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Use of monoclonal faecal elastase-1 concentration for pancreatic status assessment in cystic fibrosis patients

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Abstract

Objective: To assess the concentration of faecal elastase-1 (EL-1) in pediatric patients with cystic fibrosis with mutation ΔF508.

Methods: Cross-sectional study with samples collected consecutively from 51 patients aged 4 months to 17 years old (mean 9.11±4.74); 32 (62.8%) patients were male. Clinical-demographic data were collected, as well as data on the type of mutation. Exocrine pancreatic insufficiency was established by the activity of faecal EL-1 < 200 µg/g. EL-1 was quantified through the monoclonal ELISA method (ScheBo Biotech AG, Germany). Pancreatic supplements were used in 46 (90.2%) patients.

Results: Forty-one (80.4%) patients presented with pancreatic insufficiency (EL-1 fecal < 100 µg/g): 17 (41.5%) were homozygous, 14 were heterozygous (34.1%) and 10 were non-ΔF508 (24.4%). Regarding the mutation, there was a statistically significant association of homozygosity with faecal EL-1 concentration < 100 µg/g (p = 0.010). All patients considered to be pancreatic insufficient (n = 41) by the test were using pancreatic supplements. Ten (19.6%) presented faecal EL-1 > 200 µg/g, and 5/10 (50%) used enzymes.

Conclusions: The activity of faecal EL-1 < 100 µg/g, indicating pancreatic insufficiency, was observed in 17/17 (100%) of homozygous patients, as expected, and was less frequent in patients who were heterozygous for ΔF508 and in patients without the mutation. There was no association of faecal EL-1 concentration with age and sex of patients. The test was standardized, is easy to execute, and can be used to assess the pancreatic status of patients with cystic fibrosis.

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Introduction

Cystic fibrosis (CF) is the most common potentially fatal genetic disease affecting the Caucasoid population.[1] CF is characterized by chronic obstructive pulmonary disease, meconium ileus, exocrine pancreatic insufficiency and high sweat electrolyte concentration.[2] Diagnosis is conducted through the sweat test (Gibson-Cooke method), in which chlorine values < 40 mmol/L are considered normal and values ≥ 60 mmol/L are altered.[3] The sweat test should be repeated to confirm the CF diagnosis.[4] In a recent study, Mattar et al.[5] compared the classic Gibson-Cooke test and the

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conductivity test in patients with and without CF, observing that the conductivity test was equivalent to the classic test and concluding that there was good correspondence between both tests.

In CF, there is a progressive loss of exocrine pancreatic function due to the obstruction of the intrapancreatic ducts by mucous secretion, leading to the retention of digestive enzymes and establishing a chronic inflammatory process with fibrosis and consequent loss of pancreatic function.[6] It is widely accepted that, among the various manifestations of CF, the pancreatic phenotype has the best relation with the genotype, as stated in studies by Kerem et al.[7] and Zielenski.[8] According to Iwánczak et al.,[9] there is a clear correlation between the presence of mutation ΔF508 and the severity and frequency of gastrointestinal tract manifestations. According to the literature, approximately 15% of patients with FC are pancreatic sufficient (PS),[10-12] although Messick[13] has reported that all patients with CF present with some degree of pancreatic insufficiency (PI). The evaluation of exocrine pancreatic function is a mandatory procedure for diagnosis, with the purpose of determining the need for enzyme supplements.

There is a large number of exocrine pancreatic function tests, most of which are limited in their use, whether due to high cost, low availability and/or inconvenience of collecting stool samples for a long period. The measurement of faecal elastase-1 (EL-1) has proven promising, which is shown by the results that reveal a 90-100% sensitivity and a 96-100% specificity as indicators of exocrine pancreatic insufficiency.[14-16]

The objectives of this study were to assess and quantify the faecal EL-1 concentration in patients with CF, with mutation ΔF508, and compare the values of faecal EL-1 in patients with CF with and without mutation ΔF508.

Patients and methods

A cross-sectional study was conducted with patients suffering from CF, of both sexes, aged 4 months to 17 years old, who were being followed up at the pediatric pneumology ward of Hospital de Clínicas de Porto Alegre (HCPA), Porto Alegre, Brazil. The present study was approved by this institution's Research Ethics Committee, and was conducted between September, 2007 and November, 2008. Samples were collected consecutively, and the 51 individuals analyzed accounted for approximately 34% of patients with CF being followed up at the ward.

Inclusion criteria for the study were as follows: a) Patients with CF of both sexes, aged 1 month to 18 years old, who were being followed up at the pediatric pneumology ward of HCPA; b) Presence of CF, with diagnosis confirmed through two sweat sodium and chloride tests or by the presence of two mutations associated with the clinical diagnosis.

Exclusion criteria included: patients taking drugs to regularize bowel habits; patients with presence of enterostomy/colostomy; liquid stool three or more times a day, during the two weeks preceding the examination; and not signing the informed consent form. Data was collected during admission and/or routine outpatient follow-up of patients with CF. The follow-up routine at the HCPA pediatric pneumology outpatient clinic includes appointments with the medical team every two months and an annual check-up that includes additional tests. In order to conduct this study, we invited patients who come to the outpatient clinic for their annual check-up; demographic data were obtained from the patient records and the dose of pancreatic supplement was informed by the patient's guardians. At the time of signing the informed consent form, the guardians received the stool container.

The analysis of the mutation to CF was conducted at the Medical Genetics Service of HCPA, the following mutations were researched: ΔF508, G542X, R553X, G551D and N1303K. Real-time PCR was the DNA sequencing method used (it is the standard method at the HCPA Genetics Service).

The collected stool samples were stored at the Laboratory of Experimental Gastroenterology and Hepathology, in a freezer, at the temperature of -20 °C, until the time of testing. According to the manufacturer's guidelines (ScheBo Biotech AG, Germany), the material could be stored for up to one year, if stored in this manner.

Faecal EL-1 was quantified using monoclonal sandwich ELISA, which uses two monoclonal antibodies against different epitopes of human pancreatic elastase. The cutoff point was established at 200 µg/g, with patients whose values were above 200 µg/g classified as pancreatic sufficient and patients whose faecal EL-1 values were below 200 µg/g as pancreatic insufficient. Values ranging between 100 and 200 µg/g were considered as probable mild PI, whereas values below 100 µg/g were considered as severe PI, according to the manufacturers' guidelines. Eighty-one stool samples from 51 patients were analyzed. Thirty parts were duplicated from 10 patients in each group.

Statistical analysis

Sample size was calculated to detect a difference of 1 standard deviation between the faecal elastase levels in the 3 groups of patients studied (group 1: patients homozygous for mutation ΔF508; group 2: patients heterozygous for mutation ΔF508; group 3: patients without mutation ΔF508), considering $\alpha = 0.05$, 80% power. A minimum number of 17 patients in each group was established.

The quantitative variables were described through mean values and standard deviation (symmetric distribution) or median and range/interquartile range (non-symmetric

distribution). The qualitative variables were described through absolute and relative frequencies.

In order to evaluate the association between qualitative variables, this study used Pearson's chi-square test or Fisher's exact test. For continuous quantitative variables regarding pancreatic insufficiency, the study used the Mann-Whitney test.

The adopted level of significance was of 5%, and analyses were conducted in the SPSS, version 13.0.

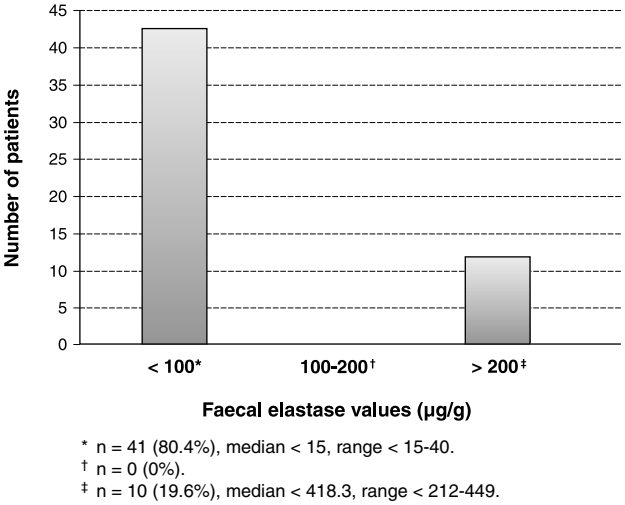

Figure 1 - Faecal elastase values categorized in three ranges in the population under study

Results

The study included 51 patients with CF diagnosis who were followed-up during stay and at the pediatric pneumology outpatient clinic of HCPA. The sample was made up by three groups: G1 = 17 patients homozygous for mutation ΔF508; G2 = 17 patients heterozygous for mutation ΔF508; and G3 = 17 patients without mutation ΔF508.

There was a 62.7% (n = 32) predominance of males. Patient age ranged for 4 months to 17 years, with a mean of 9.11 years (±4.74 years).

Admitted patients accounted for 88.2% (45/51) of the studied sample, whereas 11.7% (6/51) were outpatients. Reasons for admission to hospital were: routine annual check-up (25/51:55.5%); pulmonary exacerbation (10/51:22.2%); fever (6/51:13.3%); low weight (3/51:6.7%); and asthma crisis (1/51:2.2%). The median of days of hospital stay was 19, ranging from 11 to 65 days.

Enzyme replacement therapy was used in 46 (90.2%) patients. According to information obtained from the people in charge, the median of administered enzyme used was 5,346.88 units of lipase per kg/day (510-15,652). There was no significant difference in the dose of enzyme per kg/day among the different patient groups (Kruskal-Wallis test; p = 0.457).

There were 10/51 (19.6%) patients with faecal EL-1 concentrations above 200 µg/g, who were considered as PS. In 41/51 (80.4%), the faecal EL-1 test presented values below 100 µg/g and patients were considered to be PI. There were no patients with faecal EL-1 values between 100 and 200 µg/g (Figure 1).

In relation to PS patients, 3/10 (30%) were heterozygous for ΔF508, and 7/10 (70%) patients did not present with mutation ΔF508, whether homozygous or heterozygous. Regarding the sample of PI patients, 17/41 (41.5%) were homozygous for mutation ΔF508, 14/41 (34.1%) were heterozygous for ΔF508, and 10/41 patients (24.4%) did not present with mutation ΔF508.

There was a statistically significant association between mutation ΔF508 and the faecal EL-1 concentration < 100 µg/g (Table 1). All homozygous for ΔF508 (17/17) presented with severe PI. The group that was heterozygous for ΔF508 showed similar proportions of faecal EL-1 concentrations.

As shown in Table 1, all patients with faecal EL-1 concentration < 100 µg/g used enzymes. Fromt he 10 patients considered sufficient by the faecal EL-1 test, 5 (50%) were undergoing enzyme replacement therapy. The enzyme dose per kg/day was similar among groups with faecal EL-1 concentration < 100 µg/g and > 200 µg/g, as seen in Figure 2. There was no association of sex and age group with fecal EL-1 concentration < 100 µg/g (Table 1).

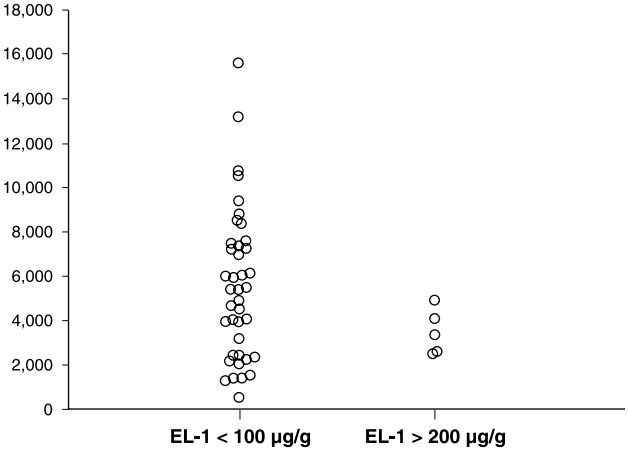

Figure 2 - Quantity (u) of lipase kg/day in patients with faecal elastase-1 concentration < 100 µg/g e > 200 µg/g

Table 1 - Association of age, sex and mutation ΔF508 with elastase concentration

Variables	Pancreatic insufficiency n (%)*		p
	Severe (EL < 100 µg/g) n = 41	Absent (EL < 200 µg/g) n = 10	
Sex			
Male	26 (63.4)	6 (60.0)	1.000†
Female	15 (36.6)	4 (40.0)	
Age group			
< 2 years	5 (12.2)	2 (20.0)	0.474‡
2-9 years	15 (36.6)	5 (50.0)	
10-17 years	21 (51.2)	3 (30.0)	
Mutation (ΔF508)			
Homozygous	17 (41.5)	0 (0.0)	0.010‡
Heterozygous	14 (34.1)	3 (30.0)	
Absent	10 (24.4)	7 (70.0)	
Enzyme replacement therapy			
Yes	41 (100.0)	5 (50.0)	0.001†
No	0 (0.0)	5 (50.0)	
Units of lipase per kg/day (median)	5,427.1 (510-15,652)	3,381.1 (2,520-4,932.0)	0.280§

EL = elastase.
* There are no values for mild pancreatic insufficiency (100 < EL < 200 µg/g) (n = 0).
† Fisher's exact test.
‡ Pearson's chi-square test.
§ Mann-Whitney's test.

Discussion

EL-1 was initially isolated by Mallory & Travis,[17] in 1975, and it was originallty called protease E. EL-1 is a specific human digestive protease, synthesized in the acinar cells and secreted in the duodenum, through the pancreatic duct. EL-1 is synthesized as zymogen and the mature enzyme has a molecular weight of 28 kDa.[18] During intestinal transit, EL-1 is bonded mainly with bile salts and, in contrast with other pancreatic enzymes, it is not degraded during the passage through the intestins.[19]

This proteolytic enzyme is measured in stool through an ELISA test, which is widely accepted in clinical laboratories.[11] Faecal EL-1 is considered more sensitive and specific than other tests currently used to detect exocrine pancreatic insufficiency.[20] Weintraub et al.,[12] in their study to correlate exocrine pancreatic function tests in pancreatic sufficient CF patients, consider the faecal EL-1 to be a test of little value for these patients, suggesting that the stool fat coefficient has a better performance. In the 2010 European consensus about the early treatment of children diagnosed with CF after neonatal screening, the authors suggest that, at the time of diagnosis, children should have their pancreatic function assessed by faecal EL-1. And if these patients present normal faecal EL-1 at the time of diagnosis, it is essential that several assessments are carried out during the first year of life.[21]

The ELISA test uses two highly specific monoclonal antibodies, specific for different epitopes in human EL-1.[14] The advantages of measuring this enzyme are countless: it is stable in a wide range of pH and temperature and the stool samples can be collected and transported without special preparation.[10,22] It can also be stored for up to one week at room temperature, for one month at a temperature of 4 to -22 °C, for long periods of time.[19] The test could also be useful for early detection of a PS patient reverting to an insufficiency status, and it can be monitored on a yearly basis, once the appearance of poor absorption is preceded by decline in elastase concentration.[20,23] The test is also useful to identify the residual capacity of the pancreatic status.[10] The monoclonal antibody against human elastase does not react with pig enzymes; i.e., it is species-specific. Therefore, the test can be performed while patients not undergoing enzyme replacement therapy.[10,22]

The sample of the present study was made up entirely of Caucasoid patients. There was a discreet predominance of males, as observed in previous cases.[14,16]

In terms of age, mean age of patients was 9.11±4.74 years old. By classifying patients in the 3 groups, according to the presence of the mutation, the mean age among homozygous was 9.10±4.82, among heterozygous it was 8.72±5.32, and among those without ΔF508 it was 9.52±4.28. Seven (14%) patients were under 2 years

old. There was no association between age and a higher or lower faecal EL-1 concentration. Faecal EL-1 has been associated with age only in the first weeks of life. Low values were found in premature and full term newborns. Von Seebach & Henker[24] analyzed stool samples from 28 premature children and from 27 full-term children, and observed a mean faecal EL-1 value of 63.9 µg/g in meconium, which gradually increased to 200 µg/g in 1 month, regardless of gestational age. thus, faecal EL-1 is low in the first weeks of life for children without CF and it will reach the normal levels in PS nursing infants. In the present investigation, in school-age patients ranging from 10 to 17 years old, there was a predominance of the group with faecal EL-1 concentration < 100 µg/g. Five (12.5%) children under 2 years old with faecal EL-1 concentration < 100 µg/g were found. Walkowiak et al.[25] found, in their sample, that 100% (27/27) of patients presented with PI at 12 months of age.

In the present study, 90.2% (46/51) of patients considered to be clinically PI were making proper use of pancreatic enzymes. However, five (10.8%) patients, aged 8 months to 10 years and using enzymes, presented faecal EL-1 above 200 µg/g, which characterizes PS. Borowitz et al.[26] studied 1,215 individuals from 33 locations registered at the Cystic Fibrosis Foundation Registry of New York (USA) for a period of 6 months. The mean age of patients was 13.5 years old, ranging from 1 month to 64 years. The study included the suspension of pancreatic enzymes depending on the faecal EL-1 test result being > 200 µg/g, except for individuals that presented with pancreatitis. Faecal EL-1 was < 200 µg/g in 1,074 (88.4%) individuals, 1,050 received pancreatic enzymes and 24 did not receive enzymes. These 24 patients were, therefore, incorrectly classified regarding their pancreatic functional status. Faecal EL-1 concentration was > 200 µg/g in 141 (11.6%) individuals. Sixty (42.5%) did not undergo enzyme replacement therapy and were correctly classified as PS, but 81 (57%) used pancreatic enzymes without any apparent need. Enzyme replacement therapy was interrupted for 67/81 individuals who had never presented with pancreatitis. Only 23 (34.3%) agreed to collect stool for stool fat balance and had their coefficient of fat absorption (CFA) measured 1 month after interrupting enzymes with a mean CFA of 96.1%. Prior to this study, 181/1,215 individuals had never had an objective test to verify their pancreatic function. The hypothesis of the authors, that a considerable number of CF patients were erroneously classified, was confirmed by the faecal EL-1.

In the present study, 80.4% (41/51) of patients in which there was suspected PI presented faecal EL-1 levels below 100 µg/g, just like in the study by Galvão et al.,[27] in which faecal EL-1 showed severe PI in most cases. In our sample, 78% (40 /51) patients had EL-1 below 15 µg/g (bottom detection limit for test). As previously described by Meyts et al.,[23] 76% (19/25) of patients studied presented very low values. Similar results have been found by Phillips et al.,[18] in which faecal EL-1 concentration of patients with CF homozygous for ΔF508 (15/15) was below 15 µg/g (bottom limit for test). Cade et al.[20] studied 142 children with CF, with 93 homozygous for ΔF508, 38 heterozygous for ΔF508 and 11 non-ΔF508. Only seven patients were found PS by the test. The faecal EL-1 median for patients with PI was of 10 µg/g. There were statistically significant differences between EL values in PS and PI patients ($p = 0.0001$), PI patients and control-group ($p < 0.0001$), but there was no difference between PS patients and the control-group. Median EL values for homozygous and heterozygous do not present statistically significant differences ($p = 0.62$). In the present study, 17 patients homozygous for ΔF508 presented faecal EL-1 concentration below 100 µg/g. The same was not true to heterozygous patients. These results are in accordance with the general literature in which patients that are homozygous for ΔF508 present greater association with PI than heterozygous and non-ΔF508.[7,11] This study found very low median values for faecal EL-1 among the groups (< 15 µg/g), similarly to the study by Cade et al.,[20] which reports a median of 10 µg/g among PI patients.

Durie et al.,[28] in a study about pancreatic function tests for patients with CF, report some criteria to be observed in order for the test to be satisfactory. On the lists that follow, we have incorporated these criteria in relation to faecal EL-1 and added the negative aspects of the test.

A) Negative points:

- EL concentration is modified in the presence of intestinal lesion[29] and alterations in the gut flora.[30]
- Diarrhea samples require careful interpretation.[30]
- Inability to distinguish between primary and secondary PI.[11]
- Assesses only endogenous PI.[11]

B) Positive points:

- Good correlation between the secretin-pancreozymin tests.[26]
- Not degraded in intestinal transit.[19]
- Easily obtained specimen.[26]
- No special storage.[26]
- Good acceptance of patients.[26]
- Easy execution.[10]
- Non-invasive.[19]
- Low cost.[10]
- Does not react with pig enzymes.[26]

One limitation of the present study is the absence of comparison with a golden standard test. In the present research, it was not possible to assess the intra-assay coefficient of variation (CV) for patients who presented a low faecal EL-1 concentration (< 200 µg/g), because the values of each part were below the bottom limit of the test (15 µg/g). Meyts et al.[23] have shown that the intra-assay

CV, based on duplicates, presented low variability with approximate values of only 4.06%. Cade et al.[20] have also shown, in their study, that inter-assay and intra-assay CV are low. The authors measured the CV for faecal EL-1 in patients with CF and found values of 7.0% (intra-assay) and 7.2% (inter-assay).

Faecal EL-1 is an indirect, accurate and replicable pancreatic function test whose values are reliable in a small stool sample.

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