Effects of an Amino Acid-Based Formula Supplemented with Two Human Milk Oligosaccharides on Growth, Tolerability, Safety, and Gut Microbiome in Infants with Cow's Milk Protein Allergy

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Background:

Amino acid-based formula (AAF) is indicated for non-breastfed infants with severe cow's milk protein allergy (CMPA), multiple food allergy, and those non-responsive to extensively hydrolysed formula. Intestinal microbial dysbiosis is associated with CMPA which manifests as low levels of bifidobacteria and higher levels of gram-negative bacteria, such as Proteobacteria. Human milk oligosaccharides (HMO) are important to establish a healthy gut microbiome by providing the specific substrate for infantile *Bifidobacterium* and *Bacteroides* species.

Objectives:

The primary objective was to assess the growth of infants with moderate-to-severe CMPA fed an AAF (Alfamino HMO) containing 2'-fucosyllactose (2'-FL) and lacto-N-neotetraose (LNnT). Growth was assessed over a 4-month follow-up period by comparison with the WHO 2006 Child Growth Standard. Secondary objectives included a 4-month evaluation of linear growth, head circumference (HC), and body mass index (BMI). The study also aimed to evaluate if the AAF with 2 HMO was effective and safe for symptom control. In addition, the effect of the HMO-containing AAF on bifidobacteria and intestinal dysbiosis was evaluated.

Study design:

- This was a single-arm, open-label, non-randomised, multicenter, interventional clinical trial.
- Non-breastfed, term infants aged 1–8 months with physician-diagnosed moderate-to-severe CMPA were considered.
- Alfamino HMO study formula contained 1.0g/L of 2'FL and 0.5g/L of LNnT, 66Kcal/100ml, 2.66g protein equivalent/100kcal in the form of free amino acids, and 24% of fat as MCT.
- Infants were followed from enrollment (V0) to 4 months (V4) with monthly monitoring (V1 to V4) of growth parameters, and other clinical information. Option was given to continue the AAF to 12 months of age with a final review at V5.
- 4 stool samples were collected at V0, V1, V4, and V5. Extraction of microbial DNA was performed, and the microbiome composition compared to the Clinical Microbiomics in-house infant fecal microbiome gene catalog. A taxon set enrichment analysis (TSEA) was performed at the genus level to assess the changes of microbiota from baseline to follow-up periods (V0 vs V1, V4 and V5).
- Fecal short-chain fatty acids (SCFA), namely acetate, propionate and butyrate, were measured by gas chromatography–mass spectrometry.

Results:

- 34 infants were screened leading to final enrollment of 32 (age range 4 to 37 weeks, mean age 18.6 weeks, 37.5% (12) female).
- 29 infants completed the trial to V4. 3 infants were withdrawn (1 due to adverse event, 1 lost to follow-up and 1 due to withdrawal of parental consent).
- The mean weight gain between V0 to V4 was 18.0 ± 6.13 g per day (range 7.8–29.2 g/day). Some catch up growth was observed. On comparison of Z-Scores for weight-for age, linear growth, HC and BMI (based on WHO growth standards), normal progression of growth parameters was observed, with a minor upward trend towards V4 and V5.
- There was a significant reduction in symptoms observed over the first month (V0 to V1), with a reduction of 79.9% and 88.4% for crying and fussing, 51.7% and 90.8% for regurgitation and vomiting, and the prevalence of skin problems reduced from 25% to 6.9%.
- In respect to safety, of the 232 adverse events (AE) experienced, the majority were mild (82.8%), and of the serious AE (SAE) reported (1.3%), none were deemed related to the formula. The AAF therefore was assessed to have an excellent safety profile.
- The gut microbiome analysis showed a significant early enrichment of the genus *Bifidobacterium*, including enrichment of HMO-utilizing bifidobacteria. Conversely, there was a significant reduction in the abundance of Proteobacteria, a marker phylum of gut dysbiosis.
- Microbiome changes were associated with a significant rise in fecal SCFA concentrations, with acetate being the predominant SCFA. Butyrate levels increased in the second half of the first year of life.
- Due to the single-arm study design it was not possible to reliably distinguish between the HMO effect and other factors that may have contributed to microbiome changes (e.g., age, complementary diet/fibre, environmental factors, antibiotics).

Conclusions:

- Infants with moderate-to-severe CMPA fed Alfamino HMO grew adequately, with minor acceleration of growth parameters towards 12 months of age. Alfamino HMO was well tolerated had an excellent safety profile. Significant beneficial effects were seen in the infants' gut microbiome with an enrichment of bifidobacteria and a partial correction of the intestinal dysbiosis.