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Neonatal modulation of serum cytokine profiles by a specific mixture of anti-inflammatory neutral and acidic oligosaccharides in preterm infants

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ABSTRACT

Infections are common in preterm infants and cause differences in cytokine levels. Aim of this study was to measure cytokine levels in preterm infants during the first year of life and to determine the effect of feeding a specific non-digestible carbohydrate mixture (scGOS/lcFOS/pAOS). Furthermore, other perinatal factors in relation to these cytokine levels were analysed.

In a randomized controlled trial, preterm infants (GA <32 weeks and/or birth weight <1500 g) received a scGOS/lcFOS/pAOS mixture or a placebo (maltodextrin) between days 3 and 30 of life. Cytokine levels (IL-1β, IL-2, IL-4, IL-6, IL-8, IL-10, IL-17, IFN-γ, and TNF-α) were analysed at 5 time points during the study: before start of the study, at day 7, at day 14 and at 5 and 12 months after the start of the intervention.

In total, 55 preterm infants in the scGOS/lcFOS/pAOS group and 58 in the placebo group were included. During the neonatal period cytokine levels increased, followed by a decrease at 5 months and 12 months. Enteral supplementation of the non-digestible oligosaccharides decreased cytokine levels at day 7 but not at day 14, indicating a temporarily anti-inflammatory effect. In the neonatal period, serious infection before sampling increased all cytokine levels.

In conclusion, enteral supplementation of this specific non-digestible oligosaccharide mixture decreased cytokine levels in preterm infants at day 7 of life, although this effect disappeared thereafter.

1. Introduction

Preterm infants have an immature immune system which makes them vulnerable for infections [1]. Cytokines are a key part of the immune system, thereby playing a role in both the susceptibility and immune-defence of newborn infants towards infections. Cytokine levels in preterm infants differ from cytokine levels in term infants. In cord blood, pro-inflammatory cytokines such as Interleukin (IL)-2, IL-4, IL-5, IL-6, IL-8, IL-10 and tumor necrosis factor α (TNF-α) are higher in preterm infants than in term infants, while there are conflicting results regarding higher or even lower IL-1β levels in cord blood of preterm infants compared to term infants [2,3]. Levels of pro-inflammatory cytokines such as IL-1β, IL-6, IL-8, TNF-α and IFN-γ are higher in cord blood of preterm infants who were prenatally exposed to infection. IL-10, IL-18, IFN-γ, TGF-β and TNF-α might differentiate between infants with fungal and bacterial sepsis, later in the neonatal period [4]. Extremely low birth weight infants with blood stream infections have lower levels of IL-17 and higher levels of IL-6 and IL-8. The highest blood cytokines levels in preterm infants are found on the day of birth [5]. Levels of cytokines at birth or in the neonatal period in preterm infants are possible predictors of bronchopulmonary dysplasia, white matter brain damage and cerebral palsy [6–10].

The infant's immune system matures by exposure to intestinal microbiota [11,12]. This interaction leads to metabolic/immunologic reactions by the epithelial cells and the underlying lymphoid cells: the bacterial–epithelial 'cross talk' [13–15]. Preterm infants have a delayed intestinal colonisation and possibly as a consequence an inappropriate bacterial–epithelial 'crosstalk', resulting in an inadequate maturation of the host immune defence [13,14,16,17].

In term infants, breastfeeding decreases the incidence of infections [18]. Human milk oligosaccharides (HMOS) are part of the factors in human milk thought to be responsible for this effect [19–21]. Non-human milk oligosaccharides such as short-chain galacto-oligosaccharides (scGOS) and long-chain fructo-oligosaccharides (lcFOS) are used to mimic the functions of HMOS [22,23]. Pectin

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derived acidic oligosaccharides (pAOS) are able to act as receptors analogs and are known to inhibit the adhesion of pathogens on the epithelial surface [24]. pAOS may also directly affect the immune cells via interaction with selectins, dendritic cell specific C-type lectin, integrins and other target receptors such as Toll-like receptors [23]. We recently described that, if supplemented in sufficient amounts, the combination of neutral oligosaccharides and acidic oligosaccharides decreases the incidence of infections in preterm infants [25]. It was hypothesised that enteral supplementation of a scGOS/lcFOS/pAOS mixture consisting of neutral and acidic oligosaccharides may positively modulate the immune system.

As part of our randomized controlled trial, the aim of this study was to measure cytokine profiles during the neonatal period and during the first year of life of preterm infants. A unique analysis, which has never been done before in preterm infants, both during the neonatal period and the following year. In addition, the effect of enteral supplementation with a mixture of non-digestible neutral and acidic oligosaccharides but also the effect of other perinatal factors on serum cytokine profiles were analysed.

2. Methods

2.1. Subjects

Infants with a gestational age (GA) <32 weeks and/or birth weight (BW) <1500 g, admitted to the level III NICU of the VU University Medical Center, Amsterdam, were eligible for participation in the study. Exclusion criteria were: infants with a GA > 34 - weeks, major congenital or chromosomal anomalies, death <48 h after birth and transfer to another hospital <48 h after birth. The medical ethical review board of the hospital approved the study protocol. Written informed consent was obtained from all parents. This trial was registered at isrctn.org as ISRCTN16211826.

2.2. Randomization, blinding and treatment

The infants were randomly allocated to treatment <48 h after birth to receive either enteral 80% scGOS/lcFOS and 20% pAOS or placebo powder (maltodextrin) as previously described [25,26]. The randomisation code was broken after data analysis was performed. Supplementation of the mixture or placebo was administered in increasing doses between days 3 and 30 of life to 1.5 g/kg/day to breast milk or preterm formula. Per 100 mL, the preterm formula provided 80 kcal, 2.4 g protein (casein-whey protein ratio 40:60), 4.4 g fat and 7.8 g carbohydrate. The preterm formula did not contain oligosaccharides. When infants were transferred to another hospital before the end of the study, the protocol was continued under supervision of the principal investigator [25].

2.3. Nutritional support

Nutritional support was administered as previously described [25]. For each infant in the study a feeding schedule was proposed

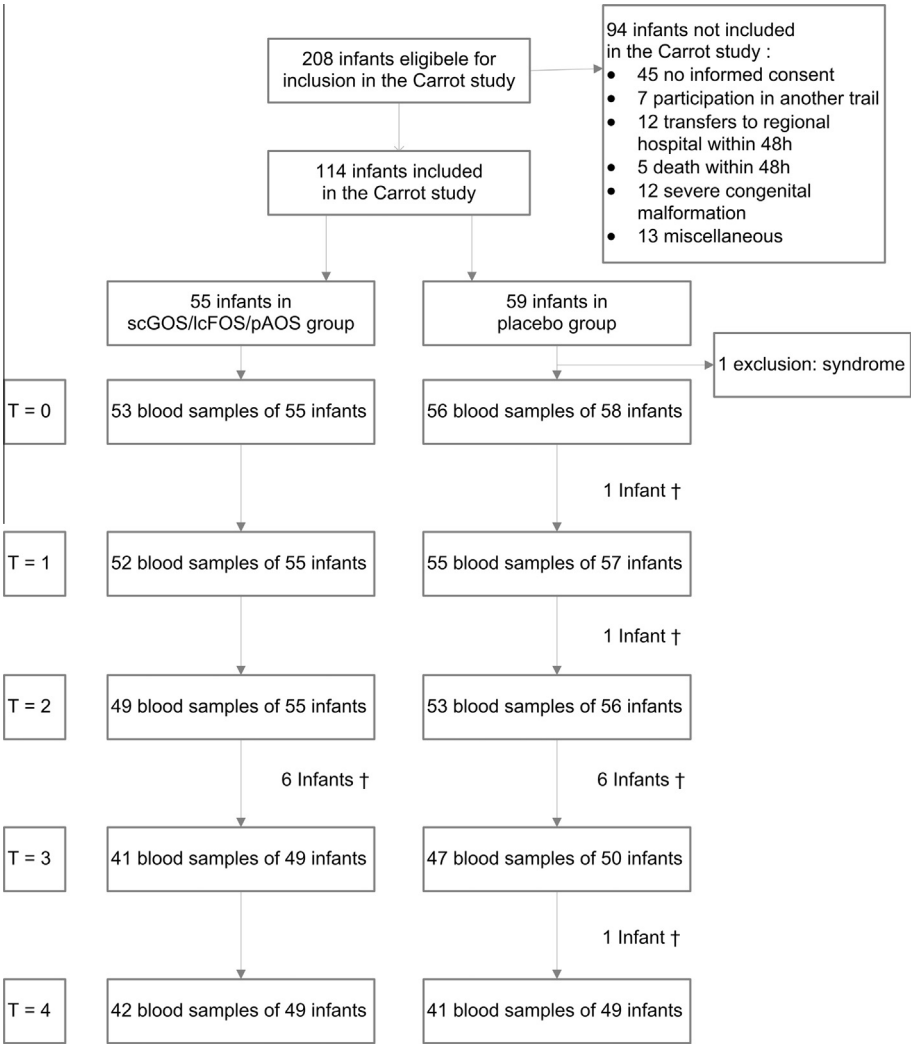

Fig. 1. Trial profile. Time points T0 = <48 h after birth, T1 = day 7, T2 = day 14, T3 = 5 months and T4 = 12 months.