BAVARIAN LONGITUDINAL STUDY OF PRETERM INFANTS: GENERAL INTELLIGENCE AND EXECUTIVE FUNCTIONING AT ADULT AGE

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AIM
Compared to term born children, being born before 32 weeks of gestation (VP) or with very low birth weight (< 1500g, VLBW) results in impairment of cognitive (IQ) and executive function (EF). This study evaluates whether these alterations can be still observed at adult age.

METHODS
Patients for the Bavarian Longitudinal Study were recruited from a geographically defined region in Southern Bavaria, being born between Jan. 1st, 1985 and March 31st, 1986. All infants born either VP or as VLBW were included and compared to a group of term newborns, born in the same hospitals and matched for sex and socioeconomic status (SES). This was the seventh longitudinal investigation at a median age of 26 years applying a set of tests to measure IQ and EF.

RESULTS
The present study group included 217 VP/VLBW and 197 control subjects. The magnitude of differences between VP/VLBW and controls (Cohen’s d) ranged from 0.83 to 0.96 for IQ and 0.46 – 0.78 for EF measures. For IQ scores VP/VLBW adults scored 0.90 – 1.27 SD lower than term adults. While term adults mostly had cognitive problems in one or two specific areas, VP/VLBW adults presented multiple problems. No significant impact for being born small for gestational age was observed. A strong effect of SES on IQ was detected both for VP/VLBW and term adults.

CONCLUSION
VP/VLBW children do not seem to outgrow their general cognitive deficits by adulthood despite all support given. Low socioeconomic status is a persisting developmental disadvantage for infants being born VP/VLBW as well as for term born infants.
AREA-WIDE LONG-TERM FOLLOW-UP OF EXTREMELY IMMATURE PRETERM INFANTS AT LOW COSTS: IS IT FEASIBLE?

Helmut Küster¹, Gabriele Damm², Wolfgang Voss³ on behalf of the Lower Saxonian preterm infant follow-up project

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Introduction:
Long-term outcome of the most vulnerable and costly neonatal patients is of general interests. Follow-up beyond 2 years has so far been mainly achieved under study conditions and for selected study centers only.

Aim:
Area-wide prospective follow-up of ELBW infants up to 10 years including quality improvement campaigns at low costs as a routine.

Methods:
Since 2004 all infants with a gestational age<28weeks treated in 23 children’s hospitals in Lower Saxony are followed at 0.5, 2, 5 and 10 years in 11 centers using BSID-II, K-ABC and SETK 3-5. Results are correlated to pre- and postnatal data and compared to a matched cohort of 305 term infants.

Results:
Of those alive, 80% were seen at 6 months and 2 years, 65% at 5 years. At 5 years 27% were tested normal, 29% had motor and/or speech deficits but were cognitive normal, 15% had an IQ of 70-84, and 29% an IQ <70 and/or CP and/or blindness. 94% had had some form of therapy during their life. In 31% a new therapy was initiated at the 5 year follow-up. Considerable differences in outcome were found when comparing the 23 participating centers. Differences were seen mainly in infants <26 weeks of gestation.

Conclusion:
Area-wide long-term follow-up is feasible. Only ¼th of the infants <28 weeks of gestation are developed age-appropriate. As a consequence a benchmarking group was established to analyze differences in treatment or organizational structure might be responsible for the differences in outcome between centers.
HEALTH PROBLEMS OF VERY LOW BIRTH WEIGHT INFANTS AT 5 YEARS OF AGE

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Background: Relatively few data exist on longer term health problems in VLBW infants (VLBWs).

Aim: We wanted to investigate differences in health status at the age of 5 years when VLBWs are compared to a national cohort of term born infants (KIGGS-Survey) in Germany.

Material and methods: As a part of the GNN follow-up, 225 parents of VLBWs were interviewed personally using a structured device containing the same questions that were also asked in the KIGGS survey of the Robert Koch Institute (RKI) of the German government. These infants (birth weight: (median, Q1-Q3): 1113 (820-1380) g; gestational age: (median, Q1-Q3): 29 (27-31) weeks) were compared to 945 infants in the KIGGS-Survey also at 5 years of age.

Results: Former VLBWs suffered less frequently from atopic dermatitis (8% vs. 13%) or allergic rhinitis (1% vs. 6%), without a difference in the incidence of asthma (4% vs. 3%) or diabetes (0.4% vs. 0.1%). Slightly more migraine (1% vs. 0%), but more general convulsions (including febrile convolution; 8% vs. 3%), heart disease (8% vs. 2%) and obstructive bronchitis (48% vs. 17%) were described. Former VLBWs were operated on more frequently (50% vs. 32%); especially drainage (typanostomy tube) of the middle ear (13% vs. 6%). But less frequently circumcisions (0% vs. 7%) were done (Table1).

Conclusion: At the age of 5 years more VLBWs suffered from obstructive bronchitis as compared to former term newborns. Allergic disease (skin, rhinitis) was observed less frequently in the VLBW group. Larger studies following health problems of VLBWs into adulthood are needed.

*Presenting author.

**Supported by a grant of the German Government (BMBF)
Table. Chronic diseases and operations at 5 years of age in VLBW infants and controls from the KIGGS study.

<table>
<thead>
<tr>
<th>Condition</th>
<th>VLBW n = 225</th>
<th>KIGGS n = 945</th>
<th>p-value</th>
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</thead>
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<tr>
<td>Allergic rhinitis</td>
<td>3 (1%)</td>
<td>56 (6%)</td>
<td>0.0033</td>
</tr>
<tr>
<td>Atopic dermatitis</td>
<td>17 (8%)</td>
<td>120 (13%)</td>
<td>0.0371</td>
</tr>
<tr>
<td>Asthma</td>
<td>9 (4%)</td>
<td>32 (3%)</td>
<td>0.6861</td>
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<td>Obstructive bronchitis</td>
<td>109 (48%)</td>
<td>162 (17%)</td>
<td>&lt;0.0001</td>
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<tr>
<td>Heart disease</td>
<td>18 (8%)</td>
<td>24 (2%)</td>
<td>0.0004</td>
</tr>
<tr>
<td>Cerebral convulsions</td>
<td>17 (8%)</td>
<td>28 (3%)</td>
<td>0.031</td>
</tr>
<tr>
<td>Diabetes</td>
<td>1 (0.4%)</td>
<td>1 (0.1%)</td>
<td>0.3478</td>
</tr>
<tr>
<td>Migraine</td>
<td>2 (1%)</td>
<td>0</td>
<td>0.0368</td>
</tr>
<tr>
<td>Need of operation</td>
<td>114 (50%)</td>
<td>306 (32%)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Adenotomie</td>
<td>32 (14%)</td>
<td>148 (16%)</td>
<td>0.68</td>
</tr>
<tr>
<td>Tonsillectomie</td>
<td>11 (5%)</td>
<td>36 (4%)</td>
<td>0.45</td>
</tr>
<tr>
<td>Tympanostomy tube</td>
<td>30 (13%)</td>
<td>59 (6%)</td>
<td>0.0007</td>
</tr>
<tr>
<td>Appendectomy</td>
<td>0</td>
<td>4 (0.4%)</td>
<td>1.0</td>
</tr>
<tr>
<td>Circumcision</td>
<td>0</td>
<td>62 (7%)</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>
INFLUENCE OF INTRAUTERINE GROWTH RESTRICTION ON POSTNATAL OSTEOPROTEGERIN CONCENTRATIONS AND AORTIC INTIMA-MEDIA THICKNESS

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Background: Intrauterine growth restriction (IUGR) is considered a risk factor for atherosclerosis and coronary artery disease in adulthood. Understanding the pathophysiology of cardiovascular disease involves the identification of novel risk factors and biomarkers. There is emerging evidence that osteoprotegerin (OPG), a member of the tumor necrosis factor-receptor superfamily, participates in the pathogenesis of atherosclerosis by amplifying the effects of inflammation and traditional risk factors.

Aim: In this case-control study we investigated whether IUGR affects postnatal OPG concentrations and the possible association between OPG levels and aortic intima-media thickness (aIMT), an index of preclinical atherosclerosis.

Methods: We studied 30 IUGR and 30 appropriate for gestational age (AGA) neonates matched for gestational age and gender. Quantitative determination of plasma OPG was performed with enzyme immunoassay on the second (DOL2) and fifth day of life (DOL5). Aortic intima-media thickness (aIMT) was measured in the distal abdominal aorta using a linear array probe, and adjusted for aortic lumen diameter.

Results: Neonates with IUGR had significantly higher OPG levels on both DOL2 and DOL5 as compared to controls (DOL2: 5.4±1.0 mmol/L vs. 4.6±1.0 mmol/L, p=0.002 and DOL5: 5.1±0.8 mmol/L vs. 3.9±0.7 mmol/L, p<0.001). Between DOL2 and DOL5, OPG concentrations did not change significantly in neonates with IUGR (p=0.087), but decreased slightly in controls (p=0.003). IUGR was also associated with increased aIMT (0.11±0.03 vs. 0.06±0.02, p<0.001). There was a positive correlation between OPG and aIMT on DOL2 (r =0.494, p<0.001), which became stronger on DOL5 (r=0.791, p<0.001; Figure).

Conclusion: We report significantly increased concentrations of OPG in IUGR neonates and a positive correlation with aIMT. The dynamics of OPG concentrations during the transitional period support the fetal origin of the cytokine. Follow-up studies with repeat OPG and aIMT measurements may be indicated to evaluate whether these findings represent a permanent effect of IUGR on the offspring.
NERVE GROWTH FACTOR/ NEUROTROPHIN 4 CONCENTRATIONS AT THE EXTREMES OF FETAL GROWTH DUE TO MATERNAL DIABETES

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²Laboratory of Clinical Biochemistry-Molecular Diagnostics, 2nd Department of Pediatrics, Athens University Medical School, Athens, Greece

Background: Diabetes during pregnancy may adversely affect fetal neurodevelopment, thereby predisposing to psychopathology in later life. Both intrauterine growth restriction (IUGR) and fetal macrosomia are associated with developmental cortical dysfunction, neurocognitive deficits and increased incidence of adult psychiatric disorders. The neurotrophins nerve growth factor (NGF) and neurotrophin-4 (NT4) are structurally related molecules, which play a crucial role in early brain development.

Aim: To compare cord blood concentrations of NGF and NT4 among large-for-gestational-age-(LGA), IUGR and appropriate-for-gestational-age-(AGA) infants born to diabetic mothers versus AGA controls of non-diabetic mothers, and correlate them with various perinatal variables.

Methods: Serum NGF and NT4 concentrations were prospectively evaluated by ELISA in 80 mixed arteriovenous cord blood samples from LGA (n=15), IUGR (n=12) and AGA (n=33) diabetic, as well as from AGA normal (controls, n=20) singleton full-term pregnancies. Fetuses were classified as LGA, IUGR or AGA, based on customized birth-weight standards, adjusted for significant determinants of fetal growth.

Results: Cord blood NGF concentrations were lower in IUGR, as compared to AGA infants (p=0.038). NT4 concentrations decreased with advancing gestational age [b=-11.966, 95%CI-21.059-(-2.873), p=0.011]. Both cord blood NGF and NT4 concentrations increased with advancing maternal age (b=0.055, 95%CI 0.016-0.094, p=0.009 and b=6.349, 95%CI: 1.711-10.986, p=0.010). In the control group, fetal NGF concentrations were lower in multiparas [b=-0.492, 95%CI-0.912-(-0.073), p=0.024].

Conclusions: IUGR fetuses exposed to maternal diabetes present with NGF deficiency, which may be implicated in the long-term IUGR-associated neurodevelopmental sequelae. Higher cord blood NT4 concentrations with decreasing gestational age may signify the well-known crucial role of NT4 in fetal brain development. Cord blood NGF and NT4 up-regulation with advancing maternal age probably represents a homeostatic response to counterbalance the age-related neuronal apoptosis. Finally, increased parity seems to adversely affect fetal NGF expression.
MODE OF DELIVERY AND DEVELOPMENT OF WHEEZING AND ALLERGIC DISORDERS IN EARLY CHILDHOOD

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BACKGROUND
It has been suggested that cesarean section (CS) is associated with increasing risk of wheezing and allergic disorders in early childhood.

AIM
To examine the relationship between mode of delivery, recurrent wheezing (RW), atopic dermatitis (AD) and food allergy (FA) during the first three years of life.

METHODS
We prospectively evaluated 459 children from birth (gestational age ≥34 weeks) to three years of age. Participants were followed-up every six months and cases of doctor-diagnosed RW (>3 episodes), AD, and FA were recorded. The effect of CS was assessed by multivariable logistic regression, after taking into account potential confounders, such as maternal age, parity, gestational age, gender, birth weight, duration of membranes rupture, exposure to tobacco smoke or antibiotics during pregnancy, exposure to antibiotics during the first six months of life, exclusive breast feeding, and parental asthma and atopy.

RESULTS
The prevalence of RW, AD, and FA was 18.7%, 13.5%, and 5.2%, respectively. Children born by CS (N=233; 50.8%) had higher probability of FA (OR 3.0 [1.1-8.4]), but not of RW (OR 1.2 [0.8-1.9]) or AD (OR 1.4 [0.8-2.5]). Parental atopy (OR 4.7 [1.9-11.7]) and higher gestational age (OR 1.5 [1.0-2.3]) were also significant risk factors for FA. The combination of CS and parental atopy was associated with significantly higher probability of FA, as compared to vaginal delivery and absence of parental atopy (OR 10.0 [3.1-32.7]). Male gender (OR 1.7 [1.1-2.8]) and parental asthma (OR 2.5 [1.3-4.8]) were the only significant and independent predictors for RW in the whole cohort. When the same analysis was repeated separately for the two genders, CS was found to be the only significant predictor of RW in girls (OR 1.9 [1.1-4.0]).

CONCLUSIONS
Our results suggest that in girls, birth by CS may predispose to wheezing disorders during early childhood. Delivery by CS seems to increase the risk of FA but not AD during the first three years of life, independently of other perinatal, familial or environmental factors.
NASAL HFOV FOR RESPIRATORY INSUFFICIENCY IN PRETERM INFANTS: A RETROSPECTIVE ANALYSIS

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Background
In many studies noninvasive ventilation (NIV) of preterm infants was superior to invasive ventilation in terms of various short-term outcome parameters. Nasal high-frequency oscillatory ventilation (nHFOV) is a new mode of NIV, but only limited data from observational studies are available so far.

Aim
To assess, (1) if nHFOV, as compared to nasal CPAP (nCPAP), is a safe mode of ventilation in preterm infants; (2) if nHFOV improves ventilation (CO₂ elimination); (3) if nHFOV reduces the rate of re-intubation.

Methods
Retrospective analysis of the clinical course of 24 preterm infants < 34 weeks gestational age (ga) between 3/2013 und 12/2014, who were treated with nHFOV for imminent failure of nCPAP or following extubation from invasive ventilation.

Results
11 out of 24 patients (29+6/7 weeks ga (median; range 24-34)) were switched from nCPAP to nHFOV at a pCO₂ of 67 (40-83)mmHg. After 2-4 hrs pCO₂ had dropped to 56 (40-60)mmHg, and after 48 hrs to 44 (39-49)mmHg. FiO₂ was 0.23 (0.21-0.30) before switch and 0.23 (21-28) after switch. 12 out of 24 patients (25+6/7 weeks ga (22-30)) were treated with nHFOV immediately after extubation at a pCO₂ of 53 (43-73)mmHg. After 2-4 hrs pCO₂ had dropped to 52 (41-72)mmHg, and after 48 hrs to 45 (41-61)mmHg. FiO₂ was 0.28 (0.21-0.45) before extubation and 0.30 (0.21-0.45) after extubation. Only 1 out of 12 patients in this group had to be re-intubated within 48 hrs for apnea and bradycardia. The only infant who was ventilated with nHOV immediately after stabilization in the delivery room had to be intubated 10 hrs later. No side effects of nHFOV, such as pneumothorax, gross abdominal distension, feeding intolerance, increased agitation, or others were noted.

Conclusions
As in other trials these preliminary results from a non-controlled pilot study show that nHFOV in preterm infants with increased pCO₂ is effective and safe, and might improve alveolar ventilation better than nCPAP. As one would expect, when using the same mean airway pressure oxygen requirement did not improve. Due to the low rate of re-intubation in this cohort no conclusion can be drawn regarding this outcome parameter. However, mechanism of action, optimum ventilator settings, indications, and patient groups, who qualify for this new treatment option, still have to be identified by randomized trials.
STANDARDIZATION OF HEATED HUMIDIFIED HIGH FLOW NASAL CANNULA (H3F) IMPROVES PATIENT CARE.

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Background

H3F is a promising alternative to nasal CPAP, providing more comfort to the baby and to breastfeeding mothers.

Aim

Based on our previous experience with H3F, we adjusted our protocol by increasing all algorithms by 1 L/m in order to further improve its efficacy.

Methods

Two hundred sixty (260) patients were divided into 4 algorithms of care based on their weight at the start of the therapy: Group A: $<$1000g (at 5L/m), Group B: 1000-1500g (at 6L/m), Group C: 1500-2000g (at 7L/m) and Group D (8L/m). The population was subdivided into 3 therapeutic categories: first intention therapy, post nasal CPAP and post tracheal extubation. Each algorithm had a weaning period variable from 24 to 48 hours based on weight and on clinical stability. Failure was considered when H3F was increased by one L/m above the protocol and the patient did not respond.

Results  Table 1.

Success was identified as no need for respiratory support when H3F was initiated. In all categories, best results were obtained among the 79 infants with IUGR, with a success rate of 83%. The most frequent reasons for H3F failure were sepsis (56%) and feeding intolerance (8%). A 12.5% improvement was observed in the $<$1500g infants when compared to our previous algorithms.

Conclusions

Our new algorithms improved the outcome of our patients. In our unit, H3F has become the first line of therapy for infants $<$1250g and a very successful modality for infants $<$1250g as a second intention therapy, (post nasal-CPAP or post-extubation). Most of the failures in $<$1250g were associated with complications commonly seen among very premature infants.
<table>
<thead>
<tr>
<th>Weight Range</th>
<th>1st Intention</th>
<th>Post CPAP</th>
<th>Post extubation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td># patients</td>
<td>% success</td>
<td># patients</td>
</tr>
<tr>
<td>&lt; 1000g</td>
<td>2</td>
<td>50%</td>
<td>6</td>
</tr>
<tr>
<td>1000-1500g</td>
<td>17</td>
<td>70%</td>
<td>24</td>
</tr>
<tr>
<td>1500-2000g</td>
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<td>28</td>
</tr>
<tr>
<td>&gt;2000g</td>
<td>49</td>
<td>92%</td>
<td>20</td>
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<tr>
<td>Total # of patients</td>
<td>89</td>
<td>78</td>
<td>93</td>
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LOW CONCENTRATIONS OF CLUB CELL SECRETORY PROTEIN (CC16) IN GASTRIC FLUID AT BIRTH IS ASSOCIATED WITH LUNG INFLAMMATION AND MORE SEVERE LUNG DISEASE IN VERY PRETERM INFANTS

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Introduction
CC16 is an anti-inflammatory protein secreted by club cells in the distal airways of the lung, diffusing into the circulation along a concentration gradient. Concentrations of CC16 in amniotic fluid increase dramatically during gestation. After birth, a surge of CC16 occurs in the lungs as well as in the circulation, less pronounced in preterm infants. Maternal chorioamnionitis is associated with lower concentrations of CC16 in the trachea, and persistent low plasma concentrations have been reported in infants who develop BPD.

Patients and methods
Descriptive study in 61 very preterm infants with a mean (SD) gestational age at birth of 25.8 (1.9) weeks and birth weight of 850 (275) g. CC16 was measured by ELISA in plasma at birth (n=47) and at 24 h of age (n=61), in gastric aspirate fluid (GAF) at birth (n=32) and in tracheal aspirate fluid (TAF) at 24 h of age in ventilated infants (n=35). Concentrations in TAF were adjusted for total protein content. IL-1β, TNF-α, and MMP-9 were analyzed by a multiplex assay (Luminex).

Results
Concentrations of CC16 in GAF and in TAF were median (range) 230 (5-671) and 97 (1.6-834) ng/mL. Median plasma CC16 at birth was 4.3 (1.1-13.1) ng/mL and increased significantly to 9.9 (1.7-41.4) ng/mL at 24 h of age; p<0.001. CC16 concentrations in GAF and TAF correlated positively with CC16 concentrations in cord blood and in blood at 24 h of age (all p < 0.05). CC16 concentrations in GAF showed an inverse correlation with IL-1β (r = -0.49, p = 0.028; figure), TNF-α (r = -0.48, p = 0.034) and MMP-9 (r = -0.53, p = 0.015) in TAF. Concentrations of CC16 in GAF correlated inversely with FiO2 at 6 h of age (r = -0.45, p = 0.009), with arterial PCO2 levels at 24 h of age (r = -0.4, p = 0.03), and with number of ventilator days (r = - 0.44, p = 0.012). Concentrations of CC16 in TAF correlated inversely with mean airway pressure at 24 h (r = -0.52, p = 0.012).

Conclusions
Low levels of CC16 in gastric fluid and in trachea were associated with more inflammation in the lungs and with an increased need for respiratory support in the early neonatal period. An imbalance between pulmonary anti-inflammatory and pro-inflammatory proteins may be of importance for injury in the immature lung.
HIGH VERSUS THERAPEUTIC DOSES OF CAFFEINE AND/OR KETOROLAC EFFECTS ON VASOACTIVE MEDIATORS IN HUMAN RETINAL ENDOTHELIAL CELLS EXPOSED TO HYPOXIA AND INTERMITTENT HYPOXIA

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Background. Caffeine (C) and ketorolac (K) may individually improve retinopathy of prematurity. Moreover, C and/or K synergistically decrease the severity of oxygen-induced retinopathy (OIR) in neonatal rats. Hyperoxia and intermittent hypoxia (IH) induce reactive oxygen species and oxidative stress which may alter retinal vascular homeostasis leading to OIR.

Objectives. 1) to test the hypothesis that C and/or K protect human retinal endothelial cells (HRECs) via regulation of vasoactive mediators; and 2) to compare high versus therapeutic doses on vasoactive mediators in HRECs.

Materials/Methods. HRECs were exposed to normoxia, hyperoxia (50% O2), or IH (50% O2 with IH (10% O2, 8 episodes/day) for 24, 48 or 72 hrs. during which they were treated with low-dose caffeine (LoC, 10 µg/mL), ketorolac (Acuvail, 0.45% ophthalmic solution, 0.4 µg/mL, LoK); LoC+LoK, high-dose caffeine (HiC, 50 µg/mL), HiK (2.0 µg/mL), or HiC+HiK. Prostanoids (PGE2, PGF2α, 6-KetoPGF1α, the stable metabolite of PGI2; and TxB2, the stable metabolite of TxA2), and 8-isoPGF2α (biomarker for oxidative stress) were determined in the media. Cell migration and tube-forming capacities were determined. Cells were stained for superoxide anion, lipid peroxidation, cyclooxygenase (COX)-1, -2, & -3.

Results. At 24 hr., 8-isoPGF2α and all prostanoids were increased with LoC, but attenuated with LoK and LoK+LoC (p<0.01). An opposite effect was noted with HiC and/or HiK (p<0.001), particularly in RA. Similar response pattern occurred at 48 hrs. By 72 hr., the levels were lower in RA and higher in IH with HiC (p<0.001). Suppression of vasoactive mediators occurred with LoC at 72 hrs. Synergistic suppression occurred at 24 hr. in IH and 48 and 72 hr. in RA and 50% O2, but not in IH. Low doses suppressed and high doses promoted migration and tube formation. The responses of 8-isoPGF2α and all prostanoids levels correlated directly with expression of superoxide anion, lipid peroxidation and COX expression.

Conclusions. Beneficial synergistic effects of caffeine and ketorolac occur with therapeutic doses. Administration of high doses may cause an opposite stimulatory effect on biomarkers of oxidative stress and vasoactive mediators which may lead to unwanted side effects.

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TRAMADOL DISPOSITION IN EARLY INFANCY ILLUSTRATES IN VIVO DRUG TRANSPORTER ACTIVITY

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INTRODUCTION: Tramadol (M) is metabolized by cytochrome P450 [CYP] 2D6 to O-demethyl tramadol (M1). M1 is subsequently eliminated by renal route while M1 formation depends on the age-dependent CYP2D6 activity and its polymorphism. These pathways only in part explain the variability observed [1]. Tzetkov et al. more recently illustrated that Organic Cation transporter 1 (OCT1) polymorphisms affects M1, but observations on OCT ontogeny and polymorphisms are absent [2].

METHODS: A dataset of 250 plasma log M/M1 samples [1] in young infants was linked with clinical characteristics, CYP2D6 and OCT1 polymorphisms (0, 1 or 2 active alleles).

RESULTS: Fig 1a illustrates the impact of the CYP2D6 activity score (higher score results in lower log M/M1 plasma, reflecting higher M1 synthesis. Similar, samples in cases with 2 active alleles had a higher plasma, reflecting more active M1 re-uptake (Fig 1b).

CONCLUSIONS: At first exploration, OCT1 polymorphisms are already of relevance in infants, suggesting that there is already relevant phenotypic OCT1 activity. More extensive evaluation of this in vivo dataset is warranted.

RESUSCITATION WITH PASSIVE LEG RAISING OF 30° INCREASES SURVIVAL AT 4 HOURS AFTER RETURN OF SPONTANEOUS CIRCULATION IN A NEONATAL MODEL OF ASPHYXIAL CARDIAC ARREST

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Background: Passive leg-raising(PLR) was found beneficial in a larger animal model of prolonged ventricular fibrillation cardiac arrest (CA). With the present study we aimed at evaluating whether resuscitation with PLR at 30° could increase return of spontaneous circulation(ROSC) in a neonatal model of asphyxial CA.

Methods: The animals were anaesthetized and instrumented (catheters inserted in the aorta via the carotid artery and in the right internal jugular vein). The ETT was clamped and cardiac arrest was induced (heart rate(HR)< 60 bpm or a mean arterial pressure(MAP)<15 mmHg. The animals were then randomized to the control group (n=10) which were resuscitated according to the 2010 guidelines on neonatal resuscitation and the intervention group (n=8) which was resuscitated according to current guidelines plus 30° PLR. The animals were monitored for 4 hours and then were humanely euthanized.

Statistical analysis: Non-parametric tests were used for statistical analysis, due to small study sample. The Wilcoxon-Mann-Whitney non-parametric test was used for comparisons among the two groups for quantitative variables. Fisher’s exact test was used for comparisons of percentages.

Results: This is an ongoing experiment. The various recorded variables are shown in table 1. We recorded no significant difference in the rate of ROSC between groups (60% versus 75%). On the other hand we noted a significant difference in the intervention group, as all animals which achieved ROSC remained alive 4 hours post ROSC.

Conclusions: In this swine model of neonatal resuscitation, titling of the resuscitaire by 30° resulted in longer survival after ROSC.
<table>
<thead>
<tr>
<th>Parameter</th>
<th>all subjects (N=18)</th>
<th>range</th>
<th>controls (N=10)</th>
<th>intervention (N=8)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>baseline HR (bpm)</td>
<td>182.1 (11.90)</td>
<td>151 to 198</td>
<td>184.2 (9.13)</td>
<td>179.5 (14.92)</td>
<td>0.656</td>
</tr>
<tr>
<td>baseline SAP (mmHg)</td>
<td>84.2 (10.33)</td>
<td>63 to 107</td>
<td>81.4 (3.86)</td>
<td>87.7 (14.65)</td>
<td>0.179</td>
</tr>
<tr>
<td>baseline DAP (mmHg)</td>
<td>50.2 (7.98)</td>
<td>37 to 67</td>
<td>50.3 (7.63)</td>
<td>50.1 (8.93)</td>
<td>0.823</td>
</tr>
<tr>
<td>baseline MAP (mmHg)</td>
<td>66.0 (7.45)</td>
<td>49 to 85</td>
<td>65.2 (4.13)</td>
<td>67.0 (10.52)</td>
<td>0.655</td>
</tr>
<tr>
<td>baseline SpO2 (%)</td>
<td>98.0 (1.18)</td>
<td>96 to 100</td>
<td>97.8 (1.22)</td>
<td>98.2 (1.16)</td>
<td>0.457</td>
</tr>
<tr>
<td>baseline pH</td>
<td>7.39 (0.054)</td>
<td>7.31 to 7.49</td>
<td>7.37 (0.054)</td>
<td>7.42 (0.045)</td>
<td>0.098</td>
</tr>
<tr>
<td>time to ROSC (sec)</td>
<td>65.0 (54.02)</td>
<td>30 to 210</td>
<td>50 (36.33)</td>
<td>80.0 (67.52)</td>
<td>0.301</td>
</tr>
<tr>
<td>[among ROSC subjects, N=12]</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>survival after 4 hours, n (%)</td>
<td>8 (66.67)</td>
<td>-</td>
<td>2 (33.33)</td>
<td>6 (100.00)</td>
<td><strong>0.061</strong></td>
</tr>
<tr>
<td>[among ROSC subjects, N=12]</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>survival time (mins)</td>
<td>177.3 (96.24)</td>
<td>8 to 240</td>
<td>114.7 (104.6)</td>
<td>240 (0.00)</td>
<td><strong>0.022</strong></td>
</tr>
</tbody>
</table>

Table 1. Mean values (S.D.) or N (%) of parameters at baseline and regarding survival after ROSC
VERY LOW APGAR SCORE AT 1 MINUTES – WHAT IS THE INCIDENCE OF SEVERE ASPHYXIA AND OUTCOME?

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**Background:** Very low Apgar score (VLAS) at birth may have different causes, including birth asphyxia, and sequelae. The International Classification of Diseases (ICD-10) states for diagnosis P21.0: Asphyxia with 1-minute Apgar score 0-3. The level of acidosis at birth, duration of VLAS, presence and stage of hypoxic-ishaemic organ damage, incl. encephalopathy, are not specified. Prolonged (10 min) low Apgar score can indicate severe asphyxia with encephalopathy. Therapeutic hypothermia has shown to reduce unfavourable outcome.

**Aim:** The aim was to study all neonatal cases with Apgar scores 0-3 at 1 minutes to define the incidence of severe asphyxia in term newborns, eligibility and application of therapeutic hypothermia, and outcome.

**Methods:** All newborns with diagnosis of P21.0 born in 2004-2014 were identified from the database of Tartu University Hospital. Retrospective analysis of the electronic patient charts identified the details of the neonatal course (gestational age, Apgar scores at 5 and 10 minutes, pH and lactate from umbilical artery, signs of fetal distress, clinical and radiological symptoms of hypoxic-ischaemic organ damage, maternal and neonatal background conditions, treatment incl. therapeutic hypothermia). Additionally, the latest data about child developmental status collected.

**Results:** The incidence of VLAS in term newborns born at Women’s Clinic of Tartu University Hospital was 0.84% (199/23 827 births). Of 19 infants whose Apgar score remained < 6 at 10 minutes (prolonged VLAS group), 15 (80%) developed encephalopathy with clinical convulsions and were submitted for therapeutic hypothermia. Moderate, severe neurodevelopmental impairment or death occurred in 37% of the prolonged VLAS group and 6.7% of the remaining VLAS group.

**Conclusions:** The incidence of severe asphyxia among term infants with VLAS (diagnosis P 21.0) at 1 minute was 10%, of whom one third had unfavourable outcome. All eligible infants underwent therapeutic hypothermia. For the ICD-11, more appropriate coding for perinatal asphyxia should be designed.
Poor neonatal brain growth in children born extremely preterm with autism traits
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Background
Children born extremely preterm face a dramatically elevated risk of autism spectrum disorder (ASD). The relation between neonatal brain volumes and the risk of ASD in extremely preterm children remains unexplored.

Aim
To determine whether structural brain alterations could be identified long before the onset of ASD symptoms children born extremely preterm.

Study design
We studied 87 children born at <27 weeks of gestation who underwent neonatal magnetic resonance imaging (MRI) at term. Participants were considered ASD positive if they screened positive (>60) on the Social Responsiveness Scale and/or had a clinical diagnosis of autism/ASD at age 6.5 years. Brain morphometric studies (automatic segmentation, atlas-based segmentation and voxel-based morphometry) were performed in infants with high-quality MRI with no evidence of focal brain lesion (N=33).

Results
Twenty-six (29.9%) children were ASD positive and 61 (70.11%) children were ASD negative. The ASD group had a significantly higher frequency of neonatal complications than the ASD negative group. In the subgroup of children with normal MRI findings and MR data suitable for volumetric assessment, the ASD infants had reduced volumes in the temporal, occipital, insular and limbic regions, as well as in brain areas involved in social skills, behavior and salience integration.

Conclusions
Extremely preterm children born children with ASD traits had smaller neonatal brain volumes compared with typically developing extremely preterm born. These deviations were detectable on neonatal MRI and localized in regions that play a key role in core features of autism. Early detection of these structural alterations may allow to early identification and intervention.
THE HUMAN MILK PARADOX

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Background. Breast milk is very important to ensure infants a well-composed and safe diet during the first months of life. In addition, although human milk does not meet the nutritional requirements for premature infants, clinical experience indicates that feeding human milk to extremely premature infants seem to reduce morbidity and improve neurodevelopmental outcome. However, the quality of breast milk seems to be affected by an increasing amount of environmental toxins (particularly so-called Persistent, Bioaccumulative Toxins [PBTs]).

Aim. While many concerns have been raised about the negative effects this may have on term infants health, this problem has not been addressed in relation to the possibly more vulnerable extremely premature infant.

Methods. A review of the literature on this topic primarily based on scientific studies and review articles identified through a non-systematic search in PubMed was performed.

Results. The concentration of PBTs in breast milk is mainly caused by man’s position as the final link in the nutritional chain. Many breast-fed term infants have a daily intake of PBTs that exceeds limits defined for the population in general, but this information is not included in textbooks or articles on the composition of human milk. However the optimal nutritional composition of breast milk and its content of immunoactive substances still seem to protect against long-term effects of such toxicity in term infants. There are, however, no studies on the presence and disposition of PBTs in extremely low birth weight infants fed human milk.

Conclusions. There is international consensus on the need reduce the level of PBTs in the environment. Clothes and toys containing PBTs are frequently recalled from the market to prevent toxicity in children. Systems for monitoring breast milk for the presence of PBTs have also been established in several countries. Such surveillance will be a good indicator of the population’s general exposure to these toxins and may also contribute to identifying groups who should not breastfeed their infants for a long time. Studies should also be undertaken to evaluate the presence and disposition of PBTs in extremely premature infants fed human milk.
EFFECT OF HYPEROXIA ON MESENCHYMAL STEM- OR STROMAL CELLS (MSCS) FROM THE DEVELOPING HUMAN LUNG

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2 CRTD DFG-Center for Regenerative Therapies Dresden – Cluster of Excellence, Dresden, Germany
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Background: Exposure of the immature lung to oxygen concentrations higher than in utero – relative (room air) or absolute hyperoxia – represents a major risk factor for the development of bronchopulmonary dysplasia (BPD) in preterm infants.

Aim: To test the effect of oxygen on mesenchymal stem- or stromal cells (MSCs) of fetal lungs.

Methods: Resident CD146pos/GD-2neg mesenchymal stem- or stromal cells (MSCs) were isolated from human fetal lungs at the canalicular stage of development (16th – 18th week of gestation) and exposed to physiologic (5% O2) and elevated oxygen concentrations (21% and 60% O2). The effect was compared with the effect on MSCs isolated from the umbilical cord stroma.

Results: Mesenchyme of the fetal lung is abundant in MSCs. Single-cell plating of lung MSCs in physiological hypoxic and ambient oxygen atmospheres revealed profound reductions of colony-forming ability and colony size in normoxic conditions. Furthermore, when exposed to absolute hyperoxia (60% O2), MSCs lost ability to form colonies, reduced expression of stem cell-restricted proteins like Oct-4 and Sox2, proliferated and switched cytokine secretion profiles towards a pro-fibrotic, pro-inflammatory phenotype. Surface marker profiling revealed distinct changes in the immunophenotype of MSCs exposed to hyperoxia; alterations in the composition of the extracellular matrix were observed. Conversely, MSCs from the umbilical cord secreted high amounts of anti-fibrotic and lung-protecting proteins like PGE2 and stanniocalcin-1.

Conclusion: The physiological function of resident lung MSCs is affected by relative and absolute hyperoxia, suggesting a key role of these cells in the immature lung responding to extra-uterine oxygen conditions.

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MITOCHONDRIAL RESPIROMETRY OF VIALBLE PLATELETS AS A SCREENING TOOL FOR MITOCHONDRIAL DISORDERS IN NEWBORNS INFANTS

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Background and Aim: Rapid diagnosis of mitochondrial disorder is difficult in newborn infants with metabolic crisis. We studied whether respiratory chain disorders can be assessed from circulating platelets.

Methods: Four infants born in 2011-2013 presented with severe metabolic acidosis within the first days of life. Common causes of metabolic acidosis were excluded and mitochondrial disorder was suspected. Respirometry (Oroboros Oxygraph) was performed on platelets and white blood cells (WBC) by sequential addition of substrates, ADP, uncoupler, and inhibitors, (“SUIT”-protocol). Isolated mitochondria from fibroblasts cultured in a glucose medium were assessed with Blue Native-PAGE (BNGE) for respiratory chain complex assembly from three patients.

Results: Patient #1 had increased platelets respiration compared to controls (52.91 vs. 34.17±7.42 pmol O₂/s/1×10⁸). The three other patients had lower respiration detected after fully saturating mitochondria with complex I substrates malate, pyruvate and glutamate (11.71 vs. 22.17±5.13, 14.04 vs. 22.17±5.13 pmol O₂/s/1×10⁸). In addition, patient #2 and #3 had lower basal respiration (9.79 vs.19.98±4.54, 13.23 vs. 19.98±4.54 pmol O₂/s/1×10⁸). Patient #3 had lower respiration after adding complex II substrate succinate, and also lower maximal respiratory capacity. No phenotype was detected from WBC. BNGE revealed lack of respiratory chain complexes (complexes I, III, and IV) in patients #2 and #4.

Conclusion: Platelets are easily accessible by blood sampling. Respirometry on platelets compared to age matched control cohort showed different oxygen consumption directing clinical investigation towards mitochondrial disorders in a timely manner.
SURGICAL SITE INFECTIONS IN NEWBORN INFANTS

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Background – Newborn infants are at great risk of surgical site infections (SSI) because surgical conditions often occur in very low birth weight, cared for in a NICU, with previous antibiotic treatment. Even though studies on surgical site infection in this population are scarce. Our aim was to study the epidemiology of SSI in a tertiary referral NICU.

Patients and Methods – This is a retrospective study of newborn infants who underwent an intervention requiring surgical incision. Patients discharged from January the 1rst 2013 to December 31rst 2014 were enrolled. Insertion of surgical central catheters and endoscopic procedures were excluded. Each patient was enrolled only once. Surgical interventions were counted one by one and classified by body cavity. Any peri-operative antibiotics course of any duration was considered as prophylaxis or treatment.

Results – During the study period there were 327 discharge events of 297 newborn infants; 141 out of these 297 patients (47.5%) were submitted to 202 surgical interventions, up to 7 interventions/patient. Mean gestational age of operated patients was 36 weeks (23-41), mean birth weight(BW) 2420g(505-4325), 32.2% had BW<1500g; 60.3% were male. For surgery, skin was disinfect with iodine alcoholic solution. Antibiotics were administered to all patients except those classified as having clean surgery. From patients with SSI eight were operated on in the first 2 days of life and 7 between 15 and 60 days and eleven were operated on in the first 2 days of the in-hospital stay and 4 between 15 and 70. Fifteen SSI were diagnosed in 15 patients accounting for 7.4% of surgical interventions (CI 95% 4.55-11.89) and 10.6% of the patients operated on. There were 8 SSI in 2013 (5 abdominal, 3 thoracic) and 7 in 2014 (4 abdominal, 1 thoracic, 1 cranial, 1 in the back). Eleven out of the 15 SSI were suture dehiscence (5.4% of surgical incisions) respectively 4.8% and 6.1% in each year; the other 4 SSI were true infections (2%) of surgical incisions.

Comments – Up to 47.5% of our patients were operated on. Rate of SSI was higher than in previous years mainly due to a high rate of sutures dehiscence accounting for almost three quarters of SSI. We plan to continue this audit in order to assess the reason of a so high dehiscence rate.