



September 15th, 2021 08:30 - 10:30

## PARALLEL SESSION 3 - NUTRITION 1

### ID 196. Ultra-high temperature (UHT) treatment and prolonged storage of liquid infant formula induces protein modifications, gut dysfunction and inflammation in preterm pigs

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**Background:** Ultra-high temperature (UHT)-treated infant formula (IF) is increasingly being used for hospitalized infants when human milk is unavailable. UHT treatment eliminates pathogens and extends shelf life, but heating and storage may negatively affect the quality by reducing bioactivity and increasing the formation of Maillard reaction products (MRPs). We hypothesized that stored UHT-treated IF negatively affect gut health in sensitive newborns.

**Methods:** Using preterm pigs as a model for sensitive newborn infants, we fed liquid IFs subjected to indirect UHT (UHT), UHT with storage at 40°C for 60 days (SUHT) or just pasteurized (PAST). Diet bioactivity and MRP levels were determined together with markers of gut maturation and health.

**Results:** Relative to PAST, the UHT-IFs contained reduced levels of bioactive proteins (IgG, lactoferrin). Storage increased MRP levels (up to 13-fold) and non-reducible protein aggregates (SUHT vs. UHT). Furthermore, SUHT had lower antimicrobial capacity (versus *E. faecalis*, *S. epidermidis*) than PAST. Following five days of feeding, pigs fed SUHT had more diarrhea than pigs fed PAST and more signs of intestinal inflammation (necrotizing enterocolitis) than PAST and UHT pigs. UHT and particularly SUHT pigs showed lower intestinal villus heights and higher crypt depths and an increase in MPO-positive cells (monocytes and neutrophils), relative to PAST. Additionally, digestive enzyme activities (lactase, aminopeptidase N) were reduced in SUHT vs. PAST pigs, with intermediate values in pigs fed UHT. In SUHT pigs, this was accompanied by gut accumulation of MRPs (furosine and advanced glycation endproducts (AGEs), including N-ε-carboxymethyllysine (CML)) as well as the protein-cross-links lysinoalanine (LAL) and lanthionine (LAN) and RAGE-mediated inflammatory responses involved upregulation of genes involved in acute inflammatory responses and cell turnover (e.g. C3, TNFA, TNFAIP3, IL6, MCP1, CD62L, CASP3, PCNA, OLFM4, TGFB1).

**Conclusion:** Indirect UHT treatment followed by prolonged storage of IF reduced protein bioactivity and increased MRP and protein cross-link accumulation. This was associated with impaired gut maturation and function in preterm pigs in the first days of life. UHT-treatment and storage may negatively affect the quality of liquid IFs and may thereby negatively affect organ development in newborn infants, particularly those that are very diet-sensitive or immature.

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## ID 268. Necrotizing enterocolitis in neonates: early genetic markers

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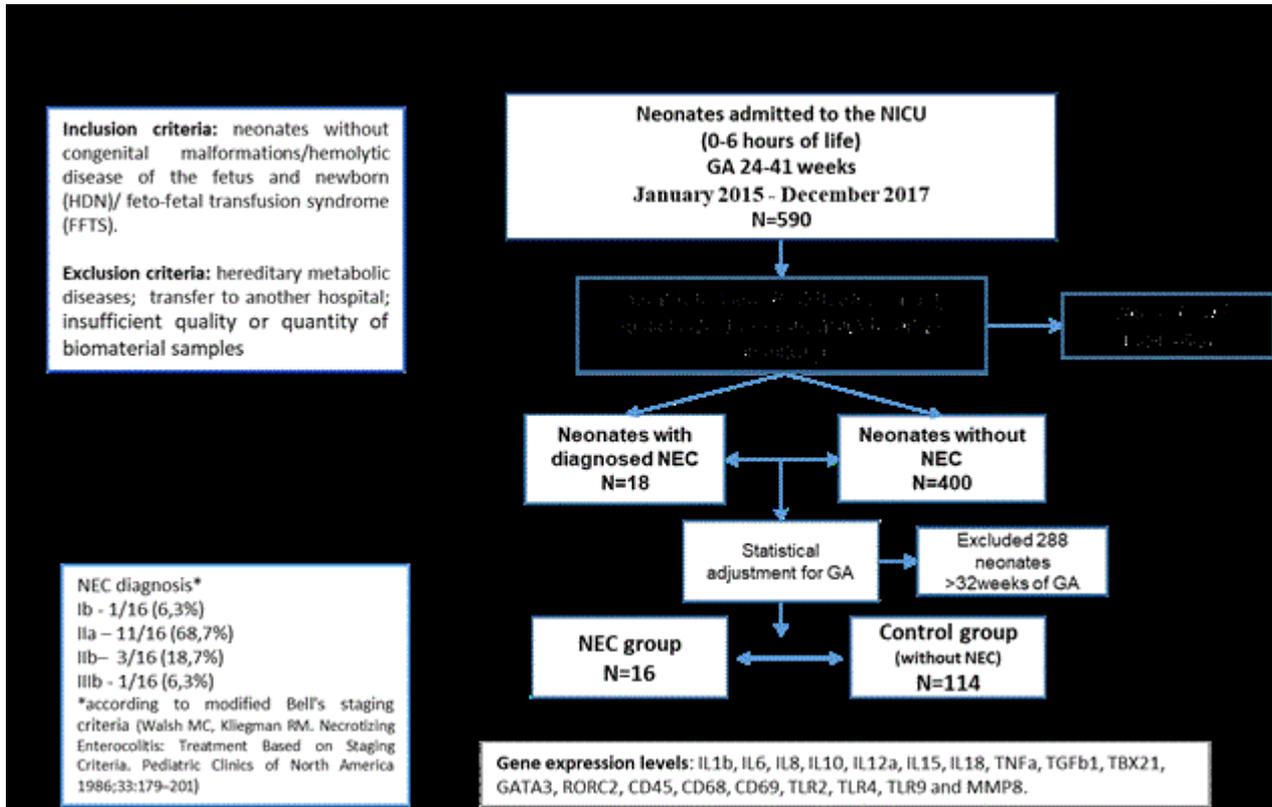
**BACKGROUND.** The most widely used tests for early necrotizing enterocolitis (NEC) detection are still non-specific. Investigating gene expression and pathophysiological pathways would allow us to find gut-associated specific biomarkers. This study aimed to reveal potential genetic predictors of NEC in neonates.

**METHODS.** The study included 590 neonates (gestational age (GA) 24-41 weeks) admitted to the NICU during the first hours of life from January 2015 to December 2017. All neonates underwent sampling of biological material (venous blood (VB) and buccal swabs (BS) before the start of medical treatment and enteral feeding. The reverse transcription method was used to measure gene expression levels: IL1b, IL6, IL8, IL10, IL12a, IL15, IL18, TNF $\alpha$ , TGF $\beta$ 1, TBX21, GATA3, RORC2, CD45, CD68, CD69, TLR2, TLR4, TLR9 and MMP8. We compared gene expression in VB and BS of neonates who developed NEC and those without NEC.

**RESULTS.** The development of NEC was noted in 25 out of 590 newborns initially included in the study (4,2%). After the technical assessment of biomaterial and exclusion of 172 newborns, there were remaining 418 patients with 18 cases of NEC. After statistical adjustment according to GA, 130 patients were remaining: 16 Neonates with NEC and 114 patients in the control group (without NEC). In premature infants, a statistically significant increase in the level of TLR4 expression in VB was associated with the development of NEC. Also, a downward trend in GATA3 was noted in neonates with NEC compared to the control. The decimal Lg (TLR4 / GATA3) then was calculated and ROC analysis showed a decent prognostic value of this criterion: threshold value of 0.74, the sensitivity -88%, and the specificity -77%; positive predictive value (PPV) - 39%, negative predictive value (NPV) - 97 %.

The same analysis in the buccal epithelium didn't show significant differences between the groups. The expression level of other genes did not differ statistically significantly between groups.

**CONCLUSION.** TLR4 gene expression measured in the first hours of life as well as Lg (TLR4/GATA3) could be considered as potential early biomarkers of subsequent NEC development; future research is needed to approve obtained data.



none declared



## ID 361. Splanchnic oxygenation below 30% as predictor for NEC in extremely preterm neonates

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**Background:** Impaired splanchnic microcirculation seems to play an important role in the pathogenesis of NEC in extremely preterm neonates. A previous study showed that a mean splanchnic oxygenation <30% is associated with increased risk to develop NEC. The aim of this study was to assess the sensitivity and specificity of 30% as cut off for splanchnic oxygenation (SrSO<sub>2</sub>) at day 2-6 of life to predict NEC in a pooled cohort of extremely preterm infants.

**Methods:** Two cohorts of extremely preterm neonates (<28 weeks GA) from two university hospitals were pooled together in a mixed cohort study. Splanchnic oxygenation was measured for 1-2 hours in extremely preterm infants at 2-6 days of life, after enteral nutrition had been introduced. Both centers used the INVOS 5100c with neonatal somasensor to perform NIRS monitoring. The primary outcome was NEC (Bell's stage >2a). Odds ratio to develop NEC was assessed with generalized linear model analysis, adjusting for center. We calculated sensitivity, specificity, positive and negative predictive values, for the SrSO<sub>2</sub> cut off 30%.

**Results:** We included 89 extremely preterm infants, 55 (61.8%) boys, median gestational age 26.2 (range 23.0-27.9). Seventeen (19%) developed NEC, 8 (18%) in center A and 9(20%) in center B. In cohort A all infants were continuously fed (n=44) while in cohort B infants were bolus-fed (n=45). There was no difference in mean SrSO<sub>2</sub> between the two centers (40.1 SD (18.5) vs 38.2 SD(20.3), p=0.65. Mean SrSO<sub>2</sub><30% was found in 33.3% of infants who did not develop NEC compared to 70.5% of those who did develop NEC (p=0.07). The Odds ratio to develop NEC, adjusted for center, with mean SrSO<sub>2</sub><30% was 4.8 (CI (1.51-15.3), P=0.008). Specificity and sensitivity of the SrSO<sub>2</sub> cut-off 30% are shown in the table. Sensitivity, specificity, positive and negative predictive values were similar in the two centers despite the different feeding strategies.

**Conclusions:** Mean SrSO<sub>2</sub><30% in extremely preterm infants between day 2-6 of life may be useful to predict who is going to develop NEC or not. The negative predictive value of mean SrSO<sub>2</sub><30% provides further proof for the importance of impaired splanchnic microcirculation in NEC pathogenesis.

Center	Both cohorts (n=89)	center A (n=44)	center B (n=45)
AUC (95% CI)	0.63 (0.47-0.78)	0.67 (0.41-0.89)	0.61(0.4-0.79)
Sensitivity (95% CI)	0.71 (0.47-0.88)	0.75 (0.38-1)	0.67 (0.33-1)
Specificity (95% CI)	0.67 (0.56-0.78)	0.72 (0.58-0.86)	0.61 (0.44-0.78)
PPV (95% CI)	0.33 (0.24-0.45)	0.38 (0.23-0.56)	0.30 (0.17-0.44)
NPV(95% CI)	0.91 (0.84-0.97)	0.93 (0.84-1)	0.88 (0.78-1)

Table: Sensitivity, Specificity, Positive predictive value ( PPV) and negative predictive value( NPV). Area under the curve (AUC) 95% confidence interval

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Table: Sensitivity, Specificity, Positive predictive value ( PPV) and negative predictive value( NPV). Area under the curve (AUC) 95% confidence interval  
None declared



## ID 399. Evaluation of late outcomes in premature patients undergoing mild therapeutic hypothermia as a treatment for necrotizing enterocolitis

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### Background

Necrotizing enterocolitis (NEC) leads to prolonged use of parenteral nutrition, generating malnutrition, and increased risk of infection through long-term venous access duration. NEC treatment with mild and controlled hypothermia is performed in our service as an additional treatment for stage 2 NEC since 2018. This study evaluated the outcomes related to the morbidity of premature infants who underwent mild therapeutic hypothermia.

### Methods

A Quality study was performed from January 2015 to March 2021. The NEC diagnosis (established by the modified Bell criteria) was made by the neonatology team and two independent neonatologists. The SQUIRE checklist was followed. The patients were divided into two groups: the control group (antibiotics and fasting) and the hypothermia group (conventional treatment and passive hypothermia for 48 hours after diagnosis). The variables studied were days of parenteral nutrition, time for the reintroduction of the milk, days of gastric residuals, time for normalization of the abdominal physical examination.

Severe neurological outcome was also assessed, considered delayed neurodevelopmental impairment and/or epilepsy and/or grade II / III intraventricular hemorrhage (Volpe classification) and/or leukomalacia at six months.

### Results

83 children were diagnosed with necrotizing enterocolitis; 53 babies underwent mild therapeutic hypothermia, in addition to conventional treatment and 30 babies received only conventional treatment. The group submitted to therapeutic hypothermia presented significant differences ( $p < 0.01$ ) in the digestive aspects: parenteral nutrition (TPN) for less time 27.4 days (SD: 30.3) versus control group 58.2 days (SD:86.7); the reintroduction of enteral feeding earlier ( $13.3 \pm 7.9$  days) versus the control group patients ( $19.9 \pm 14.9$  days), normalization of the abdominal examination 10.9 days (SD: 5.0) versus 18.8 (SD: 11.0) and decreased gastric waste earlier 8.3 days (SD:7.2) versus 13 days (SD 7.5).

The hypothermia group had a lower risk of severe neurological outcomes (RR = 0.91 (0.55; 1.49)) and a lower occurrence of death associated with NEC (36.6% vs 3.7%  $p < 0.01$ ).

### Conclusion

Preterm infants with NEC who underwent mild and controlled hypothermia had better late outcomes related to digestive and neurological outcomes when compared to patients who only received conventional therapy for NEC.



OUTCOMES	HYPOTERMIA GROUP (n=53)	CONTROL GROUP (n=30)	p VALUE
Time to reintroduce the diet (days)*	13.3 (±7.9)	19.9 (±14.9)	p < 0.01
Duration of gastric residuals (days)*	10.9 (±5.0)	18.8 (±11.0)	p < 0.01
Time for normal abdominal examination (days)*	8.3 (±7.2)	13 (±7.5)	p < 0.01
Duration of use parenteral nutrition (days)*	27.4 (±30.3)	58.2 (±86.7)	p < 0.01

\*Mean (Standard Deviation)

None declared