Background and Transparency: This newsletter was commissioned by Biofloratech Ltd, who manufacture Labinic® drops, which contain *Lactobacillus Acidophilus*, *Bifidobacterium infantis* and *Bifidobacterium bifidum.* Information intended for healthcare professionals.

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Can analysis of HMOs in preterm stool predict NEC?

Masi AC, Embleton ND, Lamb CA, et al. Human milk oligosaccharide DSLNT and gut microbiome in preterm infants predicts necrotising enterocolitis. Gut December 2020;0:1–10 doi:10.1136/ gutjnl-2020-322771

Researchers from Newcastle University, working with colleagues from Texas and San Diego, compared the stools of 33 preterm babies with NEC and 37 control babies. They examined the relative abundance of healthy probiotic bacteria, and also the concentrations of DSLNT (disialyllacto-N-tetraose), a human milk oligosaccharide (HMO). They also examined maternal secretor status (possession of active FUT2 gene for synthesis of α1-2 fucolsylated oligosaccharides) by analysing for presence of 2'-FL. Where babies had received a probiotic, it was Labinic or a similar one.

When comparing the 2 groups the researchers found that higher levels of DSLNT were associated with higher Bifidobacterium levels. Low levels of DSLNT, on the other hand, were associated with NEC and were independent of maternal secretor status. This may pave the way for the development of biomarkers which could predict the development of NEC.

Comment:

In our April 2020 newsletter we reviewed a couple of publications describing the metabolism of HMOs with a useful review of these compounds from the same San Diego group published in 2016 (**Moukarzel and Bode, Clinics in Perinatology 10.1016/j.clp.2016.11.014**).

There are hundreds of HMOs, which have structural similarities as they are based on monosaccharides. They are not digested by human gastrointestinal enzymes, but instead appear to be the nutritional source for probiotic bacteria. DSLNT appears to protect against NEC whereas the evidence that 2'FL, the most abundant HMO, offers similar protection is still unclear, although the evidence of GI protection in mice models is quite compelling. The Newcastle paper is interesting as it suggests that an individualised approach to ensuring that normal HMO levels are maintained may offer targeted benefit, where at risk babies could be supplemented to reduce their risks.

Giving supplemental HMOs routinely might work providing that the 'safe' dose range was sufficiently wide. 2'FL has been reported as being safe, and a dose finding study compared doses of 0.2g/dL and 1g/dL and found both well tolerated, with plasma concentrations highest in the breast-fed group rather than the supplemented ones. A level of 241nmol/mL

seemed to be a critical threshold for differentiating babies who developed NEC. Human milk remains the ideal choice for providing the majority of HMOs.

It does raise a thorny issue however – and that is that the production of HMOs by different mothers varies considerably. For example, this work found that up to it is estimated that up to 39% of mothers did not produce 2'FL (ie non-secretor status). In this work, this did not seem to be associated with NEC, but there is more to learn about this. Other non-genetic factors also play a part.

Work such as that published by the Newcastle group contributes to describing the "perfect" composition of HMOs in milk needed to minimise the risk of NEC and other important diseases, but of course breastmilk contains many other non-HMO constituents. However a supplement consisting of a combination of Bifidobacteria-rich probiotics (Labinic being an example) and prebiotics (e.g. HMOs), i.e. a synbiotic, would seem to be a sophisticated direction of travel to reduce the risks of NEC and improving gut health in babies.

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Can Artificial Intelligence analysis of preterm stool predict NEC?

Hooven T, Lin YC, Salleb-Aoussi A. Multiple instance learning for predicting necrotizing enterocolitis in premature infants using microbiome data.

This abstract was presented at the ACM Conference of Health, Inference and Learning by researchers at Pittsburgh and Columbia. It describes the development of an early warning, machine-learning enabled system, to synthesise data from microbiome analysis, clinical and demography, to generate daily risk predictions of NEC for individual babies. They retrospectively analysed samples 2,895 of stool collected from 161 preterm infants, 45 of whom developed NEC in a 2016 study.

Comment:

If HMO and other factors for risk-analysis are added to the microbiome evaluations, and such tests become available (even at the ward level one day) then the prediction of NEC should become even more accurate and individualised therapies may then be possible. Watch this space!

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Probiotics and babies with congenital surgical bowel dysbiosis

Rao SC, Esvaran M, Patole SK *et al*. Gut microbiota in neonates with congenital gastrointestinal surgical conditions: a prospective study. Pediatric Research 2020:88;878–886

The awareness of dybiosis is increasing amongst clinical researchers. This work looked at the dysbiosis present in term babies with congenital surgical conditions, such as diaphgramatic hernia, oesophageal and intestinal atresias, and exomphalos . They evaluated Short Chain Fatty Acid (SCFA) levels and undertook analysis of bacterial populations using 16s ribosomal RNA analysis of stool samples.

They found that, by 10-14 postnatal days, surgical babies had developed significant dysbiosis with emergence of pathogenic bacteria and reduction of probiotic bacteria. In the control infants, Bifidobacterium was the dominant probiotic bacteria in stool.

For the SCFAs, the most prevalant were acetic acid, propionic acid and butyric acid, important contributors to the acidification of stool, and were lower in the surgical babies.

Comment:

Neonatologists have been wary of using probiotics in surgical or post-surgical babies due to reports of systemic probiotic sepsis. In a meta-analysis of adult surgical patients (Lytvyn L. et al. Probiotics and synbiotics for the prevention of postoperative infections following abdominal surgery: a systematic review and meta-analysis of randomized controlled trials. J. Hosp. Infect 2016:92;130–139) post-operative infections and hospital inpatient stays were reduced. It remains to be seen if this can be translated into benefits for babies with surgical GI conditions.

It is likely that this group of babies would benefit from similar analysis to the preterm infants studied by the Newcastle group (above), so that optimal feeding and probiotic supplementation practices could be adopted.

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Probiotics and Cochrane – more trials needed.

Probiotics to prevent necrotising enterocolitis in very preterm or very low birth weight infants. Sharif S, Meader N, Oddie SJ, Rojas-Reyes MX, McGuire W. Cochrane Database of Systematic Reviews 2020, Issue 10. DOI: 10.1002/14651858.CD005496.pub5

The conclusions from the latest Cochrane Review are somewhat at odds with many other network and study meta-analyses. The Cochrane reviewers said “further, large, high-quality trials are needed to provide evidence of sufficient quality and applicability to inform policy and practice”.

They focussed exclusively on very preterm and VLBW infants, and in their analysis of trials that they considered to be high-quality only, there was a reduction in NEC (n=4597 babies) from 7% to 5.9%, but this was not statistically significant. There were no episodes of probiotic sepsis in the included studies, which is an important reassurance of safety.

Comment:

It is important for the Cochrane reviewers to distinguish higher and lower quality studies. Whilst this in itself is open to interpretation, it limits the pool of evidence available for analysis. Whilst there was a reduction in NEC, it was not signficant.

But this is not the only source of knowledge on the subject. Large and well conducted, peer-reviewed retrospective studies (such as the Norwich study from Prof. Paul Clarke's group) point to important reductions in NEC and mortality after the introduction of multistrain probiotics.

Clinicians are now in a position to know that probiotics are safe, and that they may well be effective. The clinical evidence-base is lagging behind the scientific one, and this is probably because of the prohibitive cost and complexity of running large RCTs.

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Meta-analysis - Probiotics and prebiotics in preterm babies

Effects of Probiotics in Preterm Infants: A Network Meta-analysis. Cheng Chi, Cheng Li, Nicholas Buys et al. Pediatrics. 2021;147(1):e20200706

Another very large meta-analysis published in Pediatrics, of 45 trials with over 12,300 babies included, which aimed to rank the efficacy of strains to guide clinical decision-making about choosing a probiotic for neonatal use. **This analysis concluded that combination probiotics (Lactobacillus plus Bifidobacterium combinations) were associated with lower NEC (RR 0.47 (0.27-0.79)) and lower mortality (RR 0.56 (0.34-0.84)).**

NEC was defined as Bells Stage 2 or greater (to eliminate mild or suspected cases) and they examined secondary outcomes including sepsis, time to full enteral feeds and length of inpatient stay.

They stated that "the efficacy of single probiotic supplements is limited". However they also found that the addition of a prebiotic to the probiotic was more effective than placebo for sepsis (Lactobacillus plus prebiotic RR 0.18 (0.06-0.44)) and for enteral feeding and length of stay (Bifidobacterium plus prebiotic). The combination of Lactobacillus and Bifidobacterium also was superior to placebo for time to full enteral feeds (3.97 (1.65-5.74) and reducing hospital stay (7.3 (0.99-14.13).

Comment:

There are a number of studies which consistently show that combination probiotics, with a mixture of Lactobacillus and Bifidobacteria, achieve reductions of NEC, mortality and sepsis in premature infants.

The addition of prebiotic analysis showed, in this work, that they may achieve further reduction in mortality but not NEC.

It is likely that the prebiotic helps the colonisation of the probiotics to be achieved faster but may also help to improve intestinal maturity. We now need to determine whether prebiotics are needed in infants who are on breastmilk feeds, or whether they are only required to supplement formula feed.

And if so, which one(s)?

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Thank you for reading this update, we hope you found it interesting. Please feel free to share with healthcare-professional colleagues.

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