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INTERLEUKIN-6 AND C REACTIVE PROTEIN IN SERUM AND URINE OF NEONATES

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Background: Interleukin-6 (IL-6) and C reactive protein (CRP) are frequently detectable in amniotic fluid (AF). While CRP seems to be excreted by the fetal kidneys and probably by the lungs, IL-6 is an inflammatory cytokine produced by activated macrophages and lymphocytes which gain access to the amniotic cavity during intrauterine infection. IL-6 and other proinflammatory cytokines are also found in bronchoalveolar lavage of ventilated infants and in the urine of children with urinary tract infection. However, no information is available on the urinary IL-6 (uIL-6) content of neonates.

Methods: Urine and blood samples were obtained from consecutive neonates in the first week of life. Urine was collected using sterile cotton flock. After centrifugation of the flock, urinary CRP (uCRP) was put in sterile tubes and sent immediately to the laboratory. Urinary CRP and uIL-6 were measured with a commercially available ELISA test. The sensitivity of the assay was below 10% for both measurements. The uCRP and uIL-6 values were normalized for the urinary creatinine content of every single probe. Spearman rank correlation was used for statistical purposes.

Results: Serum and urinary CRP and IL-6 were measured in 15 infants. Mean±SD gestational age at delivery and birth weight were 33.3±3.8 weeks and 1789±794 grams, respectively. Serum and uCRP was detectable in all samples with a median (range) serum concentration of 2830.2 ng/mL (113.3–187171) and 4.76 ng/mL (0.83–58.73) in the urine. No correlation was found between serum CRP and uCRP concentration. IL-6 was present in 14 (93.3%) blood samples and in 9 (60%) urine samples. The median (range) concentration of IL-6 was 3.6 pg/ml (0–542.5) in serum and 0.48 pg/ml (0–39.5) in urine, respectively. A significant correlation was found between serum IL-6 and uIL-6 ($r=0.56$; $p<0.05$). No correlation was found between uCRP and IL-6 in serum and urine.

Conclusion: IL-6 can be present in neonatal urine at birth. This suggests that fetal urine could be another source of amniotic fluid IL-6 during intrauterine inflammatory processes.

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URINARY C-REACTIVE PROTEIN IN SMALL FOR GESTATIONAL AGE NEONATES

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Background: C reactive protein (CRP) has been found in amniotic fluid (AF) and fetal urine. Elevated levels of AF-CRP have been associated with preterm birth and neonatal infection. Increased AF-CRP levels have been found at the time of genetic amniocentesis in cases which developed preeclampsia later in gestation. The aim of this study was to explore whether small for gestational age neonates (SGA) excrete more CRP.

Methods: Consecutive newborns admitted to the NICU were included in the study. SGA was defined as a birth weight below the 10th percentile. SGA neonates exposed to placental insufficiency leading to intrauterine growth restriction (IUGR), defined as abdominal circumference below the 5th percentile at prenatal sonography, were analysed separately. Each SGA/IUGR infant was matched for gestational age to an appropriate for gestational age (AGA) neonate. Neonates with infectious morbidity at delivery were excluded. Urine samples were obtained in the first week of life using sterile cotton flock. After centrifugation of the flock, CRP was collected in sterile tubes and sent immediately to the laboratory. Urinary CRP was measured with a commercially available ELISA kit. The sensitivity of the assay was below 10%. The urinary CRP values were normalized for the urinary creatinine content of every single probe. Spearman rank correlation and Mann Whitney test were used for statistical purposes.

Results: Urinary CRP was measured in 21 SGA and 21 control infants. Clinical and laboratory results are presented in the table.

Characteristics	SGA cases (n=21)	Controls (n=21)	Significance
Gestational age, weeks	33.3±3.3	33.3±3.3	NS
Birth weight, grams	1444±530	2149±800	P<0.01
IUGR	11 (52.4%)	-	NA
Day of sampling	2 (1-6)	2 (1-6)	NS
Urinary CRP (ng/ml)	85.5 (0.66-544.9)	2.8 (0.18-114.5)	P<0.01

Values are presented as mean± SD, median (range), or numbers; NS, not significant;NA, not assessed

Serum CRP values were below the detection limit (<3mg/l) in 66.7% (14/21) and 76.2% (16/21) of cases and controls, respectively. Including only IUGR infants in the analysis, the difference in urinary CRP between cases and controls [92.1 (2–544.9) vs. 3.1 (0.2–114.5); $p<0.05$] remained statistically significant.

Conclusion: SGA and IUGR infants excrete in the urine more CRP than AGA infants. This difference may be explained either by a prerenal mechanisms due to hypoperfusion during placental insufficiency or increased stimulation of renal CRP production by circulating pro-inflammatory cytokines.

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PREOPERATIVE CRANIAL ULTRASOUNDS FINDINGS IN INFANTS WITH CONGENITAL HEART DISEASE

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Background: Advances in diagnostic testing and surgical techniques have resulted in reduced mortality in neonates with congenital heart disease (CHD) and a major concern for neurological morbidity in the presence of preoperative neurological injury. **Objectives:** To determine the incidence and nature of preoperative cerebral abnormalities in neonates with CHD and to examine the relationship between cerebral abnormalities and the type of CHD. **Methods.** Retrospective study. Inclusion-criteria: 1) Neonates with CHD admitted to the NICU over a 3-year period, 2) Gestational age >35 weeks, 3) Documented preoperative cranial ultrasound available. Exclusion-criteria: 1) Small For Gestational Age, 2) Other congenital anomalies and/or chromosomal abnormalities, 3) Congenital TORCH infection. Cranial ultrasounds (CUS) were reviewed without knowledge of the cardiac defect. CHD were categorized.

Results: Fifty-one of 109 neonates with CHD met the inclusion criteria. Twenty-one patients (41%) had abnormalities on CUS. Thirteen of these (25%) had widened ventricular and/or subarachnoid spaces, 3 (6%) lenticulostriate vasculopathy, 1 (2%) calcification in the basal nuclei, and 4 (8%) neonates had acute ischemic changes. Cerebral abnormalities were found more frequently in patients with coarctation or hypoplastic left heart syndrome (HLHS) than transposition of the great arteries (TGA) (63% versus 14%).

Conclusions: There is a high incidence of preoperative cerebral ultrasound abnormalities in neonates with CHD.

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ACTIVATION OF CIRCULATING CD4+ T-CELLS IN PRETERM INFANTS WITH RDS

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Background: In preterm infants with respiratory distress syndrome (RDS), early activation of circulating phagocytes is present as a sign of systemic inflammation. Phagocytes interact closely with lymphocytes. The role of lymphocytes in the pathogenesis in RDS is unclear. The aim of this study was to evaluate lymphocyte subsets and their activation during the first postnatal week in preterm infants with and without RDS .

Methods: Peripheral blood samples from 58 preterm infants [gestational age (GA) 27.3(26.3–29.4) wks; birth weight (BW) 930(733–1200)g] were taken on postnatal days 1, 3 and 7 (d1, d3, and d7). T-lymphocyte subpopulations (CD4+, CD8+ and NK-cells) and proportions of T-cells expressing activation marker CD54 (ICAM-1) were analyzed by flow cytometry using fluorescent antibodies. Infants who had increased C-reactive protein levels (<20mg/L) were excluded from the analysis (N=10) to control activation of lymphocytes due to infection. The remaining infants were assigned to two groups according to whether they had RDS or not. The results are given as absolute cell counts (10E9/L) and proportions of CD54+CD4+ cells of CD4+T-lymphocytes (%), in medians (quartiles).

Results: 25 infants had RDS [GA 26.7(25.3–27.6)wks, BW 860(700–1060)g], and 23 infants had not [GA 32.7(30.6–33.9)wks, BW 1500(1380–1990)g]. Infants with RDS had significantly lower GA and BW than those without RDS, both $p<0.001$. Infants with RDS had significantly lower blood T-lymphocyte count on d3 than did infants without RDS ($p=0.028$). Compared with infants without RDS, infants with RDS had lower CD4+T-cell counts on d3 ($p=0.034$) and CD8+T-cell counts on d1 ($p=0.036$) and on d3, although not statistically significant ($p=0.067$). In infants with RDS on d1, d3, and d7, a greater proportion CD4+ T-cells was CD54-positive than in infants without RDS [d1: 4.3(1.4–10.0) vs. 2.1(0.8–2.8), $p=0.001$; d3: 4.0(1.4–13.0) vs 1.8(0.8–3.0)%, $p=0.009$; d7: 5.4(2.6–9.9) vs 2.5(1.7–3.9), $p=0.014$]. There was no correlation between gestational age and proportions of CD4+CD54+-cells.

Conclusion: In preterm infants with RDS, the absolute numbers of circulating T-cells are low and the peripheral blood CD4+ T-lymphocytes have more active immuophenotype than in infants without RDS. These results indicate an activation of circulating lymphocytes in RDS. The significance of T-cell activation in the inflammatory process related to RDS and development of chronic complications, such as bronchopulmonary dysplasia, remains to be elucidated.

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LOW PROPORTIONS OF PERIPHERAL BLOOD TCRÁÁ-CELLS IN NEWBORN INFANTS

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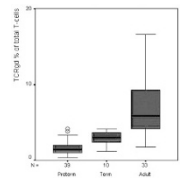
Background: Majority of peripheral blood T-cells express the áâT-cell receptor (TCRáâ) , which recognizes specific MHC-bound antigens. In the adult, 5–10% of blood T-cells express the TCRää-receptor. TCRää-cells recognize antigens in a non-MHC-restricted manner and are part of innate immunity. They are thought to act in defence against microbial pathogens and may play a role in controlling inflammation and preventing chronic inflammatory reactions. Newborn infants, especially those born very preterm, are susceptible to infections. In preterm infants inflammation may play a role in the development of chronic complications. However, little is known about the T-cell subsets in the perinatal period in newborn infants. The aim of this study was to evaluate the TCR áâ and ää -subsets in the peripheral blood in newborn preterm and term infants in comparision with adults.

Methods: Peripheral blood was drawn on the first day of life from 45 preterm infants [gest. age (GA) 28.3(10.3)wks; birth weight (BW) 1000(1780)g], 12 healthy term infants, and 33 healthy adult volunteers. From the preterm infants, follow-up blood samples were drawn at the age of 1, 2, 4, and 6 weeks. T-cell subpopulations were analyzed by flow-cytometry using fluorescent antibodies.

Results: In newborn preterm infants, the proportion of T-cells presenting TCRää was lower than in term infants [median (range) 1.5(3.8) vs 3.1(3.0) %; $p<0.001$], and in adult controls [5.9(14.8);$p<0.001$]. In term newborns the proportion of TCRää-cells was lower than in adults ($p=0.001$) (Figure). The blood TCRää-cell counts were lower in preterm infants than in term infants [0.030(0.11) vs 0.091(0.10) 10E9/L;$p<0.001$] or adults [0.08(0.25)10E9/L;$p<0.001$]. The blood TCRää-cell counts were similar in term infants and adults. The blood TCRää-cell counts gradually increased during the follow-up in the preterm infants, and at the age of 6 wks they were comparable with the term infants' level at d1 [0.081(0.28) vs 0.091(0.10)].

Conclusion: The low proportion of TCRää-cells in the peripheral blood of newborn infants may contribute to their susceptibility to infections. Furthermore, in preterm infants this may contribute to development of chronic inflammation seen in complications such as bronchopulmonary dysplasia.



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DETERMINANTS OF ALTERED ADIPOSE TISSUE DEPOSITION IN PRETERM INFANTS AT TERM

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Background and aims: Adiposity, particularly intra-abdominal, is associated with insulin resistance and type-2 diabetes. Insults in early life may programme long term risks but causal pathways are unclear. We have previously presented work to this Society showing that total adiposity in preterm infants at the age of term-equivalent is similar to term controls, but that the distribution is altered, with decreased subcutaneous (SC), and increased intra-abdominal (IA) AT. The aim of this part of the study was to explore possible determinants of total adiposity and altered AT partitioning, specifically the influence of gestational age(GA) at birth, postnatal growth, disease severity, and diet.

Methods: Infants underwent whole body magnetic resonance AT imaging at term-equivalent. Individual AT compartments were quantified and summated to determine total AT volume. Total adiposity was expressed as a percentage of body weight (%ATM); AT partitioning was expressed as SC and IA AT as a percentage of total AT volume (%SCATV, %IAATV). We documented the number of days that breast milk was received, and number of days of level 1 and 2 care (BAPM 2001) and expressed these as percentage of total days from delivery to term-equivalent (% breast milk and % level 1&2 care). We used % level 1&2 care as an index of disease severity. We expressed weight gain as weight SDS gain (SDSG) (Child Growth Foundation, U.K).

Results: We studied 38 infants (GA range 23 - 32 wk). Linear regression showed a significant correlation between %ATM and SDSG ($r= 0.396$, $p=0.014$),There was significant positive correlation between %SCATV and GA ($r= 0.388$, $p=0.016$) and SDSG ($r= 0.404$, $p=0.012$), and a significant negative correlation with %level 1&2 care ($r= -0.575$, $p<0.0001$). The negative impact of increased % level 1&2 care on %SCATV was confirmed in a multiple regression analysis allowing for GA, % breast milk and SDSG (adj. r square 31.7%, $B= -0.087$, $SE=0.028$. $p=0.004$). A multiple regression model incorporating the same variables showed increasing %IAATV with increasing % level 1&2 care (adj. r square 23.1%, $B= 0.037$, $SE=0.011$, $p=0.002$).

Discussion: We have shown that rapid postnatal weight gain is accompanied by increased adiposity. We have also shown that increased disease severity results in decreased SC AT and increased IA AT. Establishing if rapid postnatal growth is a risk factor for later obesity and if altered AT partitioning is sustained and accompanied by metabolic abnormalities should be considered research priorities.