Mechanisms Affecting the Gut of Preterm Infants in Enteral feeding trials (MAGPIE)

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Conflict of Interest

- Research funding
  - UK National Institutes for Health Research
  - Nestle Nutrition, Danone/Nutricia, Prolacta Bioscience
  - Charities (Tiny Lives, Special Trustees)

- No shares, financial holdings
Newcastle Neonatal Research Team

- Approach to studying NEC and microbiome
- Collaborative trials
  - ELFIN – Lactoferrin RCT
  - SIFT – Feed speed RCT
- Nested mechanistic studies
  - SERVIS & Great North Neonatal Biobank
  - MAGPIE (nested in ELFIN & SIFT)
- Active trials
  - INDIGO opened September 2017
How far could we go doing rubbish research?

Garbage research … how much science can you do using the rubbish bin!
Milk – food or biochemical signal?

Change in thinking - Breast milk: developmentally regulated maternal-infant biochemical signalling pathway
Gut - most important immune organ

• Microbial > human **cells**

• Microbial >>> human **genes**

• Interested in functional effects
  – Metabolites produced by bacteria and nutrients
  – Bacterial gene function
Mechanisms & effects change over the life-course

Life-course

Microbes

Nutrients

Immune system
NEC is result of abnormal interactions between these elements
Day to day practice to reduce NEC in large NICU

- Strongly promote mother’s own fresh breast milk
  - Leadership, resources, beliefs etc.
- Evidence based practice
  - Infection control, antibiotic use etc.
  - Blood transfusion?
  - Probiotics?
  - Donor milk??
  - Feeding rates?
  - Lactoferrin?

*We need large RCTs +/- meta-analyses*
Pragmatic RCT paradigm: intervention (exposure) and outcome (phenotype)

Exposures & risks

- **Modifiable**
  - Milk type
  - Antibiotics
  - Etc.

- **Not modifiable**
  - Delivery mode
  - Gestation

Phenotype

- Health
- Disease
- NEC
- Sepsis

Challenge with NEC: how does the intervention (exposure) work?
NEC complex interplay: modulating & interacting factors

Exposures & risks
- Modifiable
  - Milk type
  - Antibiotics
  - Probiotics
- Not modifiable*
  - Delivery mode
  - Gestation
  - Genetics

Phenotype
- Health
- Disease
- NEC
- Sepsis

‘Immune-type’

Genetic
Epigenetic
Immune

Microbiota

‘Gut community type’
Strong evidence: dysbiosis precedes NEC – association or causation?
NEC and microbiome

• Explosion of published studies
• Different methodologies, technical, 16s regions etc.

• Key limitation – observational design

• Challenges
  – Robust capture and definition of exposures
  – Robust definition of outcomes
  – Complexity of the NEC phenotype
Clinical research challenge in NEC

Need large pragmatic RCTs

– Almost **all** RCTs under-powered for NEC

Large RCTs designed to ‘test’ intervention with ‘proof of principle’

– Pilot work could be better (e.g. probiotic trials)

Basic clinical research in preterm baby is challenging

– Practical and ethical aspects
– Logistics & sample size
– Funding
Gut microbiomic communities - complex exposures

Observational study: what is happening, not why
Animal & enteroid models essential to improve understanding NEC mechanisms

• Cellular mechanisms – TLRs, signalling etc.

Challenges

– Microbiomic ‘milieu’ is different
– Nutritional ‘milieu’ is different
– Extremely low gestational age (<75% full gestation)
  • Impossible to mimic in animal models
– NEC antecedents are uniform & may lack validity
– NEC ‘diagnosed’ at different stage
  • Classically post-mortem

Translation to NICU is challenging
Access to gut is difficult – but this is where the action is

Gut microbial communities <-> Nutrients

Research based on stool & urine samples – multiple limitations

Challenge – make the most of the opportunity
How do nutrients modulate gut microbiota?

Gut microbial ecology

Amino acids
Urea
Lactoferrin
HMOs
Human milk oligosaccharides
Fatty acids
Carbohydrates
What nutrients and metabolites are produced?

- Short chain fatty acids, e.g. butyrate
- Amino acids
- Bile acids
- Phenols
- Vitamin B, K
- Choline

Gut microbial metabolism
Host-microbe metabolic axes

Gut microbes ↔ Metabolic & immune function

Host-microbe metabolic axes “multi-directional interactive chemical communication highway”


J Nicholson 2012
Short chain fatty acids (SCFAs)

HMO, lactate, linoleic acid etc.

Clostridia, Eubacteria Roseburia

SCFAs

Gene expression: multi-organ targets
- Promotion of Treg cells
- Cytokines
- Antimicrobial peptides
- Mucous production
- Gut brain axis
- Etc.
Choline

Complex role
- Cell membrane
- Neurotransmitter
- Methyl donor

↑ Clostridia
↓ Bacteroidetes

∴ Trimethylamine Oxide

TMAO ➔ increased risk adverse cardiovascular events
Summary schema

Dietary nutrients affect...

Different types of bacteria which...

Differences in pattern of metabolites......

Limitless range of health and disease
The potential range & complexity is enormous

Breast milk proteins, HMOs, FAs

>500 bacterial species in preterm

>50,000 metabolites

RCTs at least allow us to control for 1 exposure!

Interaction in each baby is unique
Nutrients & metabolites & microbes

- RCTs are essential to change practice
  - But progress will be very slow
  - Registry based trials (e.g. WHEAT) are important addition

- Observational studies & animal models continue to be essential to aid understanding of basic biology

- Nesting ‘non-interventional’ mechanistic studies within RCTs – unique opportunity
Great North Baby Biobank

• Started 2010
  – N=570 preterm infants <32 weeks gestation
  – >75 cases with NEC (includes referrals)
  – Daily stool & urine samples
  – 0.5mL breast milk (only where sufficient)
  – Salvaged blood
    • ‘Waste’ specimens & discarded blood spots
  – Resected gut tissue (if operation needed)
• Governance
• Logistics
• Archiving
• Funding
Twins at Risk of NEC & Sepsis

12 twins, 1 triplet set

Triplet 1

Triplet 2

Triplet 3
Twins at Risk of NEC & Sepsis

- Twins - different exposures but similar stool microbiota
- Suggests shared environmental (milk) and/or genetic factors

12 twins, 1 triplet set
Breast milk & stool microbiomes are similar

Suggests breast milk components (microbes & nutrients) are major determinant of stool microbes
Patterns in twins with/without NEC

Diversity index

NEC diagnosis

Diversity is different (NEC twin v healthy twin) in the week prior to NEC, but earlier on not much difference observed.
Patterns in twins with/without NEC

Explore impact of antibiotics – not always ‘predictable’
Changes in taxa pre-NEC

Twin 139 - NEC

Twin 140 – no NEC

NEC diagnosis

Klebsiella

E coli
• 40 babies longitudinal changes
• % of different taxa
• Sampled regularly on NICU

OUT = Operational Taxonomy Unit

**Core OTU** 0-100% of reads
  • Klebsiella, E coli, Entero faecalis, S aureus (dashed line)
**Satellite OTU** 0-10%
  • Bifido, Strep, Pseudomonas, Bacteroides, Clostridium etc. (solid line)
Baby #234
- 99% of this baby's stool for first 5 days of life = Klebsiella
  ...but he was always completely well!

Chaotic!
Every baby is unique
Impossible to identify the ‘diseased’ individual
Preterm Gut Community Type (PGCT) clustering heatmap

Cluster into one of 6 types independent of demographics based on 16s

Relative abundance
Common
Uncommon

Stewart, CJ. et al. Microbiome 2017

The Newcastle upon Tyne Hospitals
NHS Foundation Trust
Preterm Gut Community Type (PGCT) clustering heatmap

Cluster into one of 6 types independent of demographics based on 16s

Each sample is of one type
Babies change types - daily/weekly

Stewart, CJ. et al. Microbiome 2017
Preterm Gut Community Type (PGCT) clustering heatmap

Cluster into one of 6 types independent of demographics based on 16s

Next we represent 6 PGCTs each by a colour dots

- PGCT 1
- PGCT 2
- PGCT 3 etc.

Stewart, CJ. et al. Microbiome 2017
PGCTs in NEC and LOS

NEC cases
- NEC_199
- NEC_180
- NEC_161
- NEC_139
- LOS_251
- LOS_181

LOS cases
- LOS_166
- LOS_130

Controls
- Control_253
- Control_241
- Control_234
- Control_232
- Control_229
- Control_228
- Control_224
- Control_223
- Control_215
- Control_209
- Control_208
- Control_207
- Control_06
- Control_03
- Control_188
- Control_186
- Control_182
- Control_176
- Control_168
- Control_167
- Control_159
- Control_156
- Control_153
- Control_152
- Control_143
- Control_131
- Control_117

PGCT3 = Staph dominated
Less common after week 3

PGCT6 = Bifido & diverse
Present throughout in controls
Not present pre-NEC or LOS
PGCTs ‘instability’

More PGCT transitions in NEC ($P = 0.043$)

Interpretation - babies with NEC more ‘unstable’ communities
Pre-NEC infant
Healthy infant
Other rubbish opportunities

- Metabolome
- Salvaged serum
- Virome & bacteriophage
- Retrieved gut tissue
Metabolomics

• Complex & multiple techniques
  – GCMS, LCMS, NMR etc.

• Different samples
  – Stool → microbial metabolism
  – Urine & plasma → host metabolism

• Multiple methodological, technological and identity challenges
Stool Volatile Organic Compounds (VOCs)

- Gaseous, carbon based compounds
- Individual patterns are unique
  - “Smellprint”
- Preterm and NEC
  - Changes appear pre-NEC
Volatile organic compounds

Twin 2 with NEC

Twin 1 healthy

GCMS

Loss of VOC in NEC

Rapid
Cheap
Bedside development
‘Pattern recognition’
Newcastle Neonatal Research Team

- Microbiome – learning about:
  - opportunities
  - methodologies & analyses
  - logistic & technical challenges

- Focus on nutrition and NEC

- Clinician desire to make a difference
  - Collaborate on large scale RCTs
  - Prove to funding bodies ‘we can do this’
UK Neonatal Nutrition Network

- Formed in 2003 - initially 6 neonatologists
- Now >100 active members
  - Multi-disciplinary, educational meetings
  - Enabled development of research studies
  - SIFT – feeding rates (CI Jon Dorling)
  - ELFIN – lactoferrin (CI Bill McGuire)
• <32 weeks gestation or <1500g
  – Fast (30 ml/kg/day)
  – Slow (18 ml/kg/day) increase in feed volumes

• Outcome
  – survival without moderate or severe disability at 24 months
Gestational Age at Delivery

~1000 infants <28 weeks

The Newcastle upon Tyne Hospitals
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Time to reach full milk feeds
Kaplan-Meier survival estimates

Days from trial entry

Slow increase (18 ml/kg/day)
Fast increase (30 ml/kg/day)
Short-term outcomes at hospital discharge

• N=2804 recruited ahead of schedule

• **No difference** in NEC or sepsis
  – No difference in gestational age sub-groups

• Faster feed group
  – Shorter duration of PN
  – Earlier attainment full feeds

• 2 year outcome data awaited
Lactoferrin

- Antimicrobial glycoprotein
- Colostrum, breast milk, tears, saliva
- Acid proteolysis → lactoferricin
Lactoferrin - high in colostrum

Lactoferrin concentration

Cow
Mature human
Colostrum

Structure is highly conserved
Lactoferrin functions

• Direct antimicrobial effects
  – bacterial, viral, fungal

• Modification of host immune response
  – Gut lymphoid tissue

• Direct epithelial effects
Enteral lactoferrin in Neonates (ELFIN)

• Large NIHR collaborative RCT
• 25 NICUs across UK

• Recruited n=2203 (closed September 2017)

• Primary outcome = sepsis; secondary = NEC
ELFIN

A multi-centre randomised placebo-controlled trial of prophylactic enteral lactoferrin supplementation to prevent late-onset invasive infection in very preterm infants.

Recruitment

Total 2200
Target: 2200

The latest infant was recruited at Royal Victoria Infirmary, Newcastle on 25 September 2017. Congratulations!

Introduction

This randomised controlled trial will evaluate whether giving very preterm infants supplemental lactoferrin (a natural antibiotic protein from cow's milk) reduces the number of serious infections. About 20% of very preterm infants (born before 32 weeks of gestation) acquire a serious infection in the neonatal unit. Better methods of preventing infection in very preterm infants are needed.
Mechanisms Affecting the Gut of Preterm Infants in Enteral feeding studies (MAGPIE)

• NIHR funded mechanistic study
• Completed N=470 direct recruits
• Additional n=108 (Biobank samples)
  – Daily stool & urine
  – Detailed clinical data (RCT captured)
  – **Randomised interventions**
  – 50,000 samples from 12 NICUs
    • Considerable logistical challenges
    • Strong peer & parent support
    • Peer reviews - some were ‘less than helpful’
Mechanisms Affecting the Gut of Preterm Infants in Enteral feeding studies (MAGPIE)

**MAGPIE**

- Stool
- Urine
- Salvaged blood
- Resected tissue

**INDIGO**

- Microbiome
- VOCs
- Metabolome
- Immune
  (Transcriptome)
Is NEC the only important outcome?

- Feed tolerance
- Full feeds
- Duration of PN
- Antibiotics
- Dysbiosis
- Sepsis
- Growth
- Metabolism
- Cognition
NEC is not the only important outcome but ..... 

NEC kills more children than all childhood leukemia and lymphoma combined
NICU smart diaper & bedside ‘omics’?

360 million diapers are changed every day

None of this health information has been used

Until Smart Diapers

DNA sequencing

eNose – Volatile Compounds

Integration of data

www.neonatalresearch.net

The Newcastle upon Tyne Hospitals
NHS Foundation Trust
Good research is a team effort

- **Newcastle Hospitals & University**
- **Dr Janet Berrington**, T Skeath, S Zalewski, J Perry, E Marrs, J Groombridge, T Sproat,
- **Dr Chris Stewart, Dr Chris Lamb**, J Kirby, S Hambleton, A Ewer, W McGuire, S Rushton, M Shirley, E Juczack, S Cummings, C Lanyon, **G Young** & many more!
- **ELFIN, SIFT, NPEU & N3 & MAGPIE**
- **Babies & parents**
- **Staff & funders**
Newcastle Neonatal Research Team

- Qualitative work with parents
- Series of studies

**Butterfly project** – single co-twin survivor
- Most often neonatal death on NICU
- Includes stillbirth co-twin loss
- ~50% of the deaths were due to NEC

*What does it feel like to be a parent?*