Fecal elastase-1 cut-off levels in the assessment of exocrine pancreatic function in cystic fibrosis

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Abstract

Background: Fecal elastase-1 (E1) test is a sensitive and specific indirect test. However, there are few data on the best cut-off level in the assessment of exocrine pancreatic function in cystic fibrosis (CF). Material and methods: In 725 CF patients and 243 healthy subjects (HS) from Greece, Russia, Poland and the United Kingdom, E1 concentrations were measured. The best cut-off levels for the discrimination between CF and HS (for whole group as well as for individual countries) were calculated. Results: The best cut-off level for the differentiation between CF pancreatic insufficiency and normal pancreatic function in HS was found to be 184 μg/g of feces. However, some inter-country differences were stated. E1 concentrations in the UK subgroup were significantly lower than those found in Polish and Russian CF patients. E1 concentrations in Greek patients were significantly higher than in the other countries. However, E1 concentrations in ΔF508 homozygotes were very similar in all studied subgroups. In conclusion: In clinical practice, instead of a single best cut-off level for the E1 test, we suggest using a range of values (160–200 μg/g). The presence of different best cut-off levels within countries is a practical consequence of the different distribution of pancreatic function.

Keywords: Cystic fibrosis; Exocrine pancreatic function; Fecal elastase-1; Genotype

1. Introduction

The assessment of exocrine pancreatic function is one of the major diagnostic procedures in the investigation of gastrointestinal disease in cystic fibrosis (CF). Exocrine pancreatic function can be assessed by either direct or indirect methods, with the direct tests, such as the secretin–cholecystokinin test having the highest sensitivity and specificity [1]. However, direct tests have some major disadvantages, which make them unsuitable for routine evaluation in children, especially in those with CF. Indirect tests are more frequently used in clinical practice because they are non-invasive, simple, less time-consuming and less expensive.

The development of new indirect tests has improved the diagnostic approach. From previous studies, the measurement of fecal elastase-1 (E1) concentration appears to be a good measure of pancreatic function [2–4]. The enzyme is highly specific for the pancreas and is not degraded during intestinal passage [5]. E1
concentration is significantly correlated to duodenal elastase-1 output [4,6], daily variations are low [3] and no degradation over a period of 1 week at room temperature has been reported [3,4]. In addition, the monoclonal antibodies against human pancreatic elastase do not cross-react with pancreatic preparations of animal origin making it unnecessary to discontinue enzyme replacement therapy in subjects studied [4].

Previous studies evaluating the E1 test have generally recruited CF patients with severe exocrine pancreatic insufficiency and small groups of healthy subjects (HS) as controls [7,8]. In larger studies, the best cut-off level has not been calculated [9,10]. A value of 200 μg/g of feces is commonly used for determining exocrine pancreatic insufficiency, although there are few data concerning CF patients [4,11]. The aim of the present study is to calculate the best cut-off level for E1 in the assessment of pancreatic exocrine function in CF patients and healthy controls. The ‘distribution’ of pancreatic function in CF patients from different European countries has been examined to assess the variability of E1 values dependent upon genetic background.

2. Material and methods

2.1. Patients

Seven hundred and twenty five CF patients (357 females and 368 males) aged 2 months to 52 years [mean ± S.E.M. (standard error of the mean): 10.1 ± 0.2] were evaluated. The diagnosis of CF was based on clinical manifestations, chloride sweat concentrations > 60 meq/l and CFTR gene analysis. Two hundred and forty three children, adolescents and adults (130 females and 113 males) aged 2 months to 52 years (mean ± S.E.M.: 11.2 ± 0.5) without gastrointestinal disease served as a control group. All subjects included in the study were from Greece (GR), Poland (PL), Russia (RU) and the United Kingdom (UK) (Tables 1 and 2).

The best cut-off levels for the discrimination between CF and HS were calculated. All calculations were performed for both the whole CF and HS groups as well as for individual countries (GR, PL and UK). The effect of the different ‘distribution of pancreatic function’ on the best cut-off level in the different countries was assessed by comparison with the whole group of HS.

The local ethics committees in each study center approved the protocol for this study.

2.2. Methods

E1 concentrations (μg/g of feces) were measured in all subjects by an enzyme-linked immunosorbent assay (ELISA; ScheBo® Tech, Giessen, Germany) [12]. As pancreatic involvement in CF has a progressive character [13], patients under the age of 2 years were recruited to the study only if they had low E1 value (<50 μg/g). Since normal reference values are not applicable to

Table 1
E1 concentrations (μg/g of feces) in HS

<table>
<thead>
<tr>
<th>Subjects (n)</th>
<th>HS-all 243</th>
<th>HS-GR 78</th>
<th>HS-PL 105</th>
<th>HS-UK 44</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>Mean ± S.E.M.</td>
<td>11.2 ± 0.5</td>
<td>10.5 ± 0.3</td>
<td>13.3 ± 0.5</td>
</tr>
<tr>
<td></td>
<td>Range</td>
<td>0.1–52.1</td>
<td>2.00–23.00</td>
<td>0.17–40.00</td>
</tr>
</tbody>
</table>

E1* (μg/g) Mean ± S.E.M. | 636.7 ± 22.0 | 454.2 ± 9.1 | 753.3 ± 29.9 | 680.3 ± 34.7 |
| Median      | 570        | 464       | 714         | 704      |

*Russian values were not included (n = 16; E1 = 1054, 736, 662, 551 and 12x ≥ 500), since they did not allow for any calculations.

No age-related differences in E1 concentrations were found. Therefore, the analysis was performed without age subgroup distribution. GR vs. PL, RU, UK P < 0.001.

Table 2
E1 concentrations (μg/g of feces) in CF patients

<table>
<thead>
<tr>
<th>Patients (n)</th>
<th>CF-all 725</th>
<th>CF-GR 78</th>
<th>CF-PL 414</th>
<th>CF-UK 136</th>
<th>CF-RU 97</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>Mean ± S.E.M.</td>
<td>10.1 ± 0.2</td>
<td>15.2 ± 0.8</td>
<td>10.2 ± 0.3</td>
<td>7.8 ± 0.4</td>
</tr>
<tr>
<td></td>
<td>Range</td>
<td>0.1–30.8</td>
<td>1.0–30.0</td>
<td>0.1–30.8</td>
<td>0.1–20.8</td>
</tr>
</tbody>
</table>

E1* (μg/g) Mean ± S.E.M. | 90.6 ± 8.3 | 119.5 ± 18.6 | 98.5 ± 12.7 | 48.4 ± 12.5 | 94.0 ± 21.0 |
| Median      | 17         | 46        | 16         | 10        | 18       |
| Range       | 0–1810     | 4–742     | 0–1810     | 0–935     | 0–1036   |

*GR vs. PL, RU, UK P < 0.001.

UK vs. GR, PL P < 0.05.
neonates [14], HS younger than 1 month were excluded from the study.

3. Statistical analysis

The statistical differences between groups were calculated using the Kruskal–Wallis test with post test. The level of significance was set at \( P < 0.05 \).

The best cut-off levels between CF patients and HS were calculated with the use of Odds ratio.

4. Results

The range of E1 concentrations in HS was from 167 to 1802 \( \mu g/g \) of feces (Table 1). The overall specificity of the E1 test was 98.8\% for the best cut-off level calculated in the present study (184 \( \mu g/g \)) and 97.5\% for the best cut-off level recommended by the manufacturer (200 \( \mu g/g \)). The distribution of E1 results was very similar in British and Polish subgroups. However, E1 concentrations found in the HS Greek population were significantly lower \((P < 0.001)\).

The range for E1 concentrations in CF patients was from undetectable to 1810 \( \mu g/g \) of feces (Table 2). The overall sensitivity was 88.1\% and 89.1\% for own and ‘commercial’ cut-off level, respectively. The distribution of E1 results was very similar in Polish and Russian subgroups. E1 concentrations in UK subgroup were significantly lower than those found in Polish and Russian subjects \((P < 0.05)\). E1 concentrations in Greek patients were significantly higher than in the other countries \((P < 0.001 in all cases)\).

The frequency of the \( \Delta F508 \) mutation was the highest among British and the lowest among Greek patients (Table 3). E1 concentrations in \( \Delta F508 \) homozygotes were very similar in all studied subgroups. The distribution of E1 results in other CF patients was similar in British, Polish and Russian patients. In Greek population, however, E1 concentrations were significantly higher than in the other subgroups \((P < 0.001 in all cases)\) (Table 4).

With the use of E1 test, the best cut-off level for the differentiation between CF pancreatic insufficiency and normal pancreatic function in HS was 184 \( \mu g/g \) of feces. This level was different for each country (Table 5). The highest value was found for Greek population (203 \( \mu g/g \)), an intermediate value for Polish (174 \( \mu g/g \)) and the lowest for the British subgroup (161 \( \mu g/g \)). For the calculation performed in relation to the whole HS group, similar inter-country differences were observed Table 5.

5. Discussion

There are only two studies [4,11], in which the best cut-off level for E1 has been previously assessed. In that of Stein et al. [4], the optimal discrimination was found for a cut-off level of 175 \( \mu g/g \). However, the study was based on non-homogenous groups of patients, comprising CF and chronic pancreatitis patients \((n = 54)\) as well as HS \((n = 53)\) and patients with non-pancreatic disorders \((n = 57)\). Walkowiak [11] in a larger CF group \((n = 91)\) defined the best cut-off level at 198 \( \mu g/g \). However, the percentage (17.6\%) of pancreatic sufficient patients incorporated within the study was high. For this present study, we have included a large cohort of CF patients and HS from different European countries. A value of 184 \( \mu g/g \) was found as a best cut-off level for the E1 test to differentiate between pancreatic insufficiency in CF and normal pancreatic function in HS. However, we have found some minor inter-country differences. Therefore, we would suggest, in clinical practice, using a range of values for E1 (for simplifi-

Table 3
\( \Delta F508 \) frequency in CF patients

<table>
<thead>
<tr>
<th>Frequency (%)</th>
<th>( \Delta F508 ) homozygotes</th>
<th>33.9</th>
<th>CF-GR</th>
<th>19.2</th>
<th>38.1</th>
<th>65.4</th>
<th>33.3</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>( \Delta F508 ) allele</td>
<td>55.8</td>
<td>CF-PL</td>
<td>46.6</td>
<td>58.9</td>
<td>79.0</td>
<td>59.2</td>
</tr>
</tbody>
</table>

Table 4
E1 concentrations (\( \mu g/g \) of feces) in \( \Delta F508 \) homozygotes and in other patients

<table>
<thead>
<tr>
<th>( \Delta F508 ) homozygotes</th>
<th>Other patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean±S.E.M.</td>
<td>Median</td>
</tr>
<tr>
<td>All 16.2±1.2</td>
<td>11</td>
</tr>
<tr>
<td>GR 16.2±4.3</td>
<td>14</td>
</tr>
<tr>
<td>PL 15.0±1.2</td>
<td>12</td>
</tr>
<tr>
<td>UK 18.3±3.0</td>
<td>9</td>
</tr>
<tr>
<td>RU 13.3±1.8</td>
<td>16</td>
</tr>
</tbody>
</table>

\( \Delta F508 \) homozygotes: n.s. Other patients: GR vs. PL, UK, RU \( P < 0.001 \).

Table 5
Best cut-off levels of E1 in the assessment of exocrine pancreatic function (CF patients with exocrine pancreatic insufficiency vs. HS)

<table>
<thead>
<tr>
<th>CF patients</th>
<th>vs. Individual country</th>
<th>vs. Whole HS group</th>
</tr>
</thead>
<tbody>
<tr>
<td>HS subgroup</td>
<td></td>
<td></td>
</tr>
<tr>
<td>All*</td>
<td>–</td>
<td>184</td>
</tr>
<tr>
<td>GR</td>
<td>203</td>
<td>201</td>
</tr>
<tr>
<td>PL</td>
<td>174</td>
<td>183</td>
</tr>
<tr>
<td>UK</td>
<td>161</td>
<td>161</td>
</tr>
</tbody>
</table>

* Russian values included \( E1 = 1054, 734, 662, 551 \) and \( 12x > 500 \).
cation 160–200 μg/g), rather than a single cut-off value, below which further investigation of pancreatic exocrine function is warranted.

The genotype predicts with a high probability the course of pancreatic disease in CF. Patients who carry two ‘severe’ mutations develop pancreatic insufficiency, whereas those who carry at least one ‘mild’ mutation usually remain pancreatic sufficient [15]. Therefore, the variable distribution of pancreatic function measured as an output of E1 in populations with different ΔF508 frequency stated in the present study is reasonable. The presence of different best cut-off levels is a practical consequence of this finding. Since no differences for ΔF508 homozygous patients were found, the higher E1 concentrations found in Greek population could be explained by the more common presence of mild mutations [15–18].

In the present study, the percentage of CF patients (11.3%) found to have normal pancreatic function (assessed with the use of E1 test) is higher than in earlier studies assessing the applicability of the E1 test [7,8]. However, we have included complete CF populations comprising both pancreatic insufficient and sufficient patients.

In CF, the concentration of chloride, bicarbonate and water in pancreatic fluid is markedly decreased [19]. However, the amount of secreted enzymes differs significantly. Exocrine pancreatic dysfunction in CF includes disturbances of water and bicarbonate secretion but may be associated with normal enzyme output [20]. Therefore, all methods based exclusively on the measurement of pancreatic enzyme concentration/output could lead to some false negative results. Moreover, as proven earlier, a small percentage of CF patients (at least temporarily) have normal pancreatic function [21]. Although E1 test is the most reliable and sensitive indirect test used in CF children [22], its practical value in the assessment of mild pancreatic insufficiency is limited [6].

In conclusion, 184 μg/g seems to be the best cut-off level for the E1 test in differentiating between pancreatic insufficiency in CF and normal pancreatic function in HS within the European population. In clinical practice, instead of the best cut-off level, we would suggest using a range of values (160–200 μg/g), dependent upon the country of origin. The presence of different best cut-off levels within countries is a practical consequence of the different distribution of pancreatic function (resulting from the different frequency of CFTR mutations in populations of different origin) and is likely to be true for all other tests of exocrine pancreatic function.

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References


