NEONATUS 2017, Poznan, Poland

Nutrition, gut and NEC!

Boris W. Kramer, MD, PhD
Neonatologist

Director of Pediatric Research
Professor of Experimental Perinatology
Maastricht University Medical Center, Netherlands

Professor of Neonatology
Institute of Women’s Health
University College London, UK

School of Oncology and Developmental Biology
Eat responsibly.
Clinical scenario

24+2 week preterm baby, 5 days old, has not yet passed stools.

Baby was born out clinical chorioamnionitis

Is fed with breast milk

Has been on antibiotics

What would you do?
Content

- Basic idea(s) – Inflammation and Development!
  Clinical data

- Animal data
  - Gut development, inflammation & immunomodulation

- Clinical perspective
Organ injury - when, where, what effect?

proinflammatory

chorioamnionitis

resuscitation

hyperoxia

fetal gut 9 months 15 min

preterm gut weeks

NEC

Hypoxia / Ischemia

- prematurity

antenatal steroids

surfactant

postnatal steroids

Nutrition

anti-inflammatory

Neonatology = where clinical care meets developmental biology

- Genetics
- Developmental Programming – epigenetics
- Stem cell populations
- Preconditioning to modulate response to a stimulus
- Systemic immune modulation
- Postnatal drug effects
- Nutrition

Ventilation → Infection → \( \text{O}_2 \) → Injury

Conception & In utero → Organ Development → Repair

“Normal” → “Adverse Outcome”
Necrotising Enterocolitis

• Inflammation & necrosis of intestine in preterm infants

• Peak incidence at 32 weeks post menstrual age

• Occurs latest in the most preterm

• Excessive inflammatory process extends the disease systemically

• Microbiota changes precede NEC

• *Up to 25% of infants will have microcephaly*

• Risks of neurodevelopment delay transcend concerns relating purely to the gut

Pathogenesis of NEC includes enteral feeding, gut ischaemia & bacterial infection

Santulli et al. *Paediatrics* 1975;55:376-87

>90% of babies who develop NEC have been fed

Genetic predisposition

Luminal environment

Intestinal immaturity

Innate immunity

Altered perfusion
2006 definition by International Society for Cellular Therapy (ISCT)
Brain development in Germ-free mice

- Reduced memory and reference memory
- Increased risk taking & exploratory behavior
- Chemistry changed:
  - Increased turnover of dopamine and noradrenaline and 5-hydroxytryptamine
  - Synaptophysin
In 1967, Abrams and Bishop showed that animals devoid of live microbes (germ-free) had decreased gut motility compared to animals harboring a conventional mouse microbiota (Abrams and Bishop, 1967).
Indigenous bacteria produce metabolites that signal to colonic enterochromaffin cells (ECs)

ECs increase Tph1 expression & 5-HT biosynthesis

Increased 5-HT is secreted luminaly & basolaterally

Increased 5-HT uptake by circulating platelets & activation after stimulation

Increased stimulation of myenteric neurons & gut motility
Necrotising enterocolitis is characterised by disrupted immune regulation and diminished mucosal regulatory (FOXP3)/effector (CD4, CD8) T cell ratios.
Neonatal immunity: bias towards tolerance?

Disease Tolerance as a Defense Strategy

Disease Tolerance – Fetal/Maternal Unit

Tolerance to damage by pathogen

Direct Damage by Pathogen
- Preterm labor
- Stillbirth
- Neonatal Sepsis
- Organ Injury

Tolerance to damage by immune system

Host resistance mechanisms: Maternal – innate and acquired Fetal - ?

Damage from host immune responses:
Maternal – preterm labor
Fetal – cytokines causing brain injury
Tolerance to Infection

Maternal
- Chorioamnion/AF
- Placenta
- Endometrium

Fetal
- Brain
- Gut/Lung
- Skin

Tolerance Capacity

Low

High
The amniotic cavity is…

- Sterile
- Non-sterile
- Something in between
Transfer of genetically labeled bacteria from the mother to the fetus in utero

Fig. 2. PCR detection of *E. fecium* JLM3 among colonies isolated from meconium. Lane 1, 100 bp ladder (Bioline, London, UK); lane 2, PCR positive control (genomic DNA obtained from transgenic soy); lanes 3–7, colonies obtained from group A mice that grew on MRS-Cm agar plates; lanes 8–12, colonies obtained from group B mice; lane 13, PCR-negative control (*E. fecium* HA1).
Facts: Ureaplasma

- 3rd smallest bacteria
- No cell wall – but difficult DNA extraction
- Urea as source of energy
- Eucaryote cells secret urea
- Bacteria adheres to cell wall
- 40% to 80% of sexually active women have ureaplasma in lower genital tract –
- 70% of sexually active men; ureaplasma parvum serovar 3 most common
Gut injury after UP chorioamnionitis

Wolfs et al., Mucosal Immunology 2013
IL-1RA affected ZO-1 expression

Wolfs et al., Mucosal Immunology 2013
Basic idea

Nikiforou et al., Inflammatory Bowel Disease, 2015
- Microbiota are metabolically active
- The metabolic activity results into volatile organic substances (VOCs)
- VOCs can be detected by gas and mass spectroscopy
Table II. Subject characteristics of the 3 subgroups NEC, sepsis, and controls

<table>
<thead>
<tr>
<th></th>
<th>NEC 13</th>
<th>Sepsis 31</th>
<th>Control 14</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex: Male (n [%])</td>
<td>4* [31]</td>
<td>18 [58]</td>
<td>11* [79]</td>
</tr>
<tr>
<td>Birth weight (median [IQR], g)</td>
<td>740 [155]</td>
<td>880 [300]</td>
<td>937 [346]</td>
</tr>
<tr>
<td>Gestational age (median [IQR], wk + d [d])</td>
<td>26 + 6 [6]</td>
<td>26 + 3 [17.5]</td>
<td>26 + 5 [15.3]</td>
</tr>
<tr>
<td>Feeding pattern (n [%])</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Breast milk ± formula</td>
<td>12 [92]</td>
<td>29 [94]</td>
<td>13 [93]</td>
</tr>
<tr>
<td>Exclusive formula</td>
<td>1 [8]</td>
<td>1 [3]</td>
<td>0 [0]</td>
</tr>
<tr>
<td>Postnatal age at T₀ (median, [IQR], d)</td>
<td>18.5† [8]</td>
<td>10† [7]</td>
<td>17† [0]</td>
</tr>
<tr>
<td>AB use before T₀ (%)</td>
<td>n = 13 (100)</td>
<td>n = 29 (94)</td>
<td>n = 13 (93)</td>
</tr>
<tr>
<td>Deceased (n [%])</td>
<td>7 [54]</td>
<td>3 [10]</td>
<td>0 [0]</td>
</tr>
<tr>
<td>Age deceased (median [d])</td>
<td>20</td>
<td>18</td>
<td>NA</td>
</tr>
</tbody>
</table>

*AB, antibiotics; NA, not applicable.
*P = .013.
†P = .005.
‡P = .008.
**Table I.** Number of fecal samples (n) in each group per selected time windows used for VOC analysis

<table>
<thead>
<tr>
<th>Window</th>
<th>NEC</th>
<th>Control</th>
<th>Sepsis</th>
</tr>
</thead>
<tbody>
<tr>
<td>T$_{-1,0}$</td>
<td>9</td>
<td>9</td>
<td>23</td>
</tr>
<tr>
<td>T$_{-3,-2}$</td>
<td>12</td>
<td>12</td>
<td>20</td>
</tr>
<tr>
<td>T$_{-5,-4}$</td>
<td>10</td>
<td>10</td>
<td>18</td>
</tr>
</tbody>
</table>

**Table IV.** Isolated pathogens n (%) from blood cultures in 31 sepsis patients

<table>
<thead>
<tr>
<th>Pathogen</th>
<th>NEC</th>
<th>Control</th>
<th>Sepsis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Staphylococcus aureus</td>
<td>2</td>
<td>[6]</td>
<td></td>
</tr>
<tr>
<td>CNS</td>
<td>21</td>
<td>[68]</td>
<td></td>
</tr>
<tr>
<td>Staphylococcus epidermidis</td>
<td>10</td>
<td>[32]</td>
<td></td>
</tr>
<tr>
<td>Staphylococcus capitis</td>
<td>5</td>
<td>[16]</td>
<td></td>
</tr>
<tr>
<td>Staphylococcus haemolyticus</td>
<td>1</td>
<td>[3]</td>
<td></td>
</tr>
<tr>
<td>Staphylococcus warneri</td>
<td>2</td>
<td>[6]</td>
<td></td>
</tr>
<tr>
<td>Combination of 2 different CNS</td>
<td>3</td>
<td>[10]$^*$</td>
<td></td>
</tr>
<tr>
<td>Escherichia coli</td>
<td>3</td>
<td>[10]</td>
<td></td>
</tr>
<tr>
<td>Enterococcus faecalis</td>
<td>1</td>
<td>[3]</td>
<td></td>
</tr>
<tr>
<td>Serratia marcescens</td>
<td>1</td>
<td>[3]</td>
<td></td>
</tr>
<tr>
<td>Candida albicans</td>
<td>1</td>
<td>[3]</td>
<td></td>
</tr>
<tr>
<td>More than 1 pathogen cultured</td>
<td>2</td>
<td>[6]$^+$</td>
<td></td>
</tr>
</tbody>
</table>

CNS, Coagulase negative *Staphylococcus*.  
$^{*}$2 × *Staphylococcus capitis* and *Staphylococcus epidermidis*; 1 × *Staphylococcus capitis* and *Staphylococcus haemolyticus*.  
$^{+}$1 × *Enterobacter cloacae* and *Staphylococcus warneri*; 1 × *Acinetobacter baumanii* and *Staphylococcus capitis*
Electronic Nose (eNose) distinguishes sepsis and NEC

De Meijs et al., J. Pediatr., 2015
eNose on faecal samples

Prospective study - 128 infants (thirteen NEC, thirty-one sepsis, eighty-four controls) - 2110 faecal samples - Analyses performed in blinded fashion - Discrimination 2-3 days before the onset of clinical NEC possible

Niemarkt et al., submitted
**Table III. Performance characteristics of fecal VOC analysis for the discrimination of NEC, sepsis, and controls**

<table>
<thead>
<tr>
<th>Time window</th>
<th>$AUC \pm 95% \text{ CI}$</th>
<th>$P$ value</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>$+LR$</th>
<th>$-LR$</th>
</tr>
</thead>
<tbody>
<tr>
<td>NEC vs control</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$T_{0,-1}$</td>
<td>$0.99 \pm 0.04$</td>
<td>$&gt;.001$</td>
<td>88.9</td>
<td>88.9</td>
<td>8.1</td>
<td>0.1</td>
</tr>
<tr>
<td>$T_{-2,-3}$</td>
<td>$0.77 \pm 0.21$</td>
<td>0.024</td>
<td>83.3</td>
<td>75.0</td>
<td>3.3</td>
<td>0.2</td>
</tr>
<tr>
<td>$T_{-4,-5}$</td>
<td>$0.65 \pm 0.25$</td>
<td>0.257</td>
<td>60.0</td>
<td>60.0</td>
<td>1.5</td>
<td>0.7</td>
</tr>
<tr>
<td>NEC vs sepsis</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$T_{0,-1}$</td>
<td>$0.64 \pm 0.18$</td>
<td>0.216</td>
<td>88.9</td>
<td>56.5</td>
<td>2.1</td>
<td>0.20</td>
</tr>
<tr>
<td>$T_{-2,-3}$</td>
<td>$0.80 \pm 0.17$</td>
<td>0.004</td>
<td>83.3</td>
<td>75.0</td>
<td>3.3</td>
<td>0.2</td>
</tr>
<tr>
<td>$T_{-4,-5}$</td>
<td>$0.52 \pm 0.23$</td>
<td>0.886</td>
<td>50.0</td>
<td>40.0</td>
<td>0.8</td>
<td>1.3</td>
</tr>
</tbody>
</table>

$+LR$, positive likelihood ratio; $-LR$, negative likelihood ratio.
Sensitivities, specificities, and positive and negative likelihood ratios are reported for the optimum cut-points.

De Meijs et al., J. Pediatr., 2015
Take to work messages:

- Antenatal exposure to inflammation is associated with adverse outcomes ("multi-organ disease of the fetus")
- Gut may be heavily injured already upon birth
- Immunomodulatory interventions for Tregs may improve structure and reduce inflammation
- eNose may help to predict / differentiate sepsis and NEC
Thank you very much

MUMC+
Prof. Dr. L.J.I. Zimmermann
Dr. J. Been
Dr. Hendrik Niemarkt
Dr. R. Jellema
Dr. Elke Kuypers
Dr. Tim Wolfs

Würzburg
Prof. Dr. C.P. Speer
Prof. Dr. S. Kunzmann

Cincinnati
Prof. Dr. A.H. Jobe
Prof. Dr. S.G. Kallapur

Perth
Prof. Dr. J. Newnham
Dr. M. Kemp

Funding
NWO VENI
School of Oncology and Developmental Biology
School of Mental Health and Neuroscience
NIH
DFG
Stichting Kindergeneeskunde