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Original Article

Age-dependent variation of fecal calprotectin in cystic fibrosis and healthy children

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Abstract

Background: Fecal calprotectin may be used as a non-invasive method to assess the effect of novel therapies on the gut in cystic fibrosis (CF).
Method: Stools from CF patients and healthy controls (HC) (0–10 years old) were prospectively collected for evaluation of temporal trends.
Results: 130 CF samples (64 subjects) and 114 HC samples (101 subjects) were collected. Overall, fecal calprotectin levels were different in CF patients and HC from 0 to 10 years ($P = 0.0002$). Fecal calprotectin in CF was significantly lower than HC from 0 to 1 years ($P = 0.03$) and demonstrated an upward trajectory until 4 years. From >4 to 10 years calprotectin was consistently higher in CF patients compared with HC ($P = 0.007$).
Conclusions: Fecal calprotectin levels in children with CF and HC were age-dependent and had distinct trajectories. Careful interpretation of calprotectin is required if used in drug trials for CF, particularly in children less than 4 years old.

Keywords: Leukocyte L1 antigen complex; Calprotectin; S100 proteins; Cystic fibrosis; Gastrointestinal inflammation; Pediatrics

1. Introduction

Children with cystic fibrosis (CF), a life-limiting autosomal recessive disease, may struggle to reach the nutritional status of their peers and achieve their potential growth [1]. This occurs despite the development and use of nutritional therapies, including pancreatic enzyme replacement therapy (PERT) and high-fat, high-calorie diets [2]. It has recently been established that the gut environment in patients with CF is abnormal; studies have found that intestinal inflammation is present and potentially contributing to suboptimal growth in this population [3–5]. It is important these intestinal issues are addressed in order to improve nutritional outcomes in CF children.

Non-invasive fecal biomarkers are increasingly being utilized in human studies in order to investigate intestinal inflammation. In CF studies, the most widely used fecal biomarker is calprotectin,

Abbreviations: CH, Christchurch Hospital; CF, cystic fibrosis; HC, healthy controls; IBD, inflammatory bowel disease; PERT, pancreatic enzyme replacement therapy; PI, pancreatic insufficient; PS, pancreatic sufficient; RWH, Royal Women's Hospital; SCFA, short-chained fatty acids; SCH, Sydney Children's Hospital

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a zinc- and calcium-binding protein, formed by the complex of S100A8 and S100A9 [3]. In the small intestine, calprotectin is released by granulocytes, such as neutrophils and eosinophils [3,6]. Fecal calprotectin is known to be elevated in patients with CF, particularly pancreatic insufficient (PI) patients [3,7]. Furthermore, fecal calprotectin levels correlated with quality of life questionnaire scores in CF [8]. Studies of fecal calprotectin in healthy children demonstrate that calprotectin is typically elevated in infancy and early childhood [9,10]. As a result the common cut-off used for fecal calprotectin (50 mg/kg) is not a reliable cutoff for children under 4 years of age [11,12].

Fecal biomarkers, such as calprotectin, may have future use in assessing the effect of novel therapies on the CF gut [13]. To our knowledge, no other study has investigated levels of fecal calprotectin in CF across different ages. The aim of this study is to compare fecal calprotectin levels in CF and healthy children from 0 to 10 years of age.

2. Materials and method

2.1. Study population

This multi-center study prospectively recruited children, aged 0 to 10 years old, with CF from Sydney, Australia (Sydney Children's Hospital (SCH)) and Christchurch, New Zealand, (Christchurch Hospital (CH)), between 2011 and 2016. All CF children fulfilled the Cystic Fibrosis Foundation diagnostic criteria [14]. Patients were excluded from the study if they had gastroenteritis, or had been administered probiotics, corticosteroids or non-steroidal anti-inflammatory drugs in the two weeks preceding sample collection. Patients were also excluded if they had a pulmonary exacerbation, or administered oral or intravenous antibiotics (except for oral azithromycin, flucloxacillin, amoxicillin, amoxicillin-clavulanate and ciprofloxacin) in the four weeks preceding sample collection. Patients with known pre-existing gut disease (such as coeliac disease) at the time of sample collection were not included.

Demographic data collected included age, gender and genotype. Exocrine pancreatic status was evaluated and characterized as pancreatic sufficient (PS) or PI, using 72-h fecal fat and/or fecal elastase-1 [15,16].

Healthy controls (HC) were recruited from Sydney (SCH and the Royal Women's Hospital (RWH)) and CH. Subjects were excluded if they had CF, inflammatory bowel disease (IBD) or other known gut disease. The study was approved by the relevant local ethics committees. Informed consent was obtained from each subject and/or caregiver(s).

2.2. Sample collection

Stool samples were collected from CF and HC subjects. Samples were initially collected and stored at −20 °C until transferred to the laboratory where they were kept at −80 °C until analysis.

2.3. Sample analysis

Calprotectin was extracted and measured from frozen stool samples using the PhiCal kit (Calpro, San Diego, CA, US), according to the manufacturer's instructions. The lower limit of detection for the assay was 19.5 mg/kg.

2.4. Statistical analysis

All statistical analysis was performed using IBM SPSS Statistics 23.0 (Chicago, Illinois, USA). Chi-square or Fisher's exact test was used to compare categorical variables. The Mann Whitney-*U* test was used to compare continuous variables as the data was determined to be non-normally distributed. Data was presented as median [IQR; range]. Linear mixed model analysis accounts for repeated samples from the same subject and was performed to determine if there was a significant difference in calprotectin concentrations across the ages 0 to 10 years, between the two groups. Graphical interpretation of the trend in calprotectin with age was used to determine age subgroups for further analysis. Linear mixed model analysis was performed for each age subgroup comparing calprotectin concentration, adjusted for age, between groups. Calprotectin >50 mg/kg was considered elevated in children >4 to 10 years of age (calprotectin is difficult to interpret in children less than 4 years, as it is naturally high in healthy infants) [11,12]. *P*-values <0.05 were considered statistically significant for all analyses.

3. Results

3.1. Subject characteristics

A total of 130 CF samples from 64 CF subjects and 114 HC samples from 101 HC subjects were collected (Table 1). A total of 16 CF PS samples (from 9 subjects) and 114 CF PI samples (from 55 subjects) were used (Table 2). Number of samples collected ranged from 1 to 5.

3.2. Fecal calprotectin in CF patients vs. HC

Overall, there was a significant difference in levels of fecal calprotectin across ages 0 to 10 years old, between CF patients

Table 1
Patient and sample characteristics for CF patients and HC.

	CF patients	HC	*P*-value
Number of patients	64	101	
Males, n (%)	33 (51.6%)	47 (46.5%)	$P = 0.53$
Females, n (%)	31 (48.4%)	54 (53.5%)	
Number of samples	130	114	
Median age at time of sample collection [IQR; range]	4.8 [1.6–6.5; 0.08–10.0]	1.2 [0.2–3.2; 0.02–8.8]	$P < 0.0001$

CF, cystic fibrosis; HC, healthy controls; IQR, interquartile range.