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Severe disease in Cystic Fibrosis and fecal calprotectin levels

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ABSTRACT

Fecal calprotectin (FC) is used to asses the presence of intestinal inflammation also in patients with Cystic Fibrosis (CF) and recent studies showed a correlation between bowel and lung disease in these patients. The aim of this study was to analyze the levels of FC in CF and correlate them with different phenotypes of disease.

We enrolled a cohort of 54 CF patients and 50 healthy controls. In these patients, calprotectin has been assayed on a stools sample using an ELISA kit.

In all patients we analyzed, FC levels were elevated above the cut-off value and significantly higher than in healthy controls. Among CF patients, FC was significantly higher in patients older than 18 years, with pancreatic insufficiency, underweight status, *Pseudomonas Aeruginosa* airways colonization, CF-related diabetes mellitus, reduced lung function, or high number of pulmonary exacerbations.

These results suggest that in patients with CF, FC levels are not only influenced by the CF enteropathy but also by the severity of the genetic disease. Since we found higher FC levels in patients with a severe phenotype (*P. Aeruginosa* airways colonization, FEV1 < 50% of predicted, pancreatic insufficiency, underweight status,) we suggest that this marker could be useful to monitor longitudinally a clinical worsening.

1. Introduction

Several years ago, Smyth at al. showed that inflammatory process in patients with cystic fibrosis (CF) was not only limited to the lungs but also present in the intestinal tract (Smyth et al., 2000). In these patients, more recent studies confirmed the presence of a correlation between bowel and lung disease (Adriaanse et al., 2015) even if this correlation has not been quantified yet.

The current literature shows that intestinal inflammation may be assessed with high sensitivity and predictive value by the measurement of fecal calprotectin (FC) (Walsham and Sherwood, 2016).

Calprotectin, a protein found mainly in neutrophils, but also in monocytes and macrophages, is released during neutrophil activation or death. Calprotectin can be measured in plasma as well as other body fluids but it is six times more concentrated in faeces than in blood. Thus FC is an inflammatory marker that is elevated in a variety of inflammatory intestinal diseases such as Crohn's disease and CF (Vaos et al., 2013). Already in 1996, Golden at al. had measured the levels of calprotectin in serum of children with CF, many of which had been affected by infectious pulmonary exacerbations at the time of measurement. Serum calprotectin levels were higher in the group with the disease compared to healthy controls. The study revealed how the calprotectin assay in the serum would provide a better evaluation of acute inflammation of the airways than conventional serum biomarkers of inflammation, such as white blood cell counts and C-reactive protein (Golden et al., 1996). Canani et al. showed a high correlation between FC with the histologic grade of mucosal inflammation. Moreover, FC resulted in the most accurate tool to detect the presence of active mucosal inflammation when compared to clinical scores and common serum markers (Canani et al., 2008). Bruzzese et al. showed that mean FC was significantly higher in CF patients than in healthy controls, also proving that intestinal inflammation is a major feature of CF and is reduced by probiotics (Bruzzese et al., 2004).

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The aim of this study was to analyze the levels of FC in CF and correlate them with different phenotypes of disease.

2. Materials and methods

2.1. Patients population and study design

We enrolled a cohort of fifty-four patients from January to December 2015 (29 males and 25 females – age range 1–52 – average age 18.6 ± 12.4–24 younger than 18 and 30 patients older than 18) with a diagnosis of CF followed in the Cystic Fibrosis Hospital Centre of Catania University. All subjects with CF were diagnosed by using accepted diagnostic criteria, including a minimum of two clinical features consistent with the diagnosis and either positive sweat test (chloride values greater than 60 mEq/L) or two disease-causing CFTR mutations (Rosenstein and Cutting, 1998). In order to increase the homogeneity of the CF population, patients with clinical signs of exacerbation (decline of lung > 10%, start of antibiotic therapy, increase in coughing, clinical signs of infection, or signs on infection in terms of C-reactive protein elevation of differential counts) were excluded from analysis.

Fifty healthy controls (HS group – 29 males and 21 females – age range 3–53 – average age 17.7 ± 12.3 – 34 younger than 18 and 16 patients older than 18) were also recruited. None of these subjects were under medical treatment.

2.2. Collection of stool, procedures and definitions

In the 54 recruited CF patients and in the 50 healthy controls, calprotectin has been assayed on a stool sample collected from each of the patients during their regular visits, by using an enzyme-linked immunosorbent assay kit (RIDASCREEN ® Calprotectin – G09036). FC levels were expressed in μg/g. We considered normal, FC levels < 100 μg/g.

All the spirometric tests used in the present study were performed in our Pulmonary Physiology Unit. Values of forced expiratory volume in 1 s (FEV1) and forced vital capacity (FVC) were expressed as% predicted of normal values adjusted for age, gender, sex height and weight. Lung disease severity was established according to FEV1 values. In particular, FEV1 > 80% of predicted was considered as normal, FEV1 > 50% < 80% of predicted as indicative of a mild respiratory impairment; FEV1 < 50% of predicted as indicative of severe respiratory impairment. After spirometry, sputum induction was performed according to a standard operating procedure (Sagel et al., 2001) and was collected into a container. This specimen was submitted for comprehensive microbiology for CF consensus guidelines (Burns et al., 1998). We defined as *Pseudomonas Aeruginosa* airways colonization, the persistent presence of *Pseudomonas Aeruginosa* for at least 6 consecutive months in the sputum (Pressler et al., 2011). We defined as *severe*, a phenotype characterized by the presence of a FEV1 permanently < 50% of predicted, a condition of chronic airways colonization by *Pseudomonas Aeruginosa*, pancreatic insufficiency and underweight status; conversely we identified as *mild* a phenotype characterized by a FEV1 > 80% of predicted, no *Pseudomonas Aeruginosa* colonization in airways and a normal weight status. The body mass index (BMI) was used for the clinical definition of the nutritional status according to recommendations of international consensus. BMI and nutritional status patients were classified as underweight if BMI was <18.5; as normal weight if BMI was ≥18,5 ≤24,9; as overweight if BMI was ≥25.0 ≤29.9; as obese if BMI was 30.0 and above (Sinaasappel et al., 2002). Pancreatic insufficiency (PI) was defined as a fecal elastase <200 mg/g faeces. All patients with PI were treated with pancreatic enzyme replacement therapy (PERT). Cystic Fibrosis-related diabetes mellitus (CFRD) was defined by 2-h glycemia ≥ 200 mg/dL in the OGTT or two fasting glycemia measurements greater than 126 mg/dL (Moran et al., 1999). We defined as pulmonary exacerbations, respiratory infectious episodes requiring antibiotic therapy. The study was approved by our local Committee for Clinical Investigation and informed consent was obtained by each patient or parent.

2.3. Statistical analyses

Statistical analyses were performed with Microsoft Office Excel and the Minitab software (version 16.0). Differences between groups were established by unpaired *t*-test or by ANOVA followed by Bonferroni's test. Sperman correlation coefficients were used to test correlation between FC and other variables. A *P* value of <0.05 was considered to be significant.

3. Results

3.1. Patients baseline characteristics

Baseline clinical characteristics of our study subjects are summarized in Table 1.

40 patients (74%) were characterized by pancreatic insufficiency, 11 patients (20%) had CFRD; 28 (52%) had underweight status (BMI < 18,5); 21 (39%) used proton pump inhibitors (PPI); 15 (35%) had a severe respiratory impairment (FEV1 < 50% of predicted); 10 patients (23%) had a mild respiratory impairment (FEV1 > 50% <80% of predicted); 18 (42%) had a normal respiratory function (FEV1 > 80% of predicted). Chronic *Pseudomonas Aeruginosa* pulmonary colonization was assessed in 22 (41%) patients; 7 patients (13%) had a severe phenotype (chronic *Pseudomonas Aeruginosa* airways colonization, FEV1 <50% of predicted, pancreatic insufficiency and underweight status); 9 patients (17%) had a mild phenotype (no *Pseudomonas Aeruginosa* airways colonization, FEV1 > 80% of predicted and normal weight status).

3.2. FC levels in different phenotypes

FC levels were significantly higher in CF patients (598.7 ± 277.5 vs 48.4 ± 34.0 μg/g, P < 0.001), than in healthy controls and all the 54 patients had abnormal FC levels.

Within CF patients, adults showed significantly higher FC levels (667.3 ± 294.9 vs 517.5 ± 231.9 μg/g, P = 0.02) as well as patients with underweight status (637.7 ± 291.7 vs 515.1 ± 231.4 μg/g, P = 0.04), pancreatic insufficiency (665.5 ± 271.7 vs 417.1 ± 197.1 μg/g, P < 0.01), *Pseudomonas Aeruginosa* airways colonization (737.3 ± 310.3 vs 496.2 ± 216.2 μg/g, P < 0.01), CFRD (865.6 ± 256.7 vs 485.5 ± 210.2 μg/g, P < 0.01). Conversely, FC levels do not seem to be related to PPI use (659.4 ± 275.5 vs 560.1 ± 275.9 μg/g, P = 0.10).

According to pulmonary function, we found that patients with a severe respiratory impairment had significantly higher FC levels (843.5 ± 271.5 for FEV1 < 50% vs 599.4 ± 264.9 for FEV1 between 50% and 80% vs 443.3 ± 181.6 μg/g for FEV1 > 80%; P < 0.01). We summarized data in Table 2.

FC levels were closely related to pulmonary exacerbations (r = 0.55; P < 0.01) but not with lipase dosage of PERT (r = 0.02; P = 0.88). Fig. 1.

Finally, in *severe phenotype* clinically defined patients, we found significantly higher FC levels (1000.7 ± 256.6 vs 339.4 ± 94.3 μg/g, P < 0.001) than in *mild phenotype* clinically defined patients. Fig. 2.