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Original article

Probiotics in cystic fibrosis patients: A double blind crossover placebo controlled study
Pilot study from the ESPGHAN Working Group on Pancreas/CF

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SUMMARY

Background: A potential positive effect of probiotics in cystic fibrosis (CF) on fecal calprotectin (FCP), pulmonary exacerbations and weight has been described in small controlled trials.
Methods: A double-blind multicenter cross-over study (2 × 4 m) was performed looking at abdominal pain, nutritional status, pulmonary function, pulmonary exacerbation, FCP and lactulose/mannitol gut permeability test. Patients kept a diary with daily scoring of abdominal pain, stool frequency and consistency as well as treatment changes.
Results: 31 CF patients entered the study of which 25 finished it. At start patients aged 9.3yrs (6.9–12.2), had a median BMI z-score of −0.5 (−1.5–0.08), height z-score of −0.4 (−1.1–0.05) and FEV1% of 100% (87.2–106.6). Median FCP at start was 61 μg/g (17–108) and gut permeability 0.079 (0.051–0.122). No significant changes were observed in the clinical parameters (BMI, FEV1%, abdominal pain, exacerbations). Despite being frequently abnormal (17/28 (61%) >50 mg/kg), FCP did not change significantly with probiotics. The proportion of patients with normal permeability was 8% during placebo and 32% during probiotic treatment ($p = 0.031$). FCP correlated to BMI z-score ($p = 0.043$) and gut permeability to abdominal pain ($p = 0.015$). The microbiome revealed a high predominance of *Actinobacteria* and *Proteobacteriae*. Probiotic supplementation did not result in a shift at the phylum nor at phylogenetic level.
Conclusion: Normalization of gut permeability was observed in 13% of patients during probiotic treatment. However, none of the previously described effects could be confirmed.

1. Introduction

Cystic Fibrosis (CF) is the most common life shortening autosomal recessive disease. It is caused by a mutation in the cystic fibrosis transmembrane conductance regulator gene. CF results in a range of gastrointestinal complications including pancreatic insufficiency causing maldigestion and slowed intestinal transit.

Current CF treatment has led to substantial nutritional status improvement, gut inflammation remains, however, an important issue [1,2]. The strong relationship between gut inflammation and the systemic inflammation warrants further research in understanding, prevention and treatment of gut inflammation [3].

Gut dysbiosis has been described in the literature as a characteristic of CF [4,5]. In CF patients, gut microbiota are influenced by nutrient malabsorption, high caloric, fat-rich diet but also frequent treatments with proton pump inhibitors, inhaled, oral and intravenous broad-spectrum antibiotics [6–8]. Probiotics have been demonstrated to reduce the number of common respiratory and

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gastrointestinal infections in hospitalized children [9]. A recent meta-analysis evaluating the efficacy of probiotics in physiologic and pathological conditions concluded that the use of probiotics was "evidence-based" in case of antibiotic- and *Clostridium difficile*-associated diarrhea and respiratory tract infections [10].

In CF, a possible clinical effect on gut inflammation, quality of life and pulmonary function was described in a meta-analysis of the literature [11]. However, since, up to now, only a limited number of small controlled studies, with differences in study design, used probiotics as well as measured outcomes are available and further studies are necessary [12–17]. On the other hand, side effects of probiotics are scarce [11].

To further elucidate these previous results, a double blind multicenter crossover study was initiated.

2. Patients and methods

2.1. Study design

We performed a double blind placebo controlled crossover study. The inclusion criteria are listed in Table 1. Patients were randomized after consent, to receive either a probiotic containing 10^{10} Colony forming units (CFU) *Lactobacillus Rhamnosus SP1* (DSM 21690) and *Bifidobacterium animalis* spp.*BLC1* (LGM23512) or placebo for 4 months, switching to the other product for 4 months, after a wash out period of 1 month (Fig. 1). These strains were provided by the company and were chosen because of their in vitro anti-inflammatory effects on peripheral blood mononuclear cells and NTBC colitis models. The strains had in vitro no antagonistic effects.

Patients kept a diary containing a daily stool frequency and consistency evaluation, abdominal pain rating as well as all treatment changes during the complete study period. An exacerbation was considered as a period where, due to increased pulmonary symptoms, a patient was treated with antibiotics. Patients were clinically evaluated and weight, height and pulmonary function were measured at start, after the first period of 4 months and after the second period of 4 months. At the same evaluation points, a stool sample for calprotectin and microbiome analysis was taken, a gut permeability test was performed at home the weekend prior to the visit and a blood sample was taken for analysis of CRP and total IgG.

The forced vital capacity (FVC) and forced expiratory volume in 1 s (FEV1) were expressed as % of predicted. Pulmonary function is expressed as % of normal and weight, height and body mass index (BMI) as z-score compared to the Flemish growth curves [http://www.vub.ac.be/groeicurven].

2.2. Laboratory analysis

The gut permeability was measured using the lactulose (5 g)/mannitol (2 g) test. The concentrations of mannitol and lactulose in urine were measured using gas chromatographic separation of polyols. Values were considered normal if the lactulose/mannitol ratio was <0.03 [18,19].

Stool samples were stored at −80° before analysis. Calprotectin was measured on the Phadia ImmunoCAP 250 analyser (Thermo Scientific, Uppsala, Sweden) according to the manufacturer's instructions [20]. Results were expressed in mg/kg. Two cut-offs for normality were considered: 50 mg/kg and 100 mg/kg.

The microbiome was analyzed using techniques extensively described elsewhere [21]. Faeces samples were prepared for DNA extraction following the normal protocol. After extraction the 16S rRNA gene v3-v4 region was amplified. DNA concentration of amplicons of interest was determined by gelelectrophoresis and illumine libraries were constructed. Sequencing was done on an Illumina MiSeq using V3 Chemistry (Illumina).

Table 1
Inclusion and exclusion criteria.

Inclusion criteria	Exclusion
Irrefutable CF diagnosis: 2 sweat test: chloride >60 meq/L	Recent CF diagnosis (<6 m)
Clinically stable: no need for acute antibiotic treatment at start of study	Start tube feeding in 6 m prior to study
Pre-pubertal children	Oral or intravenous antibiotic treatment in the last month
Able to perform pulmonary function test	Oral or intravenous steroid treatment in the last 2 months
Preventive antibiotic treatment or antibiotic inhalation is allowed as long as taken 2 h apart from probiotic and continued throughout the study	New CF complications in the last 3 months: diabetes, liver disease
Assent from the child and consent from parents or guardian	New colonization in the past 3 months

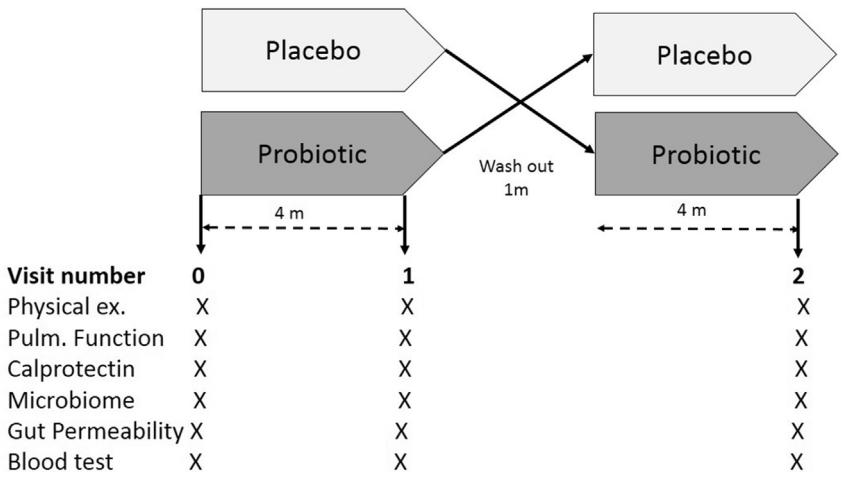

Fig. 1. Summary of the study design. (Physical ex: physical examination, Pulm. Function: pulmonary function testing).

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