time period from 15 seconds before the start of volume administration until 2 minutes after the end of IVA. The time span of 2 minutes was chosen to evaluate the final effect of the IVA. Baseline values (mean ± SD) of cHbD, ctHb and physiological variables were determined over a 15 seconds period before each single IVA during which the NIRS signal was stable. Maximal changes in cHbD, ctHb, MABP and HR were determined as mean values during a 3 seconds period of maximal deviation during the period of time between the start of each single IVA until 2 minutes after the end of IVA and compared to baseline values. Two minutes after IVA changes of all variables were determined as the mean values during a 3 seconds period and compared to pre-experiment baseline values. All changes were described separately for the right and left hemisphere of the brain. Therefore ΔcHbD is expressed as ΔcHbD_right and ΔcHbD_left, while ΔctHb is expressed as ΔctHb_right and ΔctHb_left. Maximal changes during IVA and 2 minutes after the end of IVA were compared to the baseline values by using Student-t tests. We compared changes between the right and left cerebral hemisphere with the Independent-Samples-t test. p-Values < 0.05 were considered to be statistically significant. Values are expressed as mean ± SD.

Because of the presumed effect of transfused erythrocytes on near infrared light absorption the results are described separately for erythrocytes as well as thrombocytes and NaCl 0.9%.
Results

In the 2 year study period, 10 neonates treated with va-ECMO at the Radboud University Nijmegen Medical Centre were studied. In total, the effect of 51 IVAs were observed: 26 administrations of NaCl 0.9%, 10 transfusions with thrombocytes and 15 transfusions with erythrocytes. The patients mean gestational age was 38 weeks and 6 days (range 34 weeks and 5 days to 41 weeks and 5 days). The mean birth weight was 3168 g (range 2540 to 4190 g). Six newborns were diagnosed with congenital diaphragmatic hernia, two patients had meconium aspiration syndrome, one patient had multiple pneumothoraces and one patient suffered from idiopathic persistent pulmonary hypertension. The study population consisted of 7 boys and 3 girls, the mean age at the initiation of ECMO was 2 days (range 0 to 9 days). The mean volume of the IVAs was 42 ± 11 ml, corresponding to 13 ± 4 ml.kg⁻¹. IVA was administered to the patients with a mean velocity of 0.41 ± 0.25 ml.s⁻¹. Figure 1 shows the typical pattern of cHbD, ctHb, MABP and HR during IVA until 2 minutes after the end of IVA in one patient.

Baseline values, calculated over a 15 seconds period before IVA showed a baseline variability for cHbD (mean ± SD) of 0.04 ± 0.03 µmol·100g⁻¹ and for ctHb 0.02 ± 0.01 ml·100g⁻¹ respectively in the right hemisphere and for cHbD of 0.06 ± 0.05 µmol·100g⁻¹ and for ctHb of 0.03 ± 0.02 ml·100g⁻¹ respectively in the left hemisphere. Baseline values of the measured variables, maximal changes and changes at 2 minutes after the end of IVA as compared to the baseline values are presented in table 1. Changes of variables were not different between the right and the left hemisphere. During IVAs saO₂ remained between the desired values. During administration of NaCl 0.9% and thrombocytes, cHbD, MABP and ctHb increased to maximum values that were statistically significant higher than the baseline values. After 2 minutes cHbD and MABP were still higher than the baseline values before IVA. The HR decreased during the administration of NaCl 0.9% and thrombocytes. The lowest value was statistically significant different from the baseline values. The HR was still lower than the baseline value 2 minutes after the end of IVA. Administration of erythrocytes resulted in an increase of cHbD, ctHb and MABP. Its maximum value was statistically significant higher than the baseline value. After 2 minutes, values were still higher than baseline values. The HR decreased during the erythrocytes administration with a minimum value that was statistically different from the baseline value. The HR at 2 minutes after the end of IVA was still lower than its baseline value.
**Figure 1.** Typical pattern of $cHbD$, $ctHb$, MABP and HR during IVA until 2 minutes after the end of intravascular volume administration with erythrocytes and with normal saline/thrombocytes during treatment with veno-arterial extracorporeal membrane oxygenation.

**Erythrocytes:**

**Saline/thrombocytes:**

In which $cHbD =$ oxygenation index, $ctHb =$ total concentration of hemoglobin, MABP = mean arterial blood pressure, HR = heart rate and $\Delta$ represents a change in parameters.
Table 1. Baseline values and maximal changes of \( \text{cHbD} \), \( \text{ctHb} \), MABP, HR and \( \text{saO}_2 \) during and two minutes after intravascular volume administrations in newborns treated with veno-arterial extracorporeal membrane oxygenation.

<table>
<thead>
<tr>
<th>Sal/ Thr</th>
<th>Baseline</th>
<th>Max</th>
<th>2 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>cHbD_right (( \mu \text{mol} \cdot 100 \text{g}^{-1} ))</td>
<td>n.a</td>
<td>0.20 ± 0.18 *</td>
<td>0.04 ± 0.18 NS</td>
</tr>
<tr>
<td>cHbD_left (( \mu \text{mol} \cdot 100 \text{g}^{-1} ))</td>
<td>n.a</td>
<td>0.26 ± 0.28 *</td>
<td>0.08 ± 0.22 ***</td>
</tr>
<tr>
<td>ctHb_right (( \mu \text{mol} \cdot 100 \text{g}^{-1} ))</td>
<td>n.a</td>
<td>0.12 ± 0.13 *</td>
<td>-0.08 ± 0.18 ***</td>
</tr>
<tr>
<td>ctHb_left (( \mu \text{mol} \cdot 100 \text{g}^{-1} ))</td>
<td>n.a</td>
<td>0.22 ± 0.42 **</td>
<td>0.04 ± 0.36 NS</td>
</tr>
<tr>
<td>MABP (mm Hg)</td>
<td>48 ± 12</td>
<td>12 ± 7 *</td>
<td>10 ± 7 *</td>
</tr>
<tr>
<td>HR (bpm)</td>
<td>139 ± 25</td>
<td>-17 ± 12 *</td>
<td>-11 ± 13 *</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Ery</th>
<th>Baseline</th>
<th>Max</th>
<th>2 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>cHbD_right (( \mu \text{mol} \cdot 100 \text{g}^{-1} ))</td>
<td>n.a</td>
<td>0.64 ± 0.86 ***</td>
<td>0.55 ± 0.86 ***</td>
</tr>
<tr>
<td>cHbD_left (( \mu \text{mol} \cdot 100 \text{g}^{-1} ))</td>
<td>n.a</td>
<td>0.84 ± 0.93 **</td>
<td>0.73 ± 0.93 ***</td>
</tr>
<tr>
<td>ctHb_right (( \mu \text{mol} \cdot 100 \text{g}^{-1} ))</td>
<td>n.a</td>
<td>0.27 ± 0.32 ***</td>
<td>0.15 ± 0.38 NS</td>
</tr>
<tr>
<td>ctHb_left (( \mu \text{mol} \cdot 100 \text{g}^{-1} ))</td>
<td>n.a</td>
<td>0.46 ± 0.51 **</td>
<td>0.38 ± 0.52 ***</td>
</tr>
<tr>
<td>MABP (mm Hg)</td>
<td>48 ± 11</td>
<td>15 ± 7 *</td>
<td>14 ± 8 *</td>
</tr>
<tr>
<td>HR (bpm)</td>
<td>153 ± 31</td>
<td>-27 ± 16 *</td>
<td>-20 ± 16 *</td>
</tr>
</tbody>
</table>

\( \text{cHbD} = \text{hemoglobin oxygenation index}, \text{ctHb} = \text{concentration total hemoglobin}, \text{HR} = \text{heart rate}, \text{MABP} = \text{mean arterial blood pressure}, \text{IVA} = \text{intravascular volume administration}, \text{Sal} = \text{normal saline}, \text{Ery} = \text{erythrocytes}, \text{Thr} = \text{thrombocytes}, \text{Max} = \text{maximal change during IVA} \) and \( 2 \text{ min} = \text{change} \) 2 minutes after the end of IVA. n.a = not available. All changes are compared to baseline values. *: \( p=0.000; \) **: \( p \leq 0.005; \) ***: \( p \leq 0.05; \) NS = not significant. Values are mean ± SD.

Discussion

This is the first study concerning the effects of IVA on cerebral oxygenation and hemodynamics in human newborns treated with va-ECMO. We demonstrated that IVA leads to a significant increase of \( \text{cHbD} \) and \( \text{ctHb} \) reflecting an increase in CBF and CBV. The consequence of these changes in oxygenation and hemodynamics in ECMO patients is unknown. However, it can be speculated that increase of CBF and CBV in combination with vulnerable capillary endothelium due to prolonged hypoxemia before ECMO can easily result in capillary rupture. In combination with heparinization during ECMO this may result in increased risk for development of ICH, an important complication during ECMO.

Recently we demonstrated a statistically significant relationship between the total number and volume of IVAs and the development of ICH during treatment with va-ECMO (7). In a consecutive study we showed that interruption of ECMO flow due to bladderbox alarms results in a significant decrease in \( \text{cHbD} \) and \( \text{ctHb} \) reflecting decreased CBF and CBV (8). Since bladderbox alarms are often resolved with the use of IVA, the successive events of bladderbox alarms, interruption of the ECMO flow, IVA and restoration of the ECMO flow can lead to substantial fluctuations in CBF
and CBV. All studies were performed in an ECMO system including a rollerpump and bladderbox. It is imaginable that the fluctuations of CBF and CBV caused by these successive events are a risk factor for the occurrence of cerebrovascular lesions. Several authors reported that the majority of cases of ICH occurred within 72 h after the start of ECMO (20-22). This fits with our findings, as the majority of bladderbox alarms occur in the first days of ECMO treatment, when a relatively high ECMO flow rate is required to maintain adequate saO2.

In order to reduce the risk of ICH we speculate that it might be better to administer fluid volumes as slow as possible. In our study, which reflects the daily clinical situation, the infusion velocity was dependent on the attending physician and nurse. In case of bladderbox alarms resulting in acute interruption of the ECMO pump, saline as a bolus was usually administered more quickly in order to restore the ECMO flow as soon as possible. Thrombocytes or erythrocytes were infused more slowly, because it was usually administered electively for reasons of low values in the patient. Nevertheless, this practice of thrombocytes or erythrocytes transfusion does not seem to prevent significant fluctuations in CBF and CBV. Bedside continuous monitoring of cerebral hemodynamics in infants might be helpful to determine the optimal infusion velocity for the individual patient but such device is not yet available for the clinical setting.

A hypothetical explanation for the observed effects might be found in the disturbance of cerebral autoregulation (AR). In normal, healthy infants AR maintains the cerebral blood flow over a wide range of cerebral perfusion pressures. It has been shown however, that AR is disturbed in severely ill term infants (23-25). Additional studies in newborn lambs on ECMO showed that prolonged hypoxia and/or the ECMO treatment itself significantly disturb AR (26-28). However from our data it is difficult to distinguish whether AR is disturbed, or whether the AR mechanism needs more time to regulate, since it takes about 2 seconds for a contra regulation response of CBF to occur after sudden changes in blood pressure (23,29). During the IVAs in our study the MABP continuously increased and then decreased, lacking a steady state period of 2 seconds for contra-regulation.

Some authors describe predominance of cerebral lesions in especially the right hemisphere of the brain, possibly related to ligation of the right carotid artery and right internal jugular vein, while in other studies this predominance could not be confirmed (30-36). To study possible differences between both hemispheres we used two receiving NIRS optodes. However, changes in cHbD and ctHb were not different between the right and left hemisphere of the brain.

We established this clinical observational study that did not interfere with the individual clinical practice in our ECMO patients. An important limitation of our study that reflects daily practice instead of a highly controlled laboratory setting is the difference in volume and infusion velocity of the studied IVAs. Nevertheless we were able to clearly demonstrate the effects of IVA on cHbD, ctHb and MABP.

Another important limitation is the lack of clinically relevant reference values for NIRS variables. It thus remains difficult to estimate the exact importance of the increased values of cHbD and ctHb for the individual patient. Baseline variability of cHbD has been described to range...
from -0.12 to +0.13 μmol.100g⁻¹ (37). In the same study a decrease in cHbD of > 0.3 μmol.100g⁻¹ reflects the effect of reducing saO₂ by approximately 12%. Compared to these values, mean changes in cHbD of 0.2 to 0.84 μmol.100g⁻¹ and changes for individual neonates in cHbD up to 3.48 μmol.100g⁻¹ could be physiologically important. Additionally, NIRS can only measure relative changes of cHbD and ctHb, but we are not informed about the absolute quantitative baseline values of these variables. To interpret whether changes of cHbD or ctHb are pathological, it is necessary to get the information about the quantitative baseline values.

When interpreting the value of our results, one should also take into account the different effects on hemoconcentration of adding erythrocytes versus the effect on hemodilution of adding saline and thrombocytes. Our results possibly overestimate the true increase of ctHb during administration of erythrocytes, because the increase in ctHb is partly caused by the increase in intravascular hemoglobin concentration as a result of adding erythrocytes to the circulation. On the other hand we possibly underestimate the true increase of ctHb during administration of NaCl 0.9% and thrombocytes, both of which will elicit some degree of hemodilution. Besides the magnitude we stress that changes in cHbD and ctHb occur rapidly, which might be a risk factor for cerebrovascular injury. When treating a newborn with va-ECMO one should be aware of that. In order to reduce the risk for ICH during va-ECMO treatment it might be indicated to be cautious with IVA. In conclusion, IVA on the first day during va-ECMO results in fluctuation of cerebral oxygenation and hemodynamics. Further investigation is needed to search for treatment strategies during ECMO in which these fluctuations can be reduced.
References


Chapter 4

Chapter 5

General discussion and future perspectives
General discussion

With its more routine use from the early 80’s neonatal ECMO has become an accepted modality for the treatment of severe respiratory insufficiency and pulmonary hypertension in neonates. Till today, neonatal ECMO has always been considered to be a “rescue therapy”, used after failure of other therapies. This might be explained by several reasons. First of all, ECMO is an intensive and invasive therapy and although there are nowadays more and more outcome data supporting its effectiveness, there is only one randomized trial (1). Furthermore, ECMO is not available in all hospitals. Finally the risk of ECMO related complications, especially intracranial hemorrhage and ischemia, the main causes of death and morbidity among patients treated with ECMO, might explain the current status of ECMO as a rescue therapy.

As a result of the “rescue” status of ECMO in the newborn other new techniques in the treatment of severe respiratory insufficiency and pulmonary hypertension of the newborn are generally applied before the initiation of ECMO. When these techniques fail, it might result in a delay in the initiation of ECMO, a prolonged ECMO runtime, complications related to the therapy applied and a selection of more severely ill neonates once ECMO is started. One of these new, conservative, therapies is the use of iNO. Currently there is clear evidence that iNO is effective in reducing the number of ECMO treatments, but does not reduce mortality in (near) term infants with hypoxic respiratory failure who do not have a congenital diaphragmatic hernia (1,3). It is known that the use of iNO by itself does not result in improved nor worsened neurodevelopmental outcome in term and near-term neonates with severe respiratory insufficiency (4,5). However one study demonstrated a relation between the response to iNO and neurodevelopmental outcome (6). It is not known, whether neurodevelopmental outcome is improved in those neonates kept from ECMO by the use of iNO (and other treatment modalities) and a prolonged conservative treatment, compared to those treated with ECMO after prior iNO use. Based on the results of the randomized trial of the UK collaborative ECMO group, that was discussed earlier, this might at least be questionable (2,7,8). The benefit of ECMO in comparison to conservative treatment is further confirmed in a Cochrane review from 2008 (9). However, it has to be admitted that the UK trial mentioned before is by far the strongest study in this four trials counting Cochrane review. Study objectives in (near) term infants with severe respiratory insufficiency should not only be to reduce the number of ECMO treatments, but should aim on how to improve the combined parameter of mortality and neurodevelopmental impairment. From this point of view, iNO treatment might not only be beneficial. Based on pathophysiological mechanisms (prolonged) use of iNO might play a role in the activation of the coagulation system (10-17). However, the use of iNO has also been related to increased risk for bleeding complications (18-23). Finally we stress that waiting for a response to iNO treatment might result in a delayed initiation of ECMO, especially in those neonates that do not or partly respond to iNO, occurring in up to 30% of cases (24). In this thesis a later initiation of ECMO was related to a longer ECMO runtime, but there was no significant difference in the postnatal age at the start of ECMO and duration of ECMO treatment.
between neonates treated with iNO before ECMO and those who were not treated with iNO. We did not find any significant difference in hemorrhagic complications, including ICH, between the two groups. We did, however, find a remarkable statistically significant relationship between clotting complications and/or DIC and iNO use prior to ECMO treatment. Major clot formation in the circuit can be detected by circuit inspection, but small, undetected, clots can escape into the circulation of the patient and cause (small) cerebral infarctions. Disturbance in other organ functions can also be involved in DIC. We are aware of the limitations of retrospective studies like ours, but because of ethical and practical arguments a prospective randomized trial is hard to perform and studies like ours do have a function in the search for possible risk factors. Not all accepted therapies, like iNO, should automatically be regarded to be beneficial, just because they reduce the number of ECMO treatments. Once iNO is initiated an unnecessary delay in the start of ECMO needs to be avoided as this is related to a prolonged ECMO runtime. For this reason inclusion criteria for ECMO might have to be changed and adapted in those neonates treated with iNO (25). Because more and more other new therapies for the treatment of persistent pulmonary hypertension are arising (e.g. sildenafil), it seems to be rational, that a limitation should be established in the pre-ECMO phase of conservative treatment, either for the duration or for the extent of the clinical improvement. Once this limitation has been exceeded, ECMO should be started without delay. Preferably, determination of such limitation should be supported by multicenter randomized trials.

The etiology of cerebral lesions in ECMO patients is considered to be multifactorial, as is described earlier in this thesis. It is often impossible to identify a specific cause in a given patient. Certain (technical) aspects of ECMO might form risk factors (26,27). One of these aspects is the active use of the va-bridge, a safety connection between the venous and arterial cannula of the ECMO system. For many years it has been common use to open the va-bridge intermittently, for example every 15 minutes, to prevent the development of thrombi due to blood stasis in the bridge. In earlier studies of our ECMO group Liem et al. and van Heijst et al. showed significant changes in cerebral oxygenation and hemodynamics related to the use of the va-bridge in an animal model and a clinical study by using near infrared spectrophotometry (28,29). Once opening the va-bridge, blood is shunting from the arterial cannula towards the venous cannula. This results in an acute decrease in MABP and cerebral perfusion pressure, as the ECMO flow is, especially at high flow rates, responsible for a large part of the systemic arterial blood flow in va-ECMO. In this thesis an additional clinical study using Doppler ultrasound in the pericallosal artery has also shown acute and statistically significant changes in CBFV related to the use of the va-bridge. These changes can be avoided if the va-bridge is filled with normal saline with stop-cocks on both sides. However, as surveyed at international meetings (e.g. the annual ELSO meeting, Keystone, 2010), this is not common use yet. Up to 50% of ECMO centers still performed intermittent opening of the va-bridge. In daily practice, this means that in our patient group this phenomenon would occur over 600 times per patient based on a mean ECMO run-time of 152
hours. Although a direct positive relation between the active intermittent use of the va-bridge and the occurrence of ICH and cerebral ischemia is not proven, fluctuations in CBFV due to opening of the bridge can easily be avoided. Based on our studies we would advocate further common use of the va-bridge filled with normal saline and with stopcocks located at the junction where the bridge connects to the venous and arterial tubing.

Another technical aspect of the ECMO system using a roller pump is the so-called “bladderbox”, which is situated between the venous cannula and the pump. The bladderbox controls venous drainage from the right atrium of the patient. In case of insufficient venous drainage, the bladderbox will give an alarm, slow down the pump and finally cause an acute interruption of the rollerpump resulting in interruption of arterial blood flow to the patient. If other causes of bladderbox alarms are excluded, intravascular volume administration is often given to restore the circulation through the ECMO system and the patient. We hypothesized that the sequelae of bladderbox alarms and consecutive intravascular volume administration results in significant fluctuations in cerebral oxygenation and hemodynamics. These fluctuations will occur in a brain possibly suffering from increased vascular vulnerability and disturbed cerebral autoregulation (AR). In normal, healthy infants, the brain is protected from fluctuations in CBF by the cerebral AR, which acts with a delay of 2 s after sudden changes in blood pressure. However, it has been shown that AR is disturbed in severely ill term infants (30-33). Additional studies in newborn lambs showed that prolonged hypoxia and/or ECMO treatment itself significantly disturbs AR (34-37). Several authors reported that the majority of cases of ICH occur within the first 72 h after the start of ECMO (38-40). In this episode, when most bladderbox alarms occur, the brain might be most vulnerable for intracranial hemorrhage and ischemia because of the disturbed AR.

In this thesis we first showed that the number and total volume of intravascular volume administration in the first 8 to 24 hours of va-ECMO treatment are statistically significant related to the development of ICH. These results were the basis for two following studies. In highly controlled laboratory settings bladderbox alarms were simulated in healthy lambs treated with va-ECMO. We found acute and significant changes in cerebral oxygenation and hemodynamics during and after bladderbox alarms. These changes were especially larger if bladderbox alarms were resolved by intravascular volume administration. A consecutive study in human newborns showed that even an intravascular volume administration with normal saline as well as with erythrocytes and thrombocytes without preceding bladderbox alarms, resulted in statistically significant fluctuations in cerebral oxygenation and hemodynamics that might be of clinical and physiological importance. Combining the results of the studies above, it can be speculated that a more subtle approach of bladderbox alarms and consecutive intravascular volume administrations might result in reduced risk of cerebral lesions. It might be that lower saO2 and paO2 values should be accepted during the first days of va-ECMO. Using this strategy, high ECMO flow rates can be avoided, resulting in less bladderbox alarms. If bladderbox alarms do occur, it might be interesting to study whether slower infusion rates result in less fluctuation of cerebral oxygenation and
hemodynamics in the vulnerable brain. Another possible option would be to use an ECMO system with a centrifugal pump (without a bladderbox) instead of a rollerpump. However, possible effects on the brain have to be studied for these kinds of pumps as well.

Future perspectives
The future perspectives of research in the field of ECMO should be focused in reducing mortality and morbidity of ECMO treatment. Following points should be considered.

a. As long as ICH and cerebral ischemia are still the most common important complications, future research should be focused on gaining a better understanding of pathophysiological and technical aspects related to these cerebral complications. One of these aspects is the improved understanding of factors that are causally related to disturbed autoregulation.

b. Future research should also be aimed at the place of ECMO in the treatment cascade of severe neonatal respiratory insufficiency and pulmonary hypertension. New less aggressive therapies like sildenafil should not automatically be placed ahead of ECMO with unlimited duration. Even the current commonly applied conservative treatment should be critically evaluated, whether its use will result in a delay of ECMO initiation. Unnecessary delay in the initiation of ECMO might result in longer ECMO treatments and an increased number of complications. Outcome parameters in studies considering the less aggressive therapies should not only focus on the avoidance of ECMO, but concentrate on mortality and long-term neurodevelopmental outcome.

c. A possible point in future research is the development of more individualized ECMO criteria based on factors like underlying disease and response to other treatments. It might be useful to investigate whether some underlying diseases will benefit from lower, more relaxed, criteria than other primary diseases for the initiation of ECMO. This is in contrast with the current practice, where the same criteria are used for all underlying diseases.

d. Another point of interest is the fact that vv-ECMO is considered to be more gentle for the brain, but that there is still a lack of evidence that vv-ECMO does really result in less complications and improved long term outcome. It is still unknown whether vv-ECMO has resulted in high incidence of posterior fossa hemorrhages due to more venous congestion. Till today we have missed the opportunity to perform a multi center trial in which patients are randomized and treated by va- or vv-ECMO.

e. Besides identification of and dealing with factors that are related to cerebral complications, it is important that future research should consider possible brain protective strategies like (head) cooling and neuroprotective drugs during ECMO treatment. From this point of view, results of a current randomized controlled trial by Field et al seem to be very interesting (41).
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Chapter 6

- Summary
- Samenvatting
- Dankwoord
- Curriculum vitae
Summary

Neonatal extracorporeal membrane oxygenation (ECMO) is a rescue therapy for (nearly) term newborns suffering from severe, but potentially reversible, respiratory insufficiency, often complicated by pulmonary hypertension. Next to this ECMO can be used as a bridge to diagnosis in cases of severe respiratory failure of unknown origin. Finally ECMO can be useful, as a temporary support for reversible cardiac failure, like after cardiac surgery. There are two forms of ECMO, veno-venous ECMO (vv-ECMO) and veno-arterial ECMO (va-ECMO). When va-ECMO is used, like in all studies of this thesis, blood drained from the right atrium passes an artificial lung, the membrane oxygenator, where it is oxygenated. Oxygenated blood is returned to the aorta of the patient. Neonatal ECMO is an effective therapy, but unfortunately it has its disadvantages as well. Most important disadvantage is the occurrence of cerebral lesions.

In this thesis we study risk factors for intracranial hemorrhage (ICH) and cerebral ischemia, two of the most devastating complications of ECMO leading to mortality or (long term) morbidity.

Chapter 1 gives a general introduction in ECMO. It especially stresses the ECMO technique and what is known from literature about factors related to the development of cerebral lesions. These factors can be divided in pre-ECMO, patient related factors, like hypoxia due to severe respiratory failure and factors related to the use of ECMO, like anticoagulation. At the end of chapter 1 a short introduction in near infrared spectrophotometry (NIRS) is given. This technique, in which near infrared light is used to measure oxygenated and non-oxygenated hemoglobin in the brain to determine fluctuations in cerebral oxygenation and hemodynamics, is used in two of the studies of this thesis and might be less known by the readers.

Chapter 2 presents the results of two studies concerning the possible effect of inhaled nitric oxide (iNO) used in patients before the initiation of ECMO on complications during the ECMO treatment. Since ECMO was introduced as a rescue therapy for newborns suffering from severe respiratory failure, often complicated by pulmonary hypertension, other less invasive techniques were developed to treat these children. One of these techniques is the use of iNO to treat pulmonary hypertension. Although used for its specificity as a pulmonary vasodilator, iNO has systemic effects as well. We studied whether these systemic effects, or a delay in the initiation of ECMO because of the use of iNO, might lead to an increased risk for complications of ECMO itself. From literature it is known, that iNO effectively reduces the number of ECMO treatments, but does not reduce mortality in term infants with hypoxic respiratory failure. In both studies the possible effects of iNO on hemostasis and coagulation are described. Although further research is absolutely needed to identify possible-underlying mechanisms, diffusion of NO into the systemic circulation might result in an increased risk for clotting complications as well as hemorrhagic complications. We were the first to study its relation to these complications when used before ECMO, a treatment for which the often severely ill patient will be heparinized and hemorrhage as well as clotting complications or disseminated intravascular coagulation (DIC) frequently occur. In the first study we found a positive relationship between clotting complications and/or DIC and iNO use prior to ECMO treatment. A prospective randomized control study seems to be indicated, but will be hard to perform because of medical as well as ethical considerations, as iNO has been accepted as standard therapy for newborns with severe respiratory insufficiency with pulmonary hypertension. In the second study we found that the use of iNO does not necessarily have to result in a significant delay neither in the initiation of ECMO nor in an increase in duration of the ECMO treatment. Next to this we found no statistically significant rela-
tion between the use of iNO prior to ECMO and the occurrence of hemorrhagic complications. **Chapter 3** describes the effects of clamping and unclamping of the veno-arterial bypass bridge (va-bridge) on cerebral blood flow velocity (CBFV) as measured by Doppler ultrasound in human newborns. Part of the ECMO system is a va-bridge, which connects the arterial and venous cannula when va-ECMO is used. In the event of emergencies the arterial and venous cannula can be clamped and the va-bridge opened to allow continuation of circulation through the ECMO system, while the patient is taken off from ECMO. Under normal conditions the va-bridge is clamped. Historically the va-bridge was filled with blood. To prevent clotting in the va-bridge because of blood stasis, it has been recommended to release the clamp on the va-bridge every 15 to 60 minutes during the course of ECMO. There is, however, a limited number of studies concerning the impact of this intermittent opening procedure of the blood stained bridge. In our study in 22 human newborns, Doppler ultrasound of the pericallosal artery, a terminal branch of the anterior cerebral artery, during opening of the va-bridge showed a strong and very acute decrease of the peak systolic blood flow velocity (PSV), end diastolic blood flow velocity (EDV) and time average mean blood flow velocity (TAM). All changes were statistically significant. In several cases even a backward flow, negative EDV, was found. Once the va-bridge was clamped, there was an acute recovery of all values. Changes persisted during the consecutive days of the ECMO treatment. In addition we studied the relationship between the magnitude of changes in CBFV during opening of the bridge and the ECMO flow rate. The statistically significant overall correlation between the changes in PSV, EDV and TAM and the ECMO flow rate found in our study is mainly caused by flow dependent fluctuations in CBFV at lower ECMO flows, up to 100 ml/min/kg. In addition to some studies mentioned before, our findings suggest that repetitive potentially harmful fluctuations in CBFV should be avoided. Using a va-bridge, which is filled with normal saline and has stopcocks on both sides, can easily do this. Thereby intermittent opening of the bridge is no longer necessary.

**Chapter 4** presents the findings of three studies, all concerning the possible harmful relation between bladderbox alarms, intravascular volume administration and cerebral lesions. Part of the va-ECMO system is a bladderbox, which is situated between the venous cannula and the rollerpump of the extracorporeal system. The bladderbox controls venous drainage of blood from the right atrium of the patient. If the venous drainage becomes inadequate to maintain the established flow of the ECMO system, the bladderbox will give an alarm, slow down the pump, and finally cause an acute interruption of the rollerpump resulting in interruption of blood flow to the patient. If other causes of inadequate venous drainage from the right atrium and consecutive bladderbox alarms, like disturbed position of the cannula, are ruled out, intravascular volume administration is often given to restore the circulation through the ECMO system and the patient. In the first study of chapter 4 the relation between the frequency and amount of intravascular volume administration and the occurrence of ICH was retrospectively studied in a matched case-control study with 25 newborns that developed an ICH and 40 control patients. We found a statistically significant relationship between the total number and volume of intravascular volume suppletions and the development of ICH during the treatment with va-ECMO. Neonates, receiving >8 infusions or >300 ml of volume infusion during the first 8 hours or >10 infusions during the first 24 hours of va-ECMO were statistically significant more at risk to develop an ICH. Although not necessarily causal, we hypothesized, that either the bladderbox alarms or the intravascular volume administration might cause potentially harmful fluctuations in cerebral oxygenation and hemodynamics. This might be especially true, as cerebral autoregulation seems to be disturbed in patients treated with va-ECMO.
In a second, consecutive prospective study, we analyzed the effect of bladderbox alarms on cerebral oxygenation and hemodynamics in lambs cannulated for VA-ECMO. In a controlled laboratory setting bladderbox alarms were simulated by either increasing the flow of the rollerpump of the ECMO system or by partially clamping the venous cannula. Decreasing the flow of the rollerpump, intravascular volume administration or unclamping of the venous cannula resolved bladderbox alarms. Using NIRS, we found a statistically significant decrease in cerebral oxygenation and hemodynamics as expressed by changes in hemoglobin oxygenation index (ΔHbD) and cerebral blood volume (ΔCBV) once bladderbox alarms were simulated. These changes occurred very acute. Once bladderbox alarms were resolved a recovery of cerebral oxygenation and hemodynamics was seen with an overshoot to values above baseline values before the experiments. This overshoot was much larger if intravascular volume administration was used to resolve bladderbox alarms. These acute fluctuations in cerebral oxygenation and hemodynamics might contribute to the risk for intracranial lesions. Adaptations of the ECMO circuit and/or changes in the ECMO treatment strategies might result in less bladderbox alarms and by this less intravascular volume administration.

Principle aim of the third and final study of chapter 4 is to further determine the relation between the different kinds of IVA and changes in cerebral oxygenation and hemodynamics in daily practice in human newborns. Most bladderbox alarms and consecutive intravascular volume administrations occur in the first three days of ECMO treatment. This is also the period in which the majority of ICH is found. Following the findings of the second study of this chapter we hypothesized that intravascular volume administration in human newborns causes changes in cerebral oxygenation and hemodynamics that might contribute to the risk for intracranial lesions. Intravascular volume administration can be given as normal saline, thrombocytes or erythrocytes. In total 51 moments of intravascular volume administration were observed in 10 neonates. NIRS was used to measure changes in cerebral oxygenation and hemodynamics. Changes in total Hb (ctHb) and hemoglobin oxygenation index (HbD) were calculated. These parameters represent cerebral blood volume and cerebral blood flow respectively. We demonstrated that intravascular volume administration with normal saline and thrombocytes as well as with erythrocytes resulted in rapid and statistically significant changes in ctHb and HbD. Results were equal for the right and left hemisphere of the brain. These changes, might contribute to the occurrence of cerebral lesions. Different ECMO equipment or strategies might result in less moments of intravascular volume administration. And if necessary, it might be worthy to consider lower administration velocity.

Finally chapter 5 offers the general discussion of this thesis. Main findings of the different studies are discussed in a larger context. We conclude that new therapies, introduced for the treatment of pulmonary hypertension in the newborn have to be carefully evaluated and should not automatically be placed ahead of ECMO treatment. Next to this ECMO treatment itself should be discussed for its place in the cascade of treatment in neonates with severe respiratory failure, often complicated by pulmonary hypertension. Indications for ECMO could be applied based on diagnosis and individual criteria. In the second part of this thesis it became clear that interventions and treatment strategies during ECMO do cause changes in cerebral oxygenation and hemodynamics that might contribute to cerebral lesions. More fine-tuning of the effective ECMO treatment or changes in equipment could reduce these possible risk factors for the brain.
Samenvatting

Extracorporele membraan oxygenatie (ECMO) is een reddingsbehandeling voor (bijna) voldragen pasgeborenen, met ernstige maar potentieel reversibele respiratoire insufficiëntie, vaak gecompliceerd door pulmonale hypertensie. Daarnaast kan ECMO gebruikt worden ter overbrugging tot een diagnose gesteld kan worden bij pasgeborenen met ernstig respirator falen waarbij de oorzaak onduidelijk is. Tenslotte kan ECMO behulpzaam zijn als tijdelijke ondersteuning na hartoperaties of bij kinderen met reversibel hartfalen. Er zijn twee vormen van ECMO, veno-veneze ECMO (vv-ECMO) en veno-arteriële ECMO (va-ECMO). Wanneer va-ECMO wordt gebruikt, zoals in alle studies van dit proefschrift, passeert bloed, afkomstig uit de rechter boezem van het hart, een kunstlong en wordt daarna terug geleid door een naar de grote lichaamsslagader van de patiënt. Dit proefschrift is een zoektocht naar mogelijke risicofactoren voor hersenbloeding (ICH) en hersenischemie, twee van de belangrijkste complicaties van ECMO behandeling, leidend tot mortaliteit en (lange termijn) morbiditeit.

Hoofdstuk 1 is een algemene introductie over ECMO. Speciale nadruk wordt gelegd op wat bekend is uit de literatuur over factoren die verband houden met het ontwikkelen van schade aan de hersenen. Deze factoren kunnen worden opgedeeld in factoren die in principe voor de ECMO behandeling liggen, zoals een relatief zuurstof tekort door ernstig respiratoire falen van de patiënt en factoren die gerelateerd zijn aan de ECMO behandeling zelf, zoals de antistolling die moet worden gegeven. Aan het eind van hoofdstuk volgt een korte introductie over near infrared spectrophotometry (NIRS), om dat deze techniek mogelijk minder bekend is bij de lezers. Hierbij worden frequenties van het bijna infrarode licht gebruikt om veranderingen in geoxygeneerd en niet geoxygeneerd hemoglobine te meten. Aan de hand hiervan kunnen fluctuaties in de zuurstof- en bloedvoorziening van het brein worden berekend.

Hoofdstuk 2 beschrijft de resultaten van twee studies naar het mogelijke effect van behandeling met geinhaleerd stikstof oxide (iNO) van patiënten, voordat ECMO wordt gestart, op het optreden van complicaties tijdens de ECMO behandeling. Sinds de introductie van ECMO als reddingsbehandeling van pasgeborenen met ernstig respiratoire falen, vaak gecompliceerd door pulmonale hypertensie, zijn andere, minder invasieve, technieken ontwikkeld om deze kinderen te behandelen. Eén van deze technieken is het gebruik van iNO om pulmonale hypertensie te behandelen. Hoewel primair gebruikt als specifieke verwijder van het pulmonale vaatbed, heeft iNO ook systemische effecten. Wij bestudeerden of deze systemische effecten, of een vertraging in het starten met ECMO vanwege het gebruik van iNO, zouden kunnen leiden tot een verhoogd risico op complicaties van de ECMO behandeling zelf. Vanuit de literatuur is bekend dat iNO het aantal behandelingen met ECMO effectief reduceert, maar dat de mortaliteit van op tijd geboren kinderen met hypoxisch respirator falen niet afneemt. In beide studies wordt het mogelijke effect van NO op hemostase en stolling beschreven. Hoewel verder onderzoek naar onderliggende mechanismen absoluut geïndiceerd is, is het mogelijk dat NO, dat naar de lichaamscirculatie diffundeert, een verhoogd risico geeft op zowel stollings- als bloeding complicaties. Wij hebben als eerste de dit risico onderzocht, wanneer iNO gebruikt werd voor ECMO, een behandeling waarvoor het meest ernstig zieke kind “ontstold” wordt met heparine en zowel bloeding als stollingscomplicaties en diffuse intravasale stolling (DIC) frequent optreden. In de eerste studie vonden wij een positieve relatie tussen stollingscomplicaties en/of DIC en het gebruik van iNO voor de ECMO behandeling. Een prospectieve gerandomiseerde en gecontro-
leerde studie lijkt te zijn geïndiceerd, maar zal vanwege klinische en ethische redenen moeilijk haalbaar zijn, mede omdat iNO algemeen geaccepteerd is als therapie voor de behandeling van pasgeborenen met ernstige pulmonale hypertensie. In de tweede studie vonden wij dat het gebruik van iNO niet noodzakelijk hoeft te leiden tot een significante vertraging in het starten van ECMO, noch hoeft te leiden tot een verlengde behandelingsduur met ECMO. Daarnaast vonden wij geen statistisch significante relatie tussen het gebruik van iNO voor ECMO en het optreden van bloeding complicaties.

**Hoofdstuk 3** beschrijft het effect van openen en sluiten van de veno-arteriële brug (va-bridge) op de cerebrale bloedstroom snelheid (CBFV), gemeten met Echo Doppler bij pasgeboren kinderen. Een onderdeel van het ECMO systeem is de va-bridge, die de arteriële en veneuze canule met elkaar verbindt wanneer va-ECMO wordt toegepast. In het geval van een noodsituatie kunnen de arteriële en veneuze canule afgeklemd en de va-bridge geopend worden. Hierdoor kan circulatie door het ECMO systeem blijven plaatsvinden, terwijl de patiënt op dat moment niet meer met ECMO wordt behandeld. Onder normale condities is de va-bridge gesloten. Om stolselvorming in de va-bridge ten gevolge van stilstaand bloed te voorkomen wordt aanbevolen om de va-bridge iedere 15 tot 60 minuten kortdurend te openen gedurende de gehele ECMO behandeling. Er zijn echter enkele studies die de mogelijk schadelijke gevolgen hiervan hebben onderzocht. In onze studie werd bij 22 kinderen, tijdens va-ECMO behandeling, echo Doppler onderzoek verricht van de arteria pericallosa, een eindtak van de arteria cerebi anterior. Tijdens het openen van de va-bridge vonden wij een sterke en zeer acute daling aan van de piek systolische bloedstroom snelheid (PSV), eind diastolische bloedstroom snelheid (EDV) en tijd gemiddelde bloedstroom snelheid (TAM). Alle veranderingen waren statistisch significant. In enkele gevallen vonden wij zelfs een omgekeerde, negatieve EDV. Wanneer de va-bridge weer werd gesloten vond een snel herstel van alle waardes plaats. Veranderingen bleven zich voordoen gedurende de opeenvolgende dagen van de ECMO behandeling. Ook bestudeerden wij de relatie tussen de mate van veranderingen in CBFV gedurende opening van de va-bridge en de pompsnelheid van het ECMO systeem. De statistisch significante correlatie tussen veranderingen in PSV, EDV en TAM en de pompsnelheid van het ECMO systeem wordt vooral veroorzaakt door pompsnelheid afhankelijke veranderingen in CBFV bij lagere pompsnelheden tot 100 ml/min/kg. Onze studie suggereert, in aanvulling op enkele genoemde studies, dat bij herhaling optredende, potentieel schadelijke, schommelingen in CBFV dienen te worden voorkomen. Dit kan eenvoudig bereikt worden door een va-bridge te gebruiken die gevuld is met NaCl 0,9% en aan beide kanten voorzien is van een driewegkraan.

**Hoofdstuk 4** beschrijft de bevindingen van drie studies, die allemaal betrekking hebben op de mogelijk schadelijke relatie tussen bladderbox alarmeren, intravasculaire volume suppletie en het optreden van cerebrale laesies. Onderdeel van het ECMO systeem is de zogenaamde “bladderbox”. Deze bevindt zich tussen de veneuze canule en de rollerpomp van het extracorporele systeem. De bladderbox controleert de mate van veneuze drainage uit het rechter atrium van de patiënt. Wanneer de veneuze drainage tekortschiet om de ingestelde pompsnelheid van het ECMO systeem te waarborgen, zal de bladderbox alarmeren, de rollerpomp langzamer laten draaien en tenslotte de rollerpomp acuut tot stilstand brengen. Hierbij treedt een interruptie op van de bloedstroom naar de patiënt. Wanneer andere oorzaken van inadequate veneuze drainage vanuit het rechter atrium en de daarop volgende bladderbox alarmeren, zoals een verkeerde positie van de veneuze canule, uitgesloten zijn, wordt vaak intravasculaire volume suppletie gegeven om de circulatie door het ECMO system en de patiënt te herstellen.
In de eerste studie uit hoofdstuk 4 werd de relatie tussen de frequentie en hoeveelheid van intravasculaire volume suppletie en het optreden van ICH onderzocht in een retrospectieve matched case-control studie onder 25 pasgeboren die gedurende ECMO een ICH ontwikkelden en 40 controle patiënten. Wij vonden een statistisch significante relatie tussen het totaal aantal keren dat intravasculaire volume suppletie werd toegepast en het totale volume aan suppletie enerzijds en het ontwikkelen van ICH gedurende va-ECMO anderzijds. Pasgeboren, die meer dan 8 keer, of meer dan 300 ml volume, suppletie kregen in de eerste 8 uur, of meer dan 10 keer gedurende de eerste 24 uur van de ECMO behandeling hadden een statistisch significant hogere kans om een ICH te ontwikkelen. Hoewel deze relatie niet noodzakelijkerwijs op een oorzaakelijk verband hoeft te wijzen, stelden wij de hypothese dat de bladderbox alarmen en/of de intravasculaire volume suppletie zouden kunnen leiden tot potentieel schadelijke fluctuaties in cerebrale oxygenatie en hemodynamiek. Deze hypothese zou ondersteund kunnen worden door het feit dat de cerebrale autoregulatie verstoord lijkt te zijn bij kinderen, die behandeld worden met ECMO.

In een volgende prospectieve studie werd het effect van bladderbox alarmen op de cerebrale oxygenatie en hemodynamiek onderzocht in lammeren. Deze lammeren werden allemaal behandeld met va-ECMO. In de gecontroleerde omgeving van het dierenlaboratorium werden bladderbox alarmen gesimuleerd door het ophogen van de pompsnelheid van het ECMO systeem of door het partieel afklemmen van de veneuze canule. De bladderbox alarmen werden opgeheven door de snelheid van de rollerpump weer te verlagen, intravasculaire volume suppletie te geven of de klem van de veneuze canule af te halen. Door gebruik te maken van NIRS vonden wij een significante afname van de cerebrale oxygenatie en hemodynamiek op het moment dat bladderbox alarmen werden gesimuleerd. Veranderingen werden uitgedrukt in veranderingen in de hemoglobine oxygenatie index (ΔHbD) en cerebraal bloed volume (ΔCBV). Deze veranderingen traden zeer acuut op. Wanneer bladderbox alarmen opgeheven werden vond een herstel plaats van de cerebrale oxygenatie en hemodynamiek, met een “overshoot” tot waardes boven de uitgangswaardes voor het experiment. Deze “overshoot” was veel meer uitgesproken wanneer intravasculaire volume suppletie werd gebruikt om de bladderbox alarmen op te heffen. Deze acute fluctuaties in cerebrale oxygenatie en hemodynamiek zouden kunnen bijdragen aan het risico op schade aan de hersenen. Aanpassingen van het ECMO systeem of veranderingen in het beleid rondom ECMO zouden kunnen leiden tot minder bladderbox alarmen en daardoor tot minder momenten van intravasculaire volume suppletie.

Belangrijkste doel van de derde en laatste studie van hoofdstuk 4 is om de relatie tussen de verschillende soorten intravasculaire volume suppletie en veranderingen in cerebrale oxygenatie en hemodynamiek verder te beschrijven. Dit werd gedaan bij pasgeboren behandeld met ECMO en in de dagelijkse praktijk. De meeste bladderbox alarmen en daarop volgende intravasculaire volume suppleties vinden plaats in de eerste drie dagen van de ECMO behandeling. Dit is ook de periode waarin de meeste hersenbloedingen worden gevonden. N.a.v. de resultaten van de tweede studie van dit hoofdstuk kwamen wij tot de hypothese dat intravasculaire volume suppletie bij kinderen behandeld met ECMO, leidt tot verandering in cerebrale oxygenatie en hemodynamiek, die zouden kunnen bijdragen aan het risico op schade aan de hersenen. Intravasculaire volume suppletie kan worden toegediend in de vorm van fysiologisch zout, thromboctyten transfusie of erytroctyten transfusie. In totaal werden 51 momenten van intravasculaire volume suppletie gemeten bij 10 pasgeboren. NIRS werd gebruikt om veranderingen in cere-
brale oxygenatie en hemodynamiek te meten. Veranderingen in totaal Hb (ctHB) en hemoglobine oxygenatie index (HbD) werden berekend. Deze parameters representeren respectievelijk cerebraal bloed volume en cerebrale bloed stroom. Wij toonden aan dat intravasculaire volume suppletie met zowel fysiologisch zout als met thrombocyten en erytrocyten leidt tot snelle en statistisch significante veranderingen in ctHB en HbD. Er was hierbij geen verschil tussen de rechter in linker kant van de hersenen. Deze veranderingen zouden het risico op schade aan de hersenen kunnen verhogen. Opnieuw concluderen wij dat aanpassingen aan het ECMO systeem en/of het beleid rondom ECMO zouden kunnen leiden tot minder momenten van intravasculaire volume suppletie. Het zou ook waardevol kunnen zijn om een lagere toedieningsnelheid van extra volume te bestuderen.

**Hoofdstuk 5** vormt de algemene discussie van dit proefschrift. De belangrijkste bevindingen van de verschillende studies worden bediscussieerd en in een grotere context geplaatst. Wij concluderen dat nieuwe therapiën voor de behandeling van pulmonale hypertensie van de pasgeborene zorgvuldig moeten worden geëvalueerd en niet automatisch voor ECMO behandeling geplaatst dienen te worden. Daarnaast moet er discussie plaats vinden over de plaats van ECMO in de behandeling van neonaten met ernstig respiratoir falen, vaak gecompliceerd door pulmonale hypertensie. De indicatie voor ECMO zou meer op basis van diagnose en individuele criteria gesteld kunnen worden i.p.v. universele criteria voor alle kinderen. Voorts pleiten onze studies voor meer fine-tuning van de op zich effectieve ECMO behandeling door aanpassingen aan het ECMO systeem en aanpassingen in het beleid rondom ECMO. Hierdoor zou het risico op schade aan de hersenen gereduceerd kunnen worden.
Dankwoord


Allereerst wil ik mijn promotor, Prof. R. de Groot en mijn copromotoren, Dr. K.D. Liem en Dr. A.F.J. van Heijst bedanken. Zij hebben altijd hun enthousiasme getoond om iets dat begon als een losse studie in het kader van mijn fellowship neonatologie uit te laten groeien tot een promotieproject.

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Curriculum Vitae
