Testing Impact of Perinatal Inflammation on Cerebral Autoregulation in Preterm Neonates:

Evaluation of a Noninvasive Method

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1. INCLUDED MANUSCRIPTS

This thesis is based on the following peer reviewed publications. These publications are referred to by their roman numerals:

I. Hahn GH, Christensen KB, Leung TS, Greisen G. Precision of coherence analysis to detect cerebral autoregulation by near-infrared spectroscopy in preterm infants. J Biomed Opt 2010; 15:037002.

II. Hahn GH, Heiring C, Pryds O, Greisen G. Applicability of near-infrared spectroscopy to measure cerebral autoregulation noninvasively in neonates: a validation study in piglets. Pediatr Res 2011; 70:166-170.

III. Hahn GH, Maroun LL, Larsen N, Hougaard DM, Sorensen LC, Lou HC, Greisen G. Cerebral autoregulation in the first day after preterm birth: no evidence of association with systemic inflammation. Pediatr Res 2012; 71:253-260.

IV. Hahn GH, Heiring C, Pryds O, Greisen G. Cerebral vascular effects of hypovolemia and dopamine infusions: a study in newborn piglets. Acta Paediatrica 2012; 101(7):736-42.

2. LIST OF ABBREVIATIONS

ABP:	arterial blood pressure
CA:	cerebral autoregulation
CBF:	cerebral blood flow
$Coherence_{ST}$	standardized coherence
CVR_e:	estimated cerebrovascular resistance
FIR:	fetal inflammatory response
GA:	gestational age
IL-6:	interleukin-6
IVH:	intraventricular hemorrhage
LF:	low frequency range
MIR:	maternal inflammatory response
NIRS:	near infrared spectroscopy
LDF:	laser-Doppler flowmetry
MAPB:	mean arterial blood pressure
SaO_2:	arterial oxygen saturation
VLF:	very low frequency range

3. BACKGROUND

3.1 INTRODUCTION

Neurodevelopmental impairment following preterm birth is a growing issue in neonatology: (i) the preterm delivery rate is increasing [1], (ii) improved perinatal and neonatal care has lead to improved survival after preterm birth [2] and (iii) a considerable proportion of infants who are born preterm survive with neurodevelopmental impairment [3] such as cerebral palsy and cognitive and behavioral impairments [4-6]. This trend calls for increased knowledge of mechanisms associated with brain injury following preterm birth.

This thesis is based on clinical studies of very preterm infants and experimental studies in newborn piglets with the purpose to measure cerebral autoregulation (CA) non-invasively to explore a possible association with perinatal inflammation. Also, we made use of the piglet model to study how hypovolemia and dopamine therapy affect cerebral hemodynamics.

3.2 THE VULNERABLE PRETERM BRAIN

Very preterm infants have an increased risk of brain injury. The primary lesions are intraventricular hemorrhage (IVH) and periventricular leukomalacia (PVL) [7]. IVH is a hemorrhagic lesion in the periventricular germinal matrix. This richly vascular structure – a specific feature of the immature brain - is selectively vulnerable to hemorrhage, probably because of an immature vasculature compared to other regions of the brain [8]. PVL is characterized by cyst formation with necrosis of myelinated fibers [7] located in the poorly perfused watershed areas between the long and short penetrating arteries in the immature brain [8,9].

The pathogenesis of these lesions seems to be multifactorial. Besides intrauterine inflammation [10-13], a combination of ischemia and maturation-dependent incomplete anatomic development of

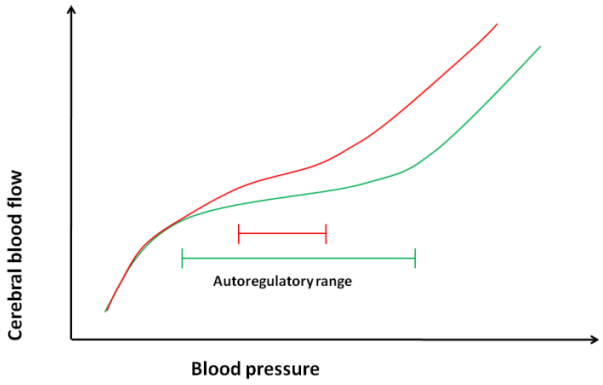

Figure 1
Schematic drawing of the concept of autoregulation. The green line represents intact cerebral autoregulation. The red line represents impaired cerebral autoregulation with a narrower autoregulatory range and a steeper slope within this range.

the cerebral vasculature is likely to play a role in the pathogenesis of both types of lesions [14,15]: (i) ischemia induces endothelial necrosis in the fragile capillaries in the germinal matrix with subsequent bleeding during reperfusion [16], and (ii) the poorly perfused white matter is obviously very vulnerable to ischemic events. Furthermore, recent experimental evidence suggests that maturation-dependent vulnerability to ischemia of oligodendrocytes progenitors rather than heterogeneity of regional cerebral blood flow (CBF) in the white matter explains the magnitude and distribution of PVL [17].

3.3 THE CONCEPT OF CEREBRAL AUTOREGULATION

CBF is influenced by arterial blood pressure (ABP), blood gases, metabolic and neurogenic factors. This thesis focuses on cerebral pressure-flow autoregulation, hereinafter simply referred to as CA.

CA is a vital feedback mechanism that acts to maintain a relative constant CBF despite fluctuations in ABP. It works within a certain range, also referred to as the autoregulatory range (Fig.1). Autoregulation is achieved by changes in cerebral vascular tone in response to changes in intravascular pressure: vasodilatation when ABP decreases and vasoconstriction when ABP increases. Outside the autoregulatory range, vasomotor adjustments are exhausted and CBF becomes pressure-passive and varies with changes in ABP. Impairment of CA causes a narrowing of the autoregulatory range, and a steeper slope within it. The clinical implication of impaired CA is that even small fluctuations in ABP are more likely to fall outside the autoregulatory range and, thus, render the cerebral circulation pressure-passive.

Lack of ethically acceptable methods to perform controlled manipulation of ABP limits the knowledge of CA in the human neonate. Animal experiments have demonstrated that the difference between the lower limit of CA and the normal resting mean ABP (MABP) is narrow in the fetal brain [18]. The difference, however increases with increasing gestational age (GA) [19]. This suggests that the vasoregulatory reserve is less in the most preterm infants. Moreover, clinical studies demonstrate a significant inverse relationship between GA and impaired CA [20,21].

The lower limit of CA is not known in preterm infants. Observational studies, however, indicate a lower breakpoint below 30 mmHg [22,23]. However, when MABP drops below the lower autoregulatory limit, it is likely that other mechanisms [24] such as increased fractional oxygen extraction [25,26] will be able to maintain cerebral tissue oxygenation and thus cellular function and integrity until a certain MABP is reached, that is the functional MABP threshold. The functional breakpoint is unknown, but may be as low as 23 mmHg as demonstrated in a group of very low birth weight infants of less than 30 weeks of gestation [26]. When MABP drops beyond this point, the ischemic threshold is reached and brain injury may occur. Again this breakpoint is unknown in preterm infants.

The position and range of the autoregulatory plateau may vary among individual infants [20,27] and according to the underlying pathological process and preexisting insults [28]. Consequently, a general threshold for hypotension seems elusive [29]. Individualized care guided by bedside monitoring of CA could enable effective targeting of cardiovascular interventions aimed at an individualized optimal ABP-range. Such autoregulation-oriented therapy might represent an advance in neonatal care.

3.4 MEASURING CEREBRAL AUTOREGULATION

3.4.1 Static versus dynamic autoregulation

CA can be subdivided into a static and a dynamic component. Static autoregulation describes the steady state response to a change in ABP, whereas dynamic autoregulation describes the immediate CBF response to a sudden change in ABP. Early studies on CA measured static CA by repeated measurements of CBF at steady state at different ABP levels or between different infants. These earlier studies were limited by a low time resolution in available methods (i.e. indicator methods such as xenon clearance and the oxygen dilution technique using near-infrared spectroscopy (NIRS)). Newer techniques with a higher time resolution, such as continuous NIRS, have enabled insight into the dynamic autoregulatory response. In human adults, the static and dynamic autoregulation are highly correlated during intact and pharmacologically abolished CA [30]. However, given that impairment of the autoregulatory process initially reduces the response time and then eventually destroys the ability to react to changes in ABP, it seems likely that dynamic CA is initially more affected than static CA if minor impairment occurs [31,32].

3.4.2 Response time

The autoregulatory response to a sudden change in ABP is effective within 2-10 s, as documented in adult humans [33] and in experimental studies [34]. Consequently, CBF measured with fast methods such as laser-Doppler flow (LDF) initially varies with changes in ABP, even when dynamic CA is actually working normally. This response time is not captured by oxygenation based flow measurements such as NIRS [35]. This is explained by the time it takes before a change in CBF is visible as a change in capillary and venous saturation.

3.4.3 Use of frequency analysis to measure dynamic autoregulation

Ideally, clinical monitoring of CA in preterm neonates should be noninvasive, continuous, bedside and precise. As static measurements provide point measurements, only the dynamic approach is applicable. Recently, several studies have used frequency analysis between changes in cerebral oxygenation as measured by NIRS and spontaneous changes in MABP to measure dynamic CA noninvasively and continuously at the bedside in preterm

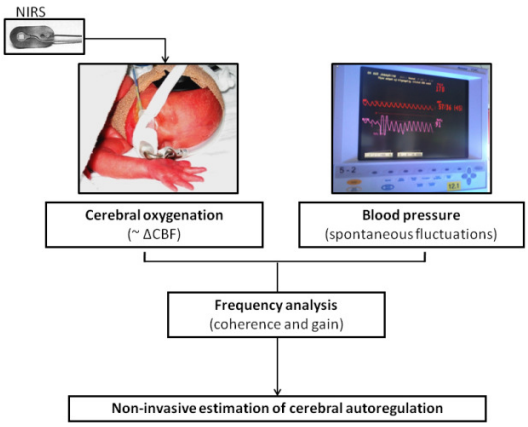

Figure 2
Schematic drawing illustrating the use of near infrared spectroscopy (NIRS) to measure dynamic cerebral autoregulation noninvasively in infants equipped with an arterial line. Changes in cerebral oxygenation are used as a surrogate measure of changes in cerebral blood flow (CBF). Coherence detects impairment, whereas gain estimates the degree of impairment.

neonates (Fig. 2) [20,21,36-39]. The repeatability and validity of this method have, however, not been assessed. By testing CA on the basis of spontaneous changes in MABP there is a considerable risk of a low signal-to-noise ratio when spontaneous fluctuations in MABP are small. In this case, variability in cerebral oxygenation is in fact dominated by "noise": (i) physiological noise (variability of arterial content of CO_2 and arterial saturation (SaO_2)) or (ii) instrumental noise. Thus, the association between the amount of spontaneous variability in MABP and the precision of estimated CA needs to be explored. Furthermore, the method needs to be validated against a conventional measure of CA. We initiated study I and II to address these issues.

The NIRS technology uses near infrared light (wavelengths from 700 to 1000 nm) to detect changes in concentration of oxygenated and deoxygenated hemoglobin. The measuring depth is 2-3 cm [40]. Placed on the head of preterm infants, the light easily transilluminates the thin layers of the skull and bone. Thus, the transmitted light is assumed to reflect cerebral oxygenation. Experimental studies have demonstrated that changes in the oxygenation index (OI), that is the difference between oxyhemoglobin and deoxyhemoglobin divided by a factor of 2 [41], reflect changes in CBF [42-44]. Consequently, continuous monitoring of OI can be used as an indicator of changes in CBF. This can, however, only be assumed if cerebral oxygen consumption and SaO_2 are unchanged [44].

CA is reflected by both its quality and its quantity, or in other words, by its presence and efficacy. Frequency analysis between spontaneous changes in MABP and NIRS derived cerebral oxygenation reflects both of them. Coherence reflects its presence, whereas gain reflects its efficacy [45,46].

Coherence represents the fraction of change in cerebral oxygenation that can be linearly explained by a change in MAPB. Similar to a coefficient of correlation, coherence ranges from 0 to 1, with 1 indicating perfect correlation and 0 complete lack of correlation at a given frequency range. Strictly speaking a coherence exceeding the threshold for significant coherence only indicates that CA is not working perfectly, whereas gain reflects the magnitude of pressure passivity. That is change in cerebral oxygenation, as a surrogate measure of CBF, per change in MAPB. A low gain would indicate that although CA was not perfect, at least the magnitude of changes in CBF was small or moderate. Similarly, a high gain would indicate that even moderate changes in MABP were associated with large changes in CBF. Unfortunately, whereas coherence is frequently reported in the literature [20,36,37,39,44,47], gain is not [21,38].

Most work concerning dynamic CA in neonates has been done in the frequency domain. Theoretically, the advantages for analysis in the frequency domain compared to simple correlation in the time domain are the following: (i) it takes account of the fact that CA may be composed of responses with different temporal properties [48], and (ii) it eliminates the effect of time lag between changes in MABP and cerebral NIRS as a consequence of cerebrovascular transit time being ~10 sec in neonates [49].

3.5 CEREBRAL AUTOREGULATION IN THE PRETERM NEONATE

3.5.1 Intact or impaired autoregulation

Several studies have investigated CA in the preterm brain, since the first demonstration of a pressure passive CBF in a group of distressed neonates in the first hours after birth [50]. Apparently, studies measuring static and dynamic CA seem to disagree about the presence of intact CA in stable preterm neonates. Generally, studies on static CA report an intact autoregulatory function [22,51-54], whereas studies on dynamic CA report a variable degree of impairment [20,21,27,36,47,55,56]. The difference may, however, be caused by methodological differences. Studies on static CA have based their conclusion of an intact CA on the fact that the lower confidence interval of the CBF-MABP reactivity encompassed zero. This approach does not exclude a considerable slope of the CA curve [57]. Studies on dynamic CA, on the contrary, are based on a large amount of data in each infant. Consequently, statistically significant correlations between CBF and MAPB (i.e. significant coherence) can be found even in case of a relative weak relation. Alternatively, the difference might simply reflect a temporal heterogeneity of CA [32].

3.5.2 Association to adverse neurological outcome

Also, studies seem to diverge on the association between impaired CA and adverse neurological outcome. Some studies have demonstrated an association between impaired CA and IVH [36,38,53], whereas others have not [20,21,27,56]. Also, Wong et al. demonstrated a significant association to neonatal death [21]. The discrepancy might have several reasons: (i) statistical uncertainty of small studies, (ii) methodological differences (timing of neuroimaging, methods of defining impaired CA etc.), (iii) differences in predisposing events causing a variable distance between the autoregulatory and the ischemic threshold in the brain or (iiii) clinical differences (impaired CA is only the basis for adverse neurological outcome, additional events, such as excessive fluctuations in ABP or blood gases, ultimately trigger brain injury [16]). Finally, it is important to emphasize that although impaired CA has been associated with adverse neurological outcome, a causal relationship has never been demonstrated. The statistical significant association may simply reflect the fact that the sickest infants tend to have (the most) impaired CA.

3.6 INFLAMMATION AND CEREBRAL AUTOREGULATION

Intrauterine inflammation is a major cause of preterm birth [58-60]. Epidemiological studies point to an association between intrau-

terine inflammation on one hand and cerebral injury and impaired neurodevelopmental outcome on the other hand [10-13,61-65]. In addition to the cytotoxic consequences of inflammation, inflammation-induced vasoparalysis, triggering a combination of impaired CA and hypotension, might partly explain this association [7].
Hypotension has generally [10,11,66-69] but not consistently [20,70-72] been associated with intrauterine inflammation. Unfortunately, only few clinical studies have addressed how intrauterine inflammation affects cerebral hemodynamics. Steady state levels of CBF [69] and NIRS derived cerebral oxygenation [72] are unaffected by histological intrauterine fetal inflammation. Additionally, Soul et al. found no association between clinically diagnosed chorioamnionitis and impaired CA [20]. By contrast, histological chorioamnionitis has been associated with decreased variability in cerebral oxygenation [67]. However, it has not been shown whether this altered cerebrovascular responsiveness includes impairment of CA. Thus, a better understanding of a possible association between histological intrauterine inflammation and impaired CA is needed. This is addressed in study III.

3.7 HYPOVOLEMIA AND CEREBRAL AUTOREGULATION
Hypovolemia is common in neonates. It occurs as a consequence of (i) traumatic delivery (placental abruption, fetomaternal hemorrhage, etc.), (ii) postnatal internal hemorrhage (intracranial, pulmonary, etc.), or (iii) excessive fluid loss from the surface, kidney or respiratory tract in very preterm infants.
Hypovolemia elicits a sympathetic response and thus systemic vasoconstriction [73]. Hypothetically, this vasoconstriction might include cerebral vasculature, and thus compromise a CA-mediated vasodilatation causing a rightward shift of the CA-curve towards higher ABP [74,75]. Consequently, CBF might become pressure passive within the perceived "normotensive-range". This effect could justify the use of volume treatment in "normotensive" infants with a medical history indicating hypovolemia. Unfortunately, data regarding the cerebrovascular effect of hypovolemia are conflicting [76] and only few studies, and solely in the adult population, have tested CA actively during hypovolemia [77-79]. Longer periods of recognized hypovolemia are uncommon in neonates. Therefore, we made use of the piglet model from study II, to address how hypovolemia affects dynamic CA (study IV).

3.8 DOPAMINE THERAPY AND CEREBRAL AUTOREGULATION
Hypotension is common in neonatal intensive care [80]. Despite widespread use, the cerebrovascular effect of dopamine – the most frequently used antihypotensive treatment in preterm neonates [81] – remains controversial. Some studies report an increase in CBF [22,82,83], where as others report no change in CBF [84,85]. The same uncertainty appears in newborn animals [86-88]. The discrepancy might, however, partly be explained by differences in the autoregulatory ability.
Theoretically, dopamine therapy can affect CBF indirectly and directly. The indirect effect is mediated by CA: If CA is working perfectly, cerebral vascular resistance is increased to keep CBF unchanged despite a dopamine induced increase in MABP. On the other hand, if CA is impaired, CBF increases in parallel with ABP. The direct effect is mediated at receptor level in the cerebral vasculature: stimulation of α-adrenergic receptors causing vasoconstriction and thus decreased CBF [89], or stimulation of dopaminergic receptors causing vasodilatation and thus increased CBF [90]. A primary effect on cerebral vasculature, however, depends on dopamine to be able to cross the blood brain barrier. This has been demonstrated in preterm infants [91] and in experimental settings [92,93]. Consequently, to study a direct cerebrovascular effect of dopamine therapy, the possible masking effect of CA needs to be negated. To our knowledge, this effect has never been considered. Consequently, we made us of the piglet model from study II, to address how dopamine therapy affects cerebral vasculature when a possible masking effect of CA is negated (study IV).

3.9 AIMS
The overall aim of this thesis was to evaluate a non-invasive method to detect and estimate CA in neonates based on frequency analysis between cerebral oxygenation as measured with NIRS and spontaneous changes in MABP, and to use this method to study a possible association with perinatal inflammation. The specific study aims were:

- To investigate whether adjusting for the varying degree of variability in ABP would lead to a more precise detection of CA (study I)
- To validate the method by comparing it with a conventional measurement of CA in newborn piglets (study II)
- To investigate a possible relationship between impaired CA and systemic inflammation in the first day after very preterm birth (study III)

Also, we made use of the piglet model to examine the following questions (study IV):

- Does hypovolemia in itself impair dynamic CA?
- Does dopamine treatment exert a direct effect on cerebral hemodynamics?

4. SUBJECTS AND STUDY DESIGN

4.1 SUBJECTS

4.1.1 Patients

Participants for study III were recruited among infants born at Department of Neonatology, Copenhagen University Hospital, Rigshospitalet, between February 2008 and March 2010. Infants with GA ≤32 were eligible if they had been fitted with indwelling arterial catheters for continuous ABP monitoring. Infants with major malformations were excluded. We attempted to recruit infants within their first day of life.
Study III was powered to detect a 1 SD difference in coherence between infants with and without fetal vasculitis. With a population SD of 0.19 36, 90% power and α-error of 0.05, 21 infants were required in each group. Consequently, with the incidence of fetal vasculitis estimated at ~35%, a sample size of 60 infants was needed.
Study I was based on a subset of the population in study III, made up of the first 22 infants, who were recruited.

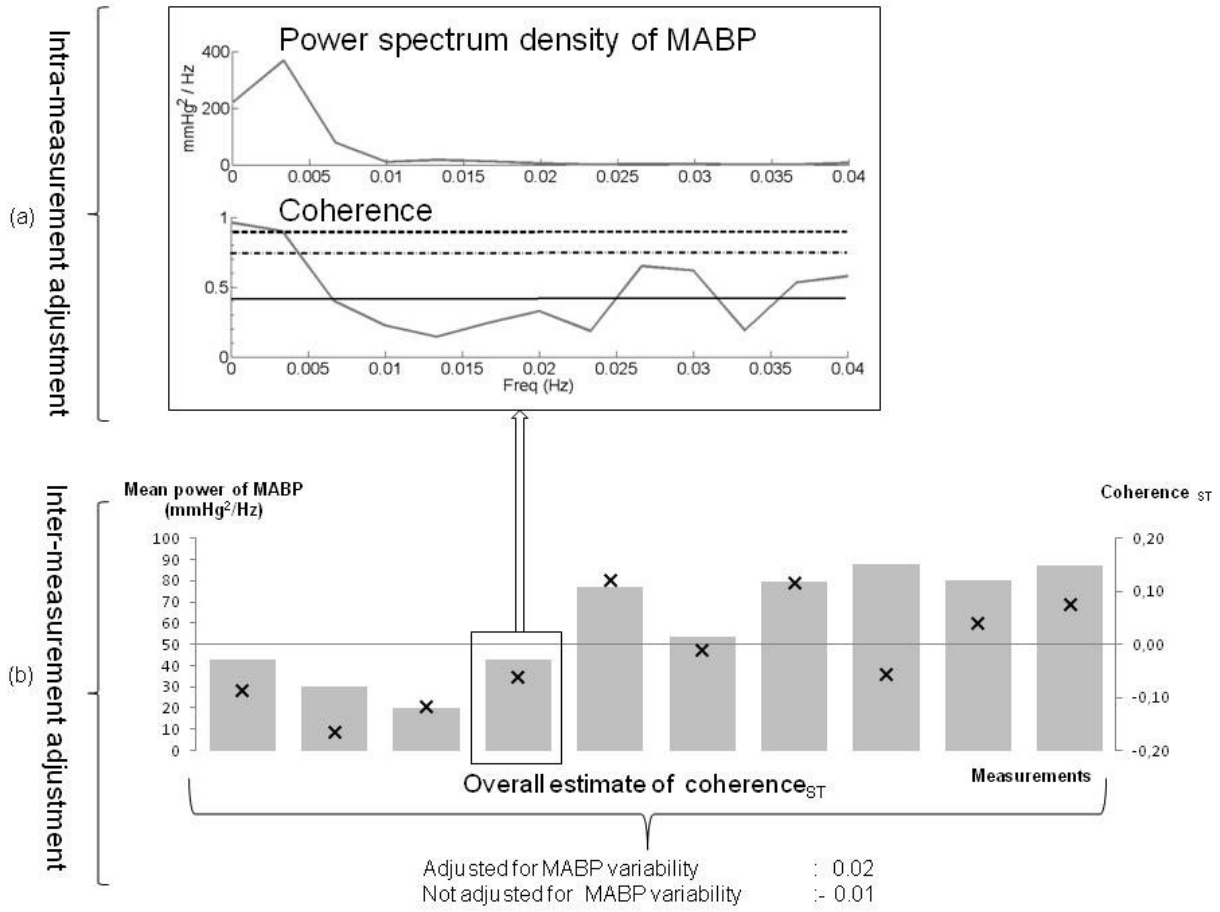

Figure 3
Example of repeated measurements in one infant illustrating the effect of (a) intra-measurements adjustment (Coh_{mean}: solid line, Coh_{wmean}: dashed-dotted line, Coh_{max}: dashed line) and (b) inter-measurements adjustment (b). In this example, the measurements with high MABP variability (reported as power of MABP and illustrated with bars) have the highest $Coherence_{ST}$ (illustrated with cross), and hence, the adjusted overall estimate of coherence is higher than the unadjusted.

4.1.2 Animals

Study II and IV were based on the same 12 piglets (median age: 1.5 (1–3) d; median weight: 1.6 (1.4 –2.0) kg). The newborn piglet is a clinically relevant and frequently applied animal model of hemodynamics in the newborn human brain: (i) the developmental stage corresponds to that of a 36- to 38-wk human infant [94], (ii) brain maturation and cerebral hemodynamics are similar to that of human infants [95] as is the thickness of the skull, and (iii) although the piglet brain is small it is big enough to allow placement of the NIRS optodes to ensure that light transverses the brain.

4.2 STUDY DESIGN

4.2.1 Clinical studies

Study I was an observational methodological study, where we examined (i) whether coherence -as a qualitative measure of CA - would be more precisely determined when changes in MABP were large rather than small, and (ii) the minimum monitoring time needed to reach a robust estimate of coherence in individual infants. To this end, we examined the effect of adjusting for the amount changes in MABP in two steps: (i) within each measurement (intra-measurement adjustment) and (ii) between repeated measurements (inter-measurements adjustment) (Fig.3). For intra-measurement adjustment we calculated coherence in three different ways: (i) as a mean value of the coherence spectrum (Coh_{mean}), (ii) as a weighted mean attaching most weight to the frequencies with highest variability in MAPB (Coh_{wmean}), and (iii) as a point measurement at the frequency with most variability in MABP (Coh_{max}). Inter-measurement adjustment was performed as a weighted analysis, where out of several measurements in each infant, those with large MABP changes were weighted higher than those with small. We hypothesized that CA stayed stable between two successive 10-min measurements, and hence, that a high repeatability reflects a high precision (that is a high signal-to-noise ratio). We chose to favor the analytical approach with the most significant difference between infants. We used Monte Carlo simulations to estimate the level of significant coherence and standardized each measurement of coherence by subtracting this value from it yielding standardized coherence ($Coherence_{ST}$). $Coherence_{ST} \geq 0$ indicates significant coherence and, thus, imperfect CA. Minimum monitoring time needed to reach a robust estimate of coherence was estimated by means of a simulation study examining the statistical power.

Study III was an observational case-control study comparing dynamic CA between (i) infants with and without placental signs

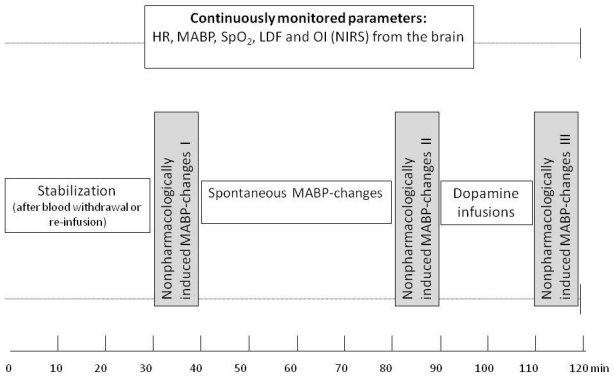

Figure 4
Experimental protocol in the newborn piglet model (*study II and IV*). The illustrated sequence was executed twice in each piglet: once during normovolemia and once during hypovolemia. See text for details.

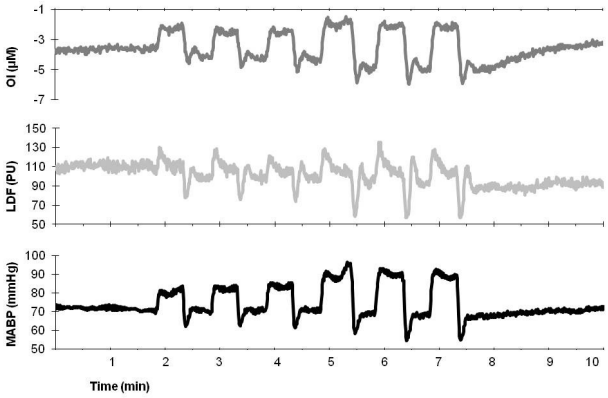

Figure 5
Recording from one piglet showing changes in cerebral laser-Doppler flow (LDF in PU, i.e. arbitrary units) (light grey lines) and cerebral oxygenation index (OI in µmol/l) (dark grey lines) to nonpharmacologically induced changes in mean arterial blood pressure (MABP) (black lines). The thoracic aorta balloon was inflated six times (three times with half its volume and three times with its maximum volume). Inflation lasted 30 sec and pauses lasting 30 sec were interpolated. Note the transient increase in cortical laser Doppler flow as response to sudden changes in MABP. This increase reflects the response time of the autoregulatory response. Cerebral oxygenation did not capture this response.

of a fetal inflammatory response (FIR), and (ii) infants with and without increased levels of the pro-inflammatory biomarker interleukin-6 (IL-6) in postnatal blood samples. This double layered design, comparing both ante- and postnatal signs of inflammation, was chosen to take account of a possible time lag between histological evidence of fetal inflammation and an ongoing systemic inflammation after birth. We attempted to perform the measurements during the first day of life. The attending physician was responsible for the treatment, and care was not altered for the purposes of our study. According to unit policy, compromised circulation was defined as MABP (in mmHg) ≤ GA (in wk) in combination of at least one clinical sign of circulatory insufficiency. The treatment guideline was: up to 2 x 10 ml saline infusion/kg, followed by dopamine (2-15µg/kg/min) and, occasionally, higher doses of dopamine, epinephrine, dobutamine, and/or glucocorticosteroid. The investigator performing all measurements and subsequent data analysis (G.H.H) was blinded as to inflammation parameters until analyses of the CA variables were completed. Likewise, the pathologist (L.L.M) and the investigator responsible for IL-analyses (N.L) were blinded as to the clinical course and CA measurements.

4.2.2 Animal studies

Study II and IV were experimental studies in newborn anaesthetized (propofol 15-25 mg/kg/h) piglets randomized to either hypovolemia followed by normovolemia or normovolemia followed by hypovolemia. Hypovolemia was induced by withdrawal of 1/3 of the estimated blood volume (weight (g) x 0.7 x 1/3). Normovolemia was re-established by re-infusing the removed blood volume.
Cerebral oxygenation (NIRS) and microvascular perfusion (laser-Doppler flow (LDF)) were monitored during spontaneous and induced changes in MAPB (Fig. 4). Changes in MABP were induced (i) nonpharmacologically by inflation of a balloon catheter placed in the thoracic part of aorta (Fig. 5), and (ii) pharmacologically with dopamine infusions (Fig.6). In study II, CA measured by means of frequency analysis between spontaneous changes in MABP and cerebral NIRS (OI) were contrasted with CA based on changes in cortical LDF during nonpharmacologically induced MABP-changes (block I and II in Fig. 4). In study IV, the cerebrovascular effect of dopamine therapy was estimated by contrasting gain during dopamine infusions with gain from the two surrounding epochs of nonpharmacologically induced MAPB-changes (block II and III in Fig. 4). Furthermore, to explore whether hypovolemia in itself impairs dynamic CA, we contrasted gain during all nonpharmacologically induced MAPB-changes (block I-III in fig. 4) in both states.
After each experiment the piglets were euthanized with Pentobarbitone (100 mg/kg iv).

5. METHODS

5.1 MEASUREMENT OF CEREBRAL AUTOREGULATION

5.1.1 Noninvasive method in clinical settings

The infants were clinically stable during measurements, where NIRS (NIRO-300, Hamamatsu Phototonics, Hamamatsu City, Japan), MABP and SaO_2 (assessed by pulse oximetry) were sampled simultaneously at 2 Hz to a laptop for off-line analysis. Data were automatically divided into 10-min epochs of uninterrupted data. Recording was stopped automatically, if changes in SaO_2 exceeded 5%. The NIRS probes were fixed in a non-transparent probe holder (interoptode distance = 4 cm) and secured to the frontotemporal or frontoparietal region of the head with a flexible bandage. Cerebral oxygenation and changes in the relative concentrations of oxygenated and deoxygenated hemoglobin were recorded and used to calculate the OI as a surrogate measure of changes in CBF.
Coherence and gain were computed in two frequency bands: the very low frequency range (VLF) (0.003 - 0.04 Hz) and the low frequency range (LF) (0.04 - 0.01 Hz), corresponding to periodic variations occurring over 25-300 and 10-25 sec, respectively (Matlab, Math Works). Epochs were detrended and subdivided into three 5-min segments with 50% overlap. A Hanning window was applied to minimize spectral leakage.

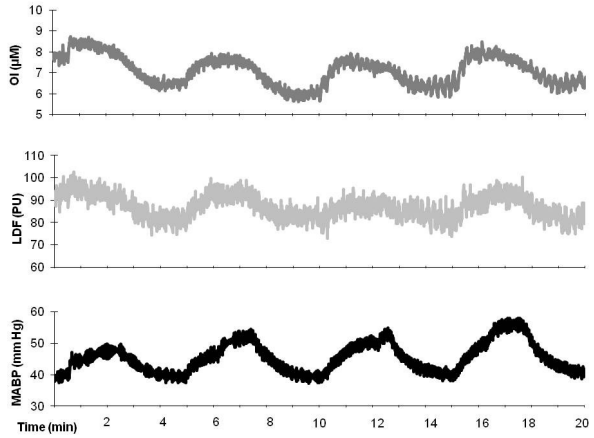

Figure 6
Recording from one piglet showing changes in cerebral laser-Doppler flow (LDF in PU, i.e. arbitrary units) (light grey lines) and cerebral oxygenation index (OI in µmol/l) (dark grey lines) to pharmacologically induced changes in mean arterial blood pressure (MABP) (black lines). MABP was raised with repeated dopamine infusions with increasing dose rates (20, 30, 40 and 50 µg/kg/min). These dose rates were chosen, since in pilot studies, lower doses did not increase MABP. Each infusion lasted 2½ min, interpolated by pauses lasting 2½ min.

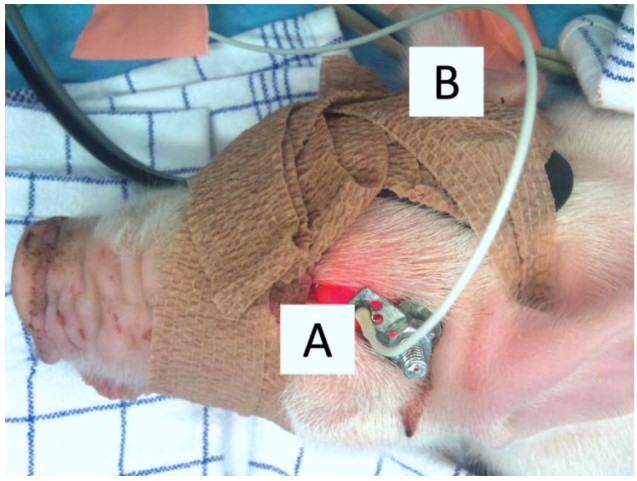

Figure 7
Picture showing the placement of the LDF probe (A) and NIRS optodes (B) on the head of a piglet. The LDF probe was placed in a metal washer that was glued to the skull enabling stabile measurements. The tip of the probe was placed in a 4-mm craniotomy in contact with dura. The NIRS optodes was placed fronto-parietal opposite the site of the craniotomy.

In study III, we used the results from study I and II to optimize the method. Accordingly, for each 10-min epoch, coherence and gain were averaged over the frequency band, and in each infant, epochs with large variation in MABP were weighted in favor of those with low. Also, based on Monte Carlo simulations in study I, the cut-off for significant coherence was ≥ 0.47 in VLF and ≥0.45 in LF. Moreover, based on results from study II, gain was only used to estimate the magnitude of impaired CA in infants with significant coherence.

5.1.2 Invasive methods in experimental settings

In study II, noninvasive measurements of CA based on frequency analysis between cerebral NIRS (NIRO-300, Hamamatsu Phototonics, Hamamatsu City, Japan) and spontaneous changes in MAPB (see above) were compared with a conventional measurement of CA based on changes in cortical LDF (Perimed 5010, Stockholm, Sweden) during nonpharmacologically induced changes in MABP (Fig. 7). We used the method described by Tiecks et al. and calculated CA capacity as percentage change in estimated cerebrovascular resistance (CVR_e) in relation to change in MABP 96. CVR_e was calculated as MAPB/laser Doppler flux. We used a mean over the last 15 sec during balloon inflations and deflations, to allow steady state to be obtained (see Fig. 5). Subsequently, CA capacity was calculated as (%ΔCVR_e/%ΔMABP) x 100%. Thus, CA capacity is expressed as percentage of full autoregulatory capacity, with perfectly working CA yielding a value of 100% and completely abolished CA yielding a value of 0%.
In study IV, frequency analysis (Matlab, Math Works) in the VLF range yielding gain between nonpharmacologically induced changes in MABP and the corresponding percentage change in LDF were used to characterize CA during normo-and hypovolemia, respectively.

5.2 MEASUREMENT OF THE CEREBRAL VASCULAR EFFECT OF DOPAMINE THERAPY

Study IV was designed to bypass a possible masking effect triggered by CA on the cerebrovascular effect of dopamine. This was accomplished by contrasting frequency gain (in the VLF range) during dopamine infusions and during nonpharmacologically induced changes in MABP (Matlab, Math Works). To study the effect on both oxygenation and perfusion, gain was estimated for both OI (gain-OI) and percentage change in LDF (LDF-gain).

5.3 INFLAMMATION

In study III, cases were identified according to signs of systemic inflammation. We used placental histology to identify antenatal (preceding) inflammation and the level of the proinflammatory cytokine IL-6 in blood samples to identify postnatal (concurrent) inflammation.

5.3.1 Placental histology

An experienced placental pathologist (L.L.M.) coded placental inflammation as suggested by Redline et al. [97]. According to this definition, FIR is diagnosed in case of neutrophils infiltrating the vessels on the fetal side of placenta (fetal vasculitis), i.e. chorionic vessels and /or umbilical vessels. A maternal inflammatory response (MIR) is diagnosed in case of neutrophilic infiltration of the placental membranes.
Given the relatively small sample size, standardized grading and staging were not analyzed. Consequently, FIR and MIR were coded as “present” or “not present”. FIR represent the most serious end of the continuum of intrauterine inflammation [98,99]. In the interests of getting well-defined groups, we decided a priori to use infants with FIR as the case group and infants with no signs of placental inflammation (no FIR or MIR) as the control group. Thus, infants whose placenta only showed signs of MIR were excluded from the placental part of study III (n=7, see Fig.12).

5.3.2 Measurement of IL-6

The postnatal level of IL-6 is considered a reliable marker of an ongoing inflammatory response [11,11,100]. Blood samples (50µl) were collected along with those being drawn for clinical indications as close as possible to the NIRS-measurements. The samples were air-dried and stored as dried blood spots in sealed plastic bags at -20°C until the end of the study. The sample taken closest in time to NIRS measurements was analyzed in duplicate for IL-6 by immunoassay (Luminex xMAP technology, Luminex) [101].
IL-6 was not normally distributed. Consequently, since from a biological perspective, the most extreme concentrations of IL-6 are the most relevant, we dichotomized the distribution into the top quartile (case group) and the lowest three quartiles (control group) [102].

5.4 CLINICAL DATA

Specific clinical data were collected by reviewing the infants´ medical charts: (i) pre- and perinatal events, (ii) clinical treatment within the first 24 h (respiratory support, surfactant, antihypotensive treatment and antibiotics), (iii) neonatal mortality (death before postnatal day 30), (iv) treatment for persistent ductus arteriosus, (v) results from cerebral ultrasound scans and (vi) blood gas and hemoglobin values measured during or closest to NIRS measurements.
The attending physician performed cranial ultrasound scans in accordance with the policy of the unit, at (i) 4–7 d postnatal, (ii) 14 d postnatal, (iii) 35 d postnatal, (iv) full-term age, or pretransfer, or predischarge or (v) as clinically indicated. The scans were recorded using Papile´s classification [103]. Based on this classification hemorrhages are graded according to the distribution of the bleeding: subependymal (grade 1), intraventricular without ventricular dilatation (grade 2), intraventricular with ventricular dilatation (grade 3), and intraventricular with parenchymal hemorrhage (grade 4). Grades 3–4 were considered severe. Cystic PVL was diagnosed in case of periventricular cysts.
In case of transcutaneous CO_2 monitoring during the NIRS measurements, we used a mean value to characterize blood CO_2. Otherwise, values from the blood sample were used.
MABP was calculated as a mean over the entire study period.

5.5 STATISTICS

In general, differences between unpaired samples were compared using Student´s t-test, Fischer´s exact test, or the Mann-Whitney U-test. Differences between paired samples were assessed using paired t-test or Wilcoxon signed-rank test. Normality was checked visually and with Shapiro-Wilk W test for normality. A two-tailed p value ≤ 0.05 was considered significant. All analyses were performed using SPSS 17.0 (SPSS Inc.), except for study I where simulation studies and ANOVA were performed in SAS (SAS 9.1, SAS Institute).
In study I, precision of the different measures of coherence (intra- and intermeasurement adjustment for ABP variability) were assessed by means of ANOVA for repeated measurements with spatial power as covariance structure. This covariance structure reckons with the fact that the variance between repeated measurements is influenced by the different time lag between measurements (due to artifacts, blood sampling, and changes in SaO_2 etc). Level of significance between infants in ANOVA was used as a measure of precision, with a low p value indicating high discrimination between infants and, thus, high precision. We used Monte Carlo simulations to estimate the level of significant coherence: OI and MAPB were reshuffled randomly 10,000 times, and the 95% percentile was taken as the 95% confidence limit of significant coherence. To estimate the minimum monitoring time needed to reach a robust estimate of CA, we examined the statistical power by means of a simulation study. Statistical power was estimated as the percentage among 1000 simulated data sets, where the null-hypothesis (no significant difference in coherence among infants) was rejected.
In study II, the Pearson correlation coefficient r was used to measure the strength of the correlation between CA-capacity and coherence and gain.
In study III, we used Mantel-Haenszel statistics to level out a confounding effect of dopamine on the association between IL-6 and hypotension. Linear regression was used to assess the association between CA and MABP.
In study IV, we assessed whether hypovolemia in itself impairs CA by means of linear regression with change in gain-LDF as dependent and change in MABP as independent variable. A statistically positive intercept would indicate an impairment of CA even when MABP is fully maintained, and thus that hypovolemia in itself impairs CA.

5.6 ETHICS

The clinical studies (study I and III) were approved by The Danish Local Ethical Committee (journal no. H-A-2007-0109) and the Danish Data Protection Agency. Written informed parental consent was obtained all infants. These studies are descriptive and did not benefit the participating infant directly. The results obtained in these studies may, however, contribute with a piece of the large puzzle of cerebral hemodynamic in preterm neonates. The overall purpose is to improve neurodevelopmental outcome in preterm infants. The experimental studies (study II and IV) were approved by the Danish Animal Experiments Inspectorate (approval ID: 2009/561-1723).

6. RESULTS AND DISCUSSION

6.1 PRECISION OF COHERENCE ANALYSIS TO DETECT CEREBRAL AUTOREGULATION BY NIRS (STUDY I)

Twenty-two infants with a median GA of 27.5 (24.2 – 29.4) wk and a median birth weight of 940 (460 - 1266) g were studied at a median postnatal age of 17 (4 - 44) h. The majority (91%) was treated with nasal-CPAP and the rest (9%) with mechanical ventilation.

6.1.2 Adjustment for variability in blood pressure

Unexpectedly, adjusting for variability in MABP within each measurement did not improve the precision. The mean coherence (Coh_{mean}) discriminated significantly between infants in both frequency bands (p = 0.001 in both VLF and LF), whereas the weighted mean (Coh_{wmean}) only discriminated significantly in LF (p value 0.09 in VLF and 0.01 in LF). Coherence at the frequency with maximum variability in MABP (Coh_{max}) did not discriminate significantly between infants. Fig.8 illustrates this difference on infant level. Inter-measurement adjustment for variability in MABP, however, increased the precision of the mean coherence (Coh_{mean}). In VLF the p value changed from 0.0013 to 0.0007 and in LF from 0.0012 to 0.0001.

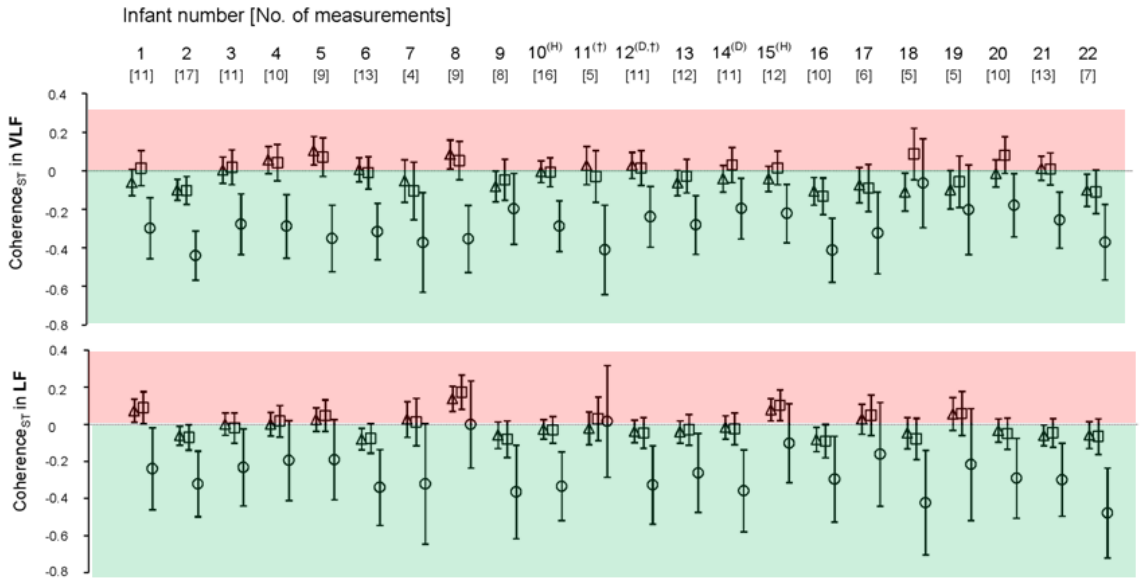

Figure 8
Estimated standardized coherence ($Coherence_{ST}$) and no. of measurements (in []) in each infant illustrating the effect of intra-measurement adjustment for variability in MAPB. Mean coherence (Coh_{mean}) [Δ], intra-measurement MABP weighted mean coherence (Coh_{wmean}) [□] and coherence at the frequency with maximum variability in MABP (Coh_{max}) [○] are shown. Error bars represent 1.96 x SE of the estimate. Infants treated with dopamine during the measurement are marked with $^{(D)}$. Infants who developed IVH grade 3-4 are marked with $^{(H)}$. Infants who died in the first month of life are marked with $^{(\dagger)}$. Green area indicates intact autoregulation ($Coherence_{ST}$ <0), red area indicates impaired autoregulation ($Coherence_{ST}$ ≥0).The mean coherence had the lowest SE.

To our knowledge, we are first to investigate the influence of variability in MABP on coherence between spontaneous fluctuations in MABP and cerebral NIRS. Our finding of an increased precision, when measurements with high variability in MABP are weighted in favor of those with small, are in line with previous studies on CA based on frequency analysis between Doppler measurements of CBF velocity and spontaneous changes in MAPB [104,105]. It has been proposed to exclude data with a minimal variability in MABP [45,104]. In practice, this approach will result in considerable data loss, as the variability in MABP can be quite low over a sizeable length of time. It is important to use data as effectively as possible when research involves vulnerable patients, such as preterm infants. Thus, our approach seems relevant for research studies comparing CA among vulnerable patients.

6.1.2 Monitoring time and frequency dependency

We investigated the minimum monitoring time needed to reach a robust estimate of coherence (i.e. a statistical power of 80%) by plotting statistical power against number of measurements for the three coherence measures in both frequency bands (Fig. 9). The minimum monitoring time was lowest for the mean coherence, where 22 (3.7 h) and 8 (1.3 h) measurements were needed in VLF and LF, respectively. The weighted mean coherence did not reach a power of 80% within 35 measurements (5.8 h). For coherence at the frequency with maximum variability in MABP, power did not change with an increasing number of measurements. Furthermore, the simulation study showed a remarkable difference in statistical power between the two frequency bands, indicating that a three times longer recording time is needed in VLF compared to LF. We speculate that this finding might be caused by the fact that other regulatory mechanisms have more influence in the VLF band. As 91% of our infants were breathing spontaneously, irregular fluctuations in arterial content of CO_2 might partly explain this finding. We are unaware of other studies exploring the minimum monitoring time needed to reach a robust estimate of coherence as a way to detect impairment of CA.
The frequency dependent relationship of coherence is plotted in Fig. 10. Even though, the difference between VLF and LF was insignificant (p = 0.3), the scatter plot clearly demonstrates lack of concordance: only 12 infants had concordant results. This might imply a frequency dependent nature of CA with infants having an

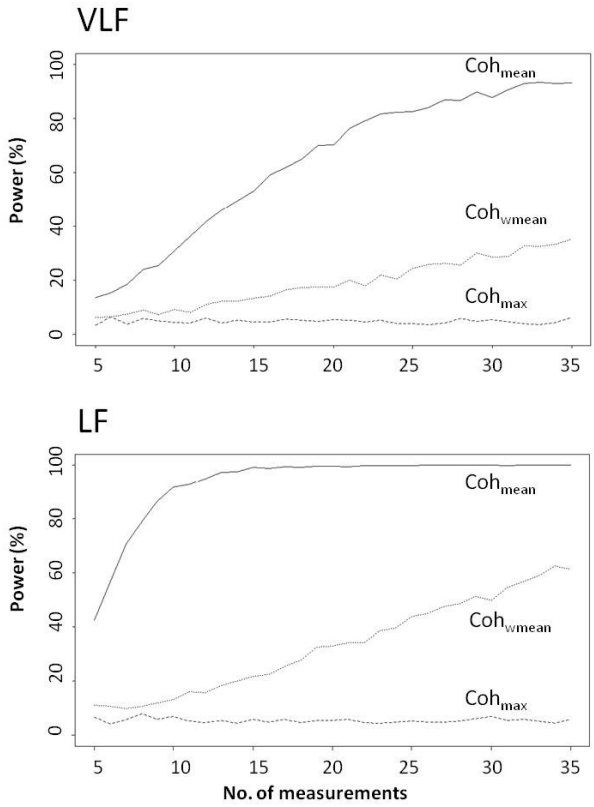

Figure 9
Graphs illustrating the minimum monitoring time needed to reach a robust estimate of coherence. Statistical power is plotted against number of simulated measurements in both frequency bands (VLF: 0.003-0.04 Hz, LF: 0.04-0.1 Hz) for the three coherence measures. Each measurement lasted 10 min.

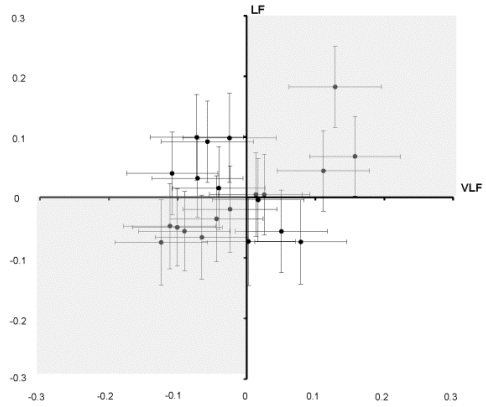

Figure 10
Scatter plot of estimated $Coherence_{ST}$ in VLF and LF illustrating the amount of concordance. Error bars represent 1.96 x SE of each infant´s $Coherence_{ST}$ [•]. The shaded areas indicate concordance.

impaired CA at higher frequencies and an unaffected one at lower frequencies (upper left quadrant in Fig. 10). Furthermore, a higher amount of physiological noise in VLF compared to LF might falsely indicate an unaffected CA at low frequencies (lower right quadrant in Fig. 10).
In conclusion, a reliable detection of CA requires several hours of monitoring. However, the precision can be improved by adjusting for the varying degree of variability in MABP between repeated measurements.

6.2 VALIDATION OF NIRS TO MEASURE CEREBRAL AUTOREGULATION NONINVASIVELY (STUDY II)
To some surprise, a high coherence between cerebral NIRS and spontaneous MABP changes was only weakly and statistically insignificantly correlated with a low CA-capacity as measured with LDF during nonpharmacologically induced MABP-changes in VLF ($r = -0.34$, $n = 24$, $p > 0.05$). Gain was also poorly and statistically insignificantly correlated with CA-capacity. However, in the subgroup of measurements with significant coherence (i.e. coherence exceeding the limit of significance), we found a strong correlation between gain and CA-capacity ($r = -0.55$, $n = 15$, $p = 0.03$) with a high gain associated with a low CA-capacity (Fig.11). In LF, all correlations were poor and insignificant, which might be due to a low spontaneous variability of MABP in this frequency range. In principle, coherence exceeding the level of significant coherence only indicates that CA is not working perfectly. Gain, on the other hand, estimates the actual magnitude of this impairment.

From this point of view, our finding was expected. Our results clearly demonstrate that detection of impaired CA based on significant coherence classifies measurements with minor impairment (high CA-capacity and low gain) together with measurements with major impairment (low CA-capacity and high gain) (Fig. 11C). Consequently, association to clinical outcome seems most reasonable for gain and not just coherence. This point is supported by findings from clinical studies. In a study by Soul et al. [20], significant coherence was frequent but not associated with IVH in preterm newborn infants. However, reanalysis demonstrated a significant association between high gain and IVH in the subgroup of infants with significant coherence [38].
In conclusion, our data validate frequency analysis between spontaneous changes in MABP and cerebral NIRS for noninvasive estimation of CA. In clinical research, larger study groups can compensate for a limited precision. For clinical use, the precision, however, seems insufficient.

6.3 INFLAMMATION AND CEREBRAL AUTOREGULATION (STUDY III)
We examined 60 infants (male/female: 36/24) with a GA of 27 (±1.3) wk and birth weight of 908 (± 258) g (mean±SD). GA and birth weight did not differ significantly between infants recruited into the study and those (i) whose parents declined participation and (ii) who were not considered for participation (Fig.12). Measurements were performed at a postnatal age of 18 (± 9.4) h and lasted 2.3 (± 0.5) h.

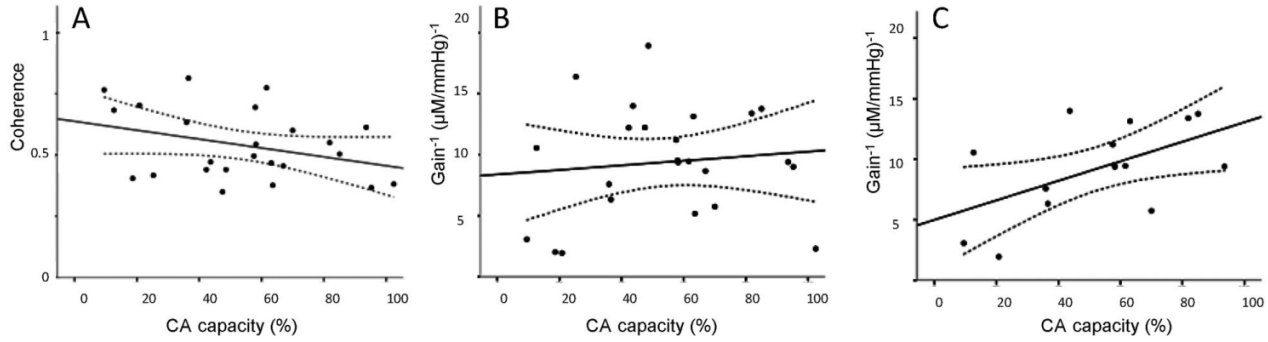

Figure 11
Scatter plots of NIRS-derived measures of CA during spontaneous changes in MABP versus LDF-derived CA-capacity during nonpharmacologically induced changes in MABP (VLF range). Gain was transformed logarithmically to obtain normality. Correlation with coherence (A) (r = -0.34, n = 24, p > 0.05) and gain (B) (r = -0.55, n = 24, p > 0.05) was weak. In the subgroup of measurements with significant coherence (C), however, gain was strongly correlated to CA capacity (r = -0.55, n = 15, p = 0.03). Dashed lines are 95% CI.

6.3.1 Overall hemodynamics

CA was weakened with decreasing MABP, as gain increased significantly with decreasing MABP adjusted for GA (i.e., MABP in mm Hg minus GA in wk) in the LF range (p = 0.02). Thus, in infants with
impaired CA, i.e. significant coherence, CBF apparently follows changes in MABP within the perceived "normotensive-range" (Fig.13). The same correlation existed in the VLF-range although this was not statistically significant (p = 0.08). This association is well described in clinical studies [20,22,27].

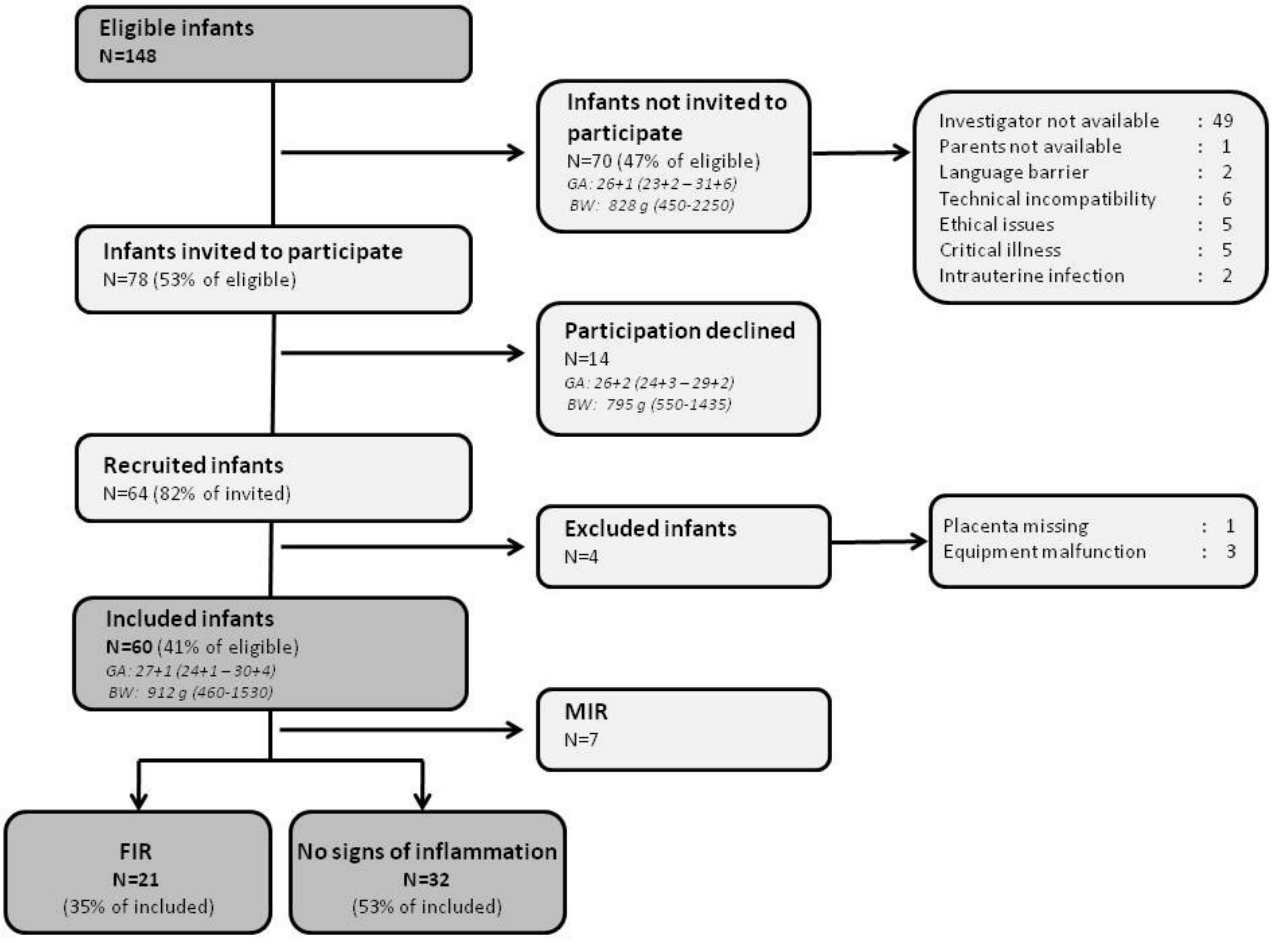

Figure 12
Flow diagram of eligible infants and placenta histology. BW, birth weight, FIR, fetal inflammatory response, GA, gestational age; MIR, maternal inflammatory response.

6.3.2 Inflammation and hemodynamics

As compared with infants in the control group, those with FIR had significantly lower GA (p = 0.001) and were significantly less

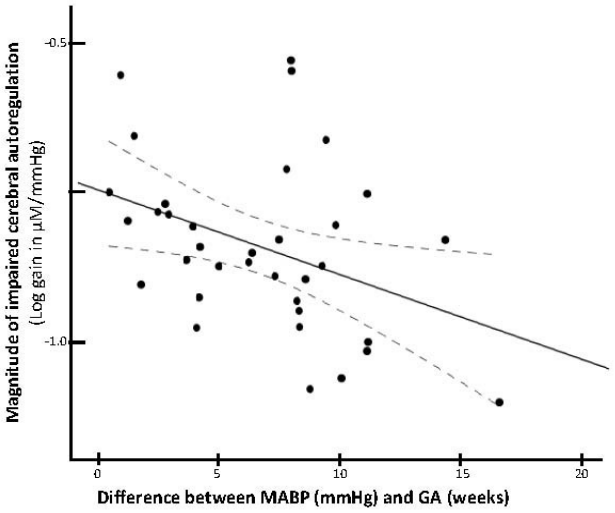

Figure 13

Association between magnitude of impaired autoregulation (i.e. gain , transformed logarithmically) and age-adjusted mean arterial blood pressure (MABP in mm Hg minus GA in wk).Linear regression revealed a significant negative association (*p* = 0.02). The broken lines represent the 95% CI.

growth-retarded (p < 0.001). In the first day of life, significantly fewer infants received surfactant (p = 0.02) and treatment for hypotension (p = 0.048) relative to controls, whereas significantly more infants received antibiotics (p = 0.006). MABP did not differ between infants with FIR and controls (Table 1). Surprisingly, IL-6 was significantly lower in infants with FIR than in the controls (52ng/l (8-448) vs. 126 ng/l (8-4,000); p = 0.01).

Infants with a high level of IL-6 in postnatal blood samples (postnatal age: 18±10.4 h) were more likely to be hypotensive (p = 0.008), for which they received volume (p = 0.035) and dopamine (p = 0.02). The association between hypotension and a high level of IL-6 persisted after controlling for dopamine therapy as a confounder (p = 0.03). Infants with a high level of IL-6 received more surfactant (p = 0.001) and were more likely to receive mechanical ventilation (p = 0.02) than controls (Table 1).

Antenatal steroids might explain why IL-6 was associated with hypotension, whereas FIR was not. Evidence exists that antenatal steroids seem to eliminate the association between FIR and hypotension [71] and between FIR and brain injury in preterm infants [106,107]. Alternatively, the difference might simply indicate that systemic inflammatory activity on the first day of life is not initiated in utero.

Autoregulatory parameters (i.e. no. of infants with significant coherence and gain in these infants), cerebral oxygenation, no. of infants with IVH, and no. of neonatal deaths were not affected by FIR or IL-6. There was, however, a trend towards a more severely impaired CA (i.e. higher gain) and a lower cerebral oxygenation in infants with FIR (Table 2).

We are first to address whether systemic inflammation is associated with impaired CA in preterm infants. Our finding of no statistically significant association between inflammation and impaired CA is in line with clinical studies showing no affect on steady state levels of CBF [69] and cerebral oxygenation [72], as well as no association between clinical evidence of chorioamnionitis and impaired CA 20. Contrary to our findings, experimental studies generally point towards an association between inflammation and impaired CA [108-110]. The discrepancy might have several reasons: (i) Lack of MABP-measurements in experimental settings. Thus, inflammation-induced hypotension might explain the finding of decreased CBF in experimental settings; (ii) a dose-dependent association [111], i.e., a higher inflammatory activity in experimental settings; (iii) lack of a clinically relevant animal model with antenatal administration of steroids [107]; and (iv) we cannot exclude that our setup with point measurements lasting approximately two hours might have missed periods during which, CA was indeed impaired.

In conclusion, postnatal inflammation was significantly associated with hypotension, and a low MABP with impaired CA. Our hypothesis could, however, not be confirmed, as neither antenatal nor postnatal signs of inflammation were significantly associated with impaired CA. In clinical terms, these results imply that, provided MABP is kept within the autoregulatory range, CA is at most moderately affected by inflammation on the first day after preterm birth. Thus, mechanisms other than impaired CA mediate the association between inflammation and brain injury.

6.4 HYPOVOLEMIA AND CEREBRAL AUTOREGULATION (STUDY IV)

As expected, blood withdrawal resulted in a significant increase in pulse rate (198±8 vs. 217±6 bpm, mean difference: 19, 95%CI: 5 to 33, p = 0.01) and a significant decrease in hemoglobin (7.7±0.4 vs. 6.8±0.3 g/dL, mean difference: -0.9, 95%CI: -0.5 to -1.4, p = 0.0004), whereas $PaCO_2$ (5.4±0.9 vs. 5.2±0.8 kPa, mean difference: -0.2, 95%CI: -0.8 to 0.5, p = 0.6) and pH did not change significantly.

Hypovolemia elicited a variable but significant decrease in MABP (68 (47-77) vs. 60 (23-73), mm Hg, p = 0.02) combined with an increasing impairment of CA (Gain-LDF increased from 1.1 (0.3-1.7) to 1.4 (0.6-3.4) %/mm Hg, p = 0.01) (Fig.14). This finding was expected, as CBF becomes pressure passive, when MABP drops below the lower autoregulatory breakpoint [20,22,27]. Linear regression with change in Gain-LDF as dependent and change in MAPB as independent factor revealed a positive intercept that did not differ significantly from zero (beta: 0.2 % pr mm Hg; 95%CI: -0.1 to 0.6). This indicates a trend but no statistically significant shift in the CA curve towards a higher MABP during hypovolemia. Only few studies have tested CA actively during hypovolemia and these studies support our result [77-79].

In conclusion, our study did not support the hypothesis that dynamic CA is affected significantly by hypovolemia itself. This does not point to rational use of volume treatment to secure CA, and thus CBF, in normotensive infants with a medical history indicating hypovolemia.

6.5 CEREBRAL VASCULAR EFFECTS OF DOPAMINE THERAPY (STUDY IV)

Dopamine infusion increased cerebral perfusion, whereas cerebral oxygenation was unaffected. Mean gain-LDF was 3.0 %/mmHg during dopamine induced changes in MABP versus 1.4 %/mmHg during nonpharmacologically induced changes in MABP (mean difference: 1.5 %/mm Hg; 95%-CI: 0.5 to 2.6; P = 0.007). The mean gain-OI was 0.12 µM/mmHg during dopamine induced changes in MABP versus 0.11 µM/mmHg during nonpharmacologically induced changes (mean difference: 0.01 µM/mmHg; 95%-CI: -0.03 to 0.05; p = 0.7) (Fig.15).

	Placental examination				Postnatal blood sample			
	FIR (n=21)	No inflammation (n=32)	p value		IL-6 in the top quartile (n=15)	IL-6 in the lower three quartiles (n=45)	p value	
Gestational age (weeks)	26+3 ± 0.2	27+4 ± 0.2	0.001	*	26+5 ± 0.3	27+1 ± 0.2	0.27	
Birth weight (g)	950 ±49	895±50	0.5		808 ± 70	941 ± 36	0.08	
Growth retardation (%)	4 ± 2.9	-18± 3.3	<0.001	*	-16± 5.3	-7± 2.7	0.09	
Sex (no of boys (%))	11 (52%)	19 (59%)	0.8		10 (67%)	26 (58%)	0.8	
Antenatal								
Prolonged rupture of membranes (no (%))	11 (52%)	7 (22%)	0.02	*	2 (87%)	17 (38%)	0.11	
Interval between membrane rupture and birth (days)	8 (1 - 53)	4 (1 - 31)	0.2		17 (3 -31)	6 (1 -53)	0.9	
Intrapartum fever (no (%))	2 (10%)	1 (3%)	0.3		1 (7%)	3 (7%)	1	
Antenatal corticosteroids (no (%))	21 (100%)	29 (91%)	0.2		15 (100%)	42 (93%)	0.6	
Delivery								
Spontaneous labour (no (%))	18 (86%)	14 (44%)	0.004	*	7 (47%)	30 (67%)	0.2	
Umbilical cord pH	7.4 ± 0.03	7.3 ± 0.01	0.1		7.3 ± 0.02	7.3 ± 0.02	1	
Apgar at 5 min	10 (6 - 10)	10 (2 -10)	0.2		10 (7-10)	10 (2-10)	0.7	
Medical therapy in the first day of life								
Dopamin (no (%))	2 (10%)	11 (34%)	0.053		8 (53%)	9 (20%)	0.02	*
Volume (no (%))	5 (24%)	15 (47%)	0.15		10 (67%)	15 (33%)	0.035	*
Dopamine or volume (no (%))	5 (24%)	17 (53%)	0.048	*	10 (67%)	17 (38%)	0.07	
Surfactant (no (%))	11 (52%)	27 (84%)	0.02	*	15 (100%)	26 (58%)	0.001	*
Antibiotics (no (%))	19 (91%)	17 (53%)	0.006	*	12 (80%)	27 (60%)	0.22	
Clinical state at NIRS measurement								
Postnatal age (h)	19.6 ± 1.7	17.4 ± 2.0	0.4		20 ± 2.3	18 ± 1.4	0.4	
MAP (mmHg)	32 ± 0.9	33 ± 0.8	0.5		31 ± 1.2	34 ± 0.6	0.02	*
MAP ≤ GA in weeks (no (%))	2 (10%)	5 (16%)	0.7		5 (33%)	2 (4%)	0.008	*
Nasal-CPAP (no (%))	15 (71%)	18 (56%)	0.4		4 (27%)	33 (73%)	0.02	*
Mechanical ventilation (no (%))	6 (29%)	14 (44%)	0.4		11 (73%)	12 (27%)	0.02	*
Inspiratory oxygen concentration (%)	23 (21 -40)	25 (21-38)	0.4		27 ± 1.4	25 ± 0.8	0.3	
Arterial oxygen saturation (%)	94 ± 0.7	93 ± 0.4	0.2		93 ± 0.8	94 ± 0.4	0.3	
Blood CO_2 (kPa)	5.6 (4.4 - 8.4)	5.9 (4.6 - 8.4)	0.3		6.3 (5.2 - 8.4)	5.6 (4.2 - 8.4)	0.01	*
Hemoglobin (mM)	8.5 ± 0.3	9.2 ± 0.2	0.04	*	9.4 ± 0.3	8.9 ± 0.2	0.16	

Table 1
Clinical characteristics according to placental examination and IL-6. Median (min-max), mean (±SEM). * indicates $p \leq 0.05$.

This study is the first of its kind, designed to circumvent a possible indirect effect of CA on the cerebrovascular effect of dopamine therapy. Also, we are first to address dopamine´s effect on both cerebral perfusion and oxygenation in the same model. Surprisingly, dopamine infusion resulted in a mismatch between cerebral perfusion and oxygenation, as perfusion increased, while oxygenation was unaffected (Fig.15). Our finding of increased perfusion indicates that dopamine elicits cerebral vasodilatation. The observed mismatch might be explained by physiological and methodological factors: (i) The NIRS-derived OI is only a valid surrogate-measure of CBF, if cerebral metabolic rate and SaO_2 remain unchanged [44]. In our study, SaO_2 was kept unchanged, thus, a dopamine-induced increase in cerebral metabolism, might explain the mismatch [112-114]. (ii) Heterogeneous flow in the cerebral microcirculation, with dopamine increasing flow in some microcirculatory territories but not in others, represents another possible physiological explanation. Increased heterogeneity increases median oxygen diffusion distance [115]. Consequently, the average cerebral oxygenation, as measured by NIRS, will be increased proportionally less. (iii) Extracranial contamination of the NIRS-signal represents a possible methodological explanation116, as dopamine induced vasoconstriction in the skin and skull might contaminate the NIRS-signal. Unfortunately, we are left with speculations.

In conclusion, our findings suggest a direct vasodilator effect of dopamine infusion with increased cerebral perfusion. This effect was not reflected in cerebral oxygenation as measured with NIRS. Speculatively, this mismatch could reflect a dopamine induced increase in cerebral metabolism or microvascular heterogeneity, or simply extracranial desaturation from dopamine induced peripheral vasoconstriction.

7. GENEREL METHODOLOGICAL CONSIDERATIONS

	Placental examination			Postnatal blood sample		
	FIR (n=21)	No inflammation (n=32)	p value	IL-6 in the top quartile (n=15)	IL-6 in the lower three quartiles (n=45)	p value
Cerebral hemodynamics						
Duration of measurement (h)	2.2 ± 0.13	2.3 ± 0.09	0.5	2.3 ± 0.1	2.3 ± 0.1	0.94
Coherence in VLF	0.46 (0.37-0.58)	0.47 (0.34-0.63)	0.9	0.46 (0.36-0.57)	0.47 (0.35-0.66)	0.7
Significant coherence in VLF (no (%))	10 (48%)	17 (53%)	0.8	7 (47%)	23 (51%)	1
Gain in VLF in measurements with significant coherence (µM/mmHg)	0.29 (0.14 - 0.50)	0.25 (0.16 - 0.31)	0.12	0.24 (0.21 - 0.28)	0..25 (0.14 - 0.50)	0.57
Coherence in LF	0.47 (0.37-0.62)	0.47 (0.35-0.68)	0.7	0.44 (0.37-0.58)	0.48 (0.35-0.68)	0.4
Significant coherence in LF (no (%))	13 (62%)	18 (56%)	0.8	7 (47%)	28(62%)	0.37
Gain in LF in measurements with significant coherence (µM/mmHg)	0.16 (0.10 - 0.30)	0.13 (0.08 - 0.28)	0.2	0.14 (0.11 - 0.28)	0.14 (0.08 - 0.30)	0.71
Cerebral oxygenation (%)	68 ± 2.2	71 ± 1.4	0.2	71 ± 1.1	70 ± 1.4	0.48
Short-term outcome						
Treatment for persistent ductus arteriosus (no (%))	12 (57%)	13 (41%)	0.3	8 (53%)	22 (49%)	1
Intraventricular hemorrhage (all) (no (%))	5 (24%)	3 (9%)	0.2	3 (20%)	7 (16%)	0.7
Intraventricular hemorrhage (server) (no (%))	4 (19%)	1 (3%)	0.07	1 (7%)	4 (9%)	1
Cystic periventricular leukomalaci (no (%))	0	0		0	0	
Neonatal mortality	3 (14%)	4 (13%)	1	4 (27%)	4 (9%)	0.1

Table 2
Cerebral hemodynamics and short-term outcome according to placental examination and IL-6. Median (min-max), mean (±SEM).

Our noninvasive method to detect and estimate CA has potential limitations. Firstly, coherence assumes linearity between changes in MABP and measures of CBF. Thus, other potential modulators of CBF, such as $PaCO_2$, are not taken into account. Fluctuations in $PaCO_2$ might produce physiological noise and, thus incorrectly low coherence, as changes in $PaCO_2$ instead of MABP drive changes in cerebral oxygenation. We tried to minimize this problem by only investigating infants, who were clinically stable. Secondly, we used the NIRS-derived OI as a proxy estimate of changes in CBF. This assumption is only reasonable as long as SaO_2 and cerebral metabolism are stable during measurements [44]. We kept the impact of variations in SaO_2 to a minimum be rejecting all data with changes in SaO_2 exceeding 5%. We regard the impact of changes in cerebral metabolism as negligible, as (i) infants were clinically stable during measurements and (ii) each measurement lasted only 10 min. Thirdly, the time delay in reaching equilibrium of oxygenation as response to changes in CBF, represent another potential problem. However, since the cerebrovascular transit time in neonates is ~10 sec [49], this does not affect measurements in our frequency range. Fourthly, there is a considerable risk of a low signal-to-noise ratio, and thus incorrectly low coherence, when spontaneous fluctuations in MABP are small. We tried to minimize this problem by weighting measurements with large variations in MABP in favor of those with small. Fifthly, we cannot exclude ascertainment bias, since indwelling catheters are used in the sickest infants, in whom invasive hemodynamic monitoring is indicated. Naturally, this limits the generalizability of our findings. The physiological study of the cerebrovascular effect of hypovolemia and dopamine therapy (study IV) was an opportunistic spin-off from the validation study (study II), where we gave in to the temptation to study clinically relevant issues, that are ethically difficult to study in neonates. Unfortunately, this clustered design limits the level of detail. It is a clear limitation that we did not study steady state responses to dopamine infusions and that we did not study possible alterations in cerebral metabolism and microvascular heterogeneity, as well as possible alterations in extracranial oxygenation. Furthermore, dopamine doses were higher than recommended in clinical practice.
Finally, even though the newborn piglet is a hemodynamic relevant and frequently applied animal model of the newborn human brain, possible interspecies differences warrants caution before extrapolating findings obtained in piglets to the human infant.

8. GENEREL CONCLUSION AND PERSPECTIVES

A reliable detection of CA by means of coherence between NIRS-derived cerebral oxygenation and spontaneous changes in MABP requires several hours of monitoring. The precision can, however, be improved by adjusting for the varying degree of variability in MABP between repeated measurements. The time needed to estimate CA reliably by means of gain is not known. Future studies ought to address this issue. Based on our validation study in newborn piglets, we conclude that this noninvasive method has potential use for detecting and estimating CA in clinical research. Low precision, however, hampers its clinical application. Theoretically, a reliable clinical monitor of CA would enable autoregulatory-oriented therapy to secure stable and adequate CBF. Providing this becomes available in the future, possible improvement in outcome, however, needs to be investigated.
A possible link between inflammation and impaired CA, might partly explain the association between inflammation and brain injury. We demonstrated that impaired CA was associated with a low MABP and a low MABP with postnatal inflammation. There was, however, no significant association between CA and either antenatal or postnatal signs of inflammation. This implies that provided MABP is kept within the autoregulatory range, CBF is at most moderately affected by variations in MABP in infants with a perinatal inflammatory condition. Thus, mechanisms other than impaired CA mediate the association between inflammation and brain injury.
In newborn piglets, hypovolemia in itself did not impair CA significantly. Thus, subject to a small study group and interspecies differences, this finding does not point to rational use of volume treatment to secure intact CA, and thus stable CBF, in normotensive infants with a medical history indicating hypovolemia.

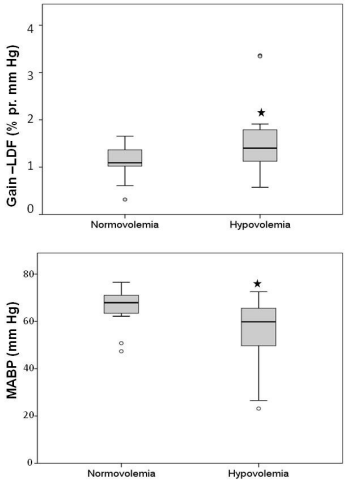

Figure 14
Boxplot of mean arterial blood pressure (MABP) and degree of impaired cerebral autoregulation (Gain-laser Doppler flowmetry (LDF)) during normo- and hypovolemia. MABP decreased significantly, while degree of impaired cerebral autoregulation increased significantly. * indicates $p \leq 0.05$ compared to normovolemic values.

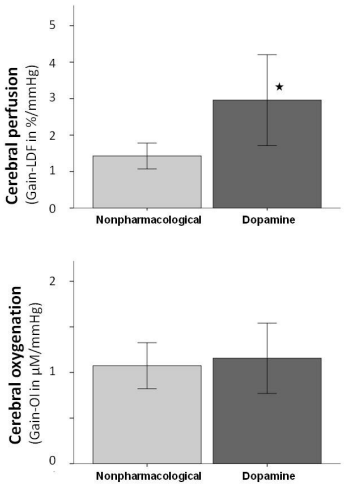

Figure 15
Effect of nonpharmacologically (light grey bar) and dopamine (dark grey bar) induced changes in MABP on cerebral perfusion (Gain-laser-Doppler-flowmetry (LDF) in %/mm Hg) and cerebral oxygenation (Gain-OI in µM/mm Hg. * indicates $p \leq 0.05$; error bars display 2 x SEM.

Dopamine is the most frequently used antihypotensive treatment in neonatal intensive care. In antihypotensive treatment, one of the primary aims is to maintain adequate oxygen supply to the brain. In spite of this, its effect on the cerebral circulation remains controversial - perhaps, partly, because former studies failed to negate a possible masking effect of CA. We made use of the piglet model to study dopamine's direct effect on cerebral hemodynamics and found that it increased cerebral perfusion but not oxygenation. This observation has formed hypothesis for continued study. Accordingly, in a larger group of newborn piglets, we are currently investigating whether dopamine affects cerebral metabolism and microvascular heterogeneity, as well as the possibility that dopamine induced vasoconstriction in the skin and skull contaminates the NIRS-derived measure of cerebral oxygenation.

9. SUMMARY

Increased preterm delivery rate and survival of preterm infants of whom a considerable proportion survive with neurodevelopmental impairment calls for better knowledge of mechanisms associated with brain injury. This thesis focuses on cerebral autoregulation and is based on clinical studies of very preterm infants and experimental studies in newborn piglets.

Maintaining adequate cerebral perfusion is critical to avoid brain injury. In healthy neonates, cerebral autoregulation ensures an almost unchanged cerebral perfusion within a narrow range of arterial blood pressures. When autoregulation is impaired, cerebral blood flow follows changes in arterial blood pressure passively. Both impaired cerebral autoregulation and perinatal inflammation have been associated with perinatal brain injury in preterm neonates. We hypothesized that impaired cerebral autoregulation might represent a hemodynamic link between inflammation and brain injury. We used an apparently well established non-invasive method based on frequency analysis between spontaneous changes in arterial blood pressure and cerebral oxygenation as measured with near-infrared spectroscopy. It turned out that the methodology was weak. This led us to evaluate the precision and validity of this method.

We monitored 22 preterm neonates and demonstrated that reliable detection of impaired cerebral autoregulation requires several hours of monitoring. However, weighting measurements with large variations in blood pressure in favor of those with small increases the precision. This reduces the required monitoring time in each infant (study I). Furthermore, we used a piglet model to validate the method against a conventional measure of cerebral autoregulation and demonstrated a significant correlation with degree of impaired autoregulation (study II).

To study a possible link between cerebral autoregulation and perinatal inflammation, cerebral autoregulation was measured in 60 infants in their first postnatal day. Fetal vasculitis was used as a marker of antenatal (preceding) inflammation. Level of interleukin-6 in postnatal blood samples was used as a marker of postnatal (concurrent) inflammation. Neither ante- nor postnatal inflammation affected cerebral autoregulation significantly. There was, however, a trend towards a more severely impaired autoregulation in infants with signs of antenatal inflammation. Postnatal inflammation was significantly associated with hypotension, and blood pressure was inversely associated with degree of impaired cerebral autoregulation (study III).

Also, we made use of our piglet model to study (i) if hypovolemia affects cerebral autoregulation, and (ii) a possible direct cerebrovascular effect of dopamine therapy. Hypovolemia without hypotension did not seem to affect autoregulation significantly. Dopamine, the most frequently used antihypotensive drug in neonates, elicited an unexplained mismatch between cerebral oxygenation and perfusion, as perfusion increased while oxygenation was unaffected (study IV). This mismatch has formed the basis for an ongoing explanatory study.

Based on the findings in the present thesis we conclude the following:

- Our non-invasive method has potential use in clinical research. However, low precision hampers its clinical application.

- In preterm infants with perinatal inflammation, cerebral blood flow is at most moderately affected by variations in arterial blood pressure, provided inflammation induced hypotension is prevented.

- In newborn piglets, hypovolemia alone did not affect cerebral autoregulation significantly, and dopamine therapy elicited an unexplained mismatch between cerebral perfusion and oxygenation.

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