EXOCRINE PANCREATIC FUNCTION TESTS

$^{13}$C-BREATH TEST AND FECAL ELASTASE
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Summary

Introduction. In this study we report our 6 years experiences with non-invasive test of exocrine pancreatic function - breath test with $^{13}$C-mixed triglycerides ($^{13}$C-MTG) and fecal elastase-1 (FELA).

Aims & Methods. The study group was 225 subjects with suspected chronic pancreatitis (CHP). The diagnosis of CHP was confirmed in 151 patients, categorised to four groups (A, B, C, D) by two aspects - morphology and function, 71 cases without CHP were used as control group. $^{13}$C-MTG test was performed with 250mg Glyceryl-1,3-diocatadecanoate-2-octanoate-1-$^{13}$C, $^{13}$C/$^{12}$C ratio were analysed by infrared NDIRS analysers (Isodiagnostika-Canada, HeliFAN-Germany). Fecal elastase 1 (FELA) were determined using ELISA with monoclonal antibody (ScheboTech, Germany).

Results. Fecal elastase as well as $^{13}$C-MTG cummulative recovery (cPDR) significantly distinguishes severe CHP (grade D) from all other groups, mild CHP (grade A) is significantly higher compared to other groups of CHP. Concordant results of $^{13}$C-MTG and FELA were found in 79.7%. The highest percentage (40.1%) of disconcordant results (low FELA, normal $^{13}$C-MTG) were in groups CHP-B and CH-D, in patients with morphological complications, resulting in low FELA, but still clinically normal functions and normal $^{13}$C-MTG.

Conclusions. Measurement of fecal elastase 1 is simple, non-invasive, robust test, which well correlates with morphological, static, extent of tissue damage. $^{13}$C-MTG breath test is better in evaluation of dynamic and kinetic aspects, real digestive ability and response to stimulation. $^{13}$C-MTG breath test is, contrary to FELA, suitable to evaluate pancreatic supplementation therapy. Two different aspects of exocrine pancreatic function could be evaluated by two laboratory methods - fecal elastase and $^{13}$C-breath test.
225 patients susp. for chronic pancreatitis, 
151 CHP confirmed by imaging methods 
ERCP - CT - US 
71 subjects - CHP excluded

### Functions

<table>
<thead>
<tr>
<th>Clinical Manifested Function Insufficiency</th>
<th>Steatorrhoea</th>
<th>Diabetes Mellitus</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>CHP</strong></td>
<td><strong>A</strong></td>
<td><strong>B</strong></td>
</tr>
<tr>
<td><strong>Number</strong></td>
<td><strong>71</strong></td>
<td><strong>50</strong></td>
</tr>
<tr>
<td><strong>Mean Age</strong></td>
<td><strong>45.4</strong></td>
<td><strong>49.8</strong></td>
</tr>
<tr>
<td><strong>Male : Female Ratio</strong></td>
<td><strong>0.87</strong></td>
<td><strong>1.00</strong></td>
</tr>
</tbody>
</table>

### Morphology

<table>
<thead>
<tr>
<th>Pancreatic Complications</th>
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</thead>
<tbody>
<tr>
<td><strong>No</strong></td>
</tr>
<tr>
<td><strong>Yes</strong></td>
</tr>
<tr>
<td>CHP - A</td>
</tr>
<tr>
<td><strong>n = 50</strong></td>
</tr>
<tr>
<td>CHP - B</td>
</tr>
<tr>
<td><strong>n = 31</strong></td>
</tr>
<tr>
<td>CHP - C</td>
</tr>
<tr>
<td><strong>n = 29</strong></td>
</tr>
<tr>
<td>CHP - D</td>
</tr>
<tr>
<td><strong>n = 41</strong></td>
</tr>
</tbody>
</table>

### CHP Groups

<table>
<thead>
<tr>
<th>CHP Group</th>
<th>NON</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number</td>
<td>71</td>
<td>50</td>
<td>31</td>
<td>29</td>
<td>41</td>
</tr>
<tr>
<td>Age (Mean)</td>
<td>45.4</td>
<td>49.8</td>
<td>48.1</td>
<td>56.1</td>
<td>51.1</td>
</tr>
<tr>
<td>Male : Female Ratio</td>
<td>0.87</td>
<td>1.00</td>
<td>2.10</td>
<td>1.42</td>
<td>7.20</td>
</tr>
</tbody>
</table>
NDIRS ANALYSERS

POCT (POINT OF CARE TESTING) FOR $^{13}$C - BREATH TESTS

ISOMAX 4000
Isodiagnostika
2 channel system
(Canada)

HeliFAN plus, FAN
4 channel system
(Germany)

NDIRS Opto-acoustic
detector unit
(Lehrer & Luft type)

NDIRS MEASUREMENT
(NON-DISPERSIVE INFRARED SPECTROSCOPY)
**13C-MTG BREATH TEST**

**TEST PROCEDURE**
- TWO SAMPLE BAGS AFTER OVERNIGHT FASTING
- PANCREATIC SUBSTITUTION THERAPY 3DAY EXCLUDED
- **STIMULATION MEAL**
  - 4 CRISP SLICES, MAIZE WITH FIBRES
    (WITHOUT CHOLESTEROL, GLUTEN-FREE)
  - 2 x 10g RAMA (VEGETABLE FAT WITHOUT MILK PROTEINS)
- TEST SUBSTANCE ADMINISTRATION - 250mg $^{13}$C-MTG
  STIRRED INTO VEGETABLE FAT
- HOURLY BREATH-BAG SAMPLING (1 - 6 hr)

**TEST ANALYTICS**
- DOB MEASUREMENT OF EACH SAMPLE $^{13}$CO$_2$ : $^{12}$CO$_2$ (in %oo)
  $T_x$ SAMPLE AGAINST $T_0$ (DOB = Delta Over Baseline)

**EVALUATION OF PANCREATIC INSUFFICIENCY**
- BSA CALCULATED (BASED ON WEIGHT, HEIGHT)
- BMR AND CO$_2$ PRODUCTION CALCULATED (MS Excel)
- CUMMULATIVE RECOVERY FOR 6 HOURS CALCULATED
FECAL ELASTASE

STOOL SAMPLES - SUBJECTS
Fecal samples were freezed and stored at -70 °C.
368 samples were routinely analysed for elastase-1 by
monoclonal elastase-1 ELISA, 213 patients were classified by A-B-C-D groups, 183 subjects with susp. CHP were tested with 13C-MTG as well.

MONOCLONAL ELASTASE-1 ELISA
Schebo ELISA routinely used since 1999
Monoclonal antibody to elastase IIA isotype
Normal results > 200 μg/g, severe insufficiency < 100 μg/g

POLYCLONAL ELASTASE ELISA
 Bioserv ELISA compared to Schebo test in 277 subjects
Polyclonal antibody to elastase IIIA and IIIB isotypes
Normal results > 200 μg/g, severe insufficiency < 100 μg/g
222 breath tests with $^{13}$C-mixed triglyceride substrate
71 control subjects (NON-CHP), 151 CHP classified as: A-B-C-D
368 fecal elastase determined by monoclonal ScheboTech ELISA ELASTASE-1 (isotype elastase-II A)
155 control subjects (NON-CHP), 213 CHP classified as: A-B-C-D
277 fecal samples
ScheBo and BioServ ELISA methods
Consistent results in 0 - 100 - 200 scale
Consistency 225/277 = 81.2%
Correlation coefficient
Spearman's $r = 0.751$
STATIC & DYNAMIC ASPECTS

cPDR 6hr $^{13}$C (%)

$\mu$g cut-off FELA

30 % cut-off MTG

NORMAL

CHP-A
CHP-B
CHP-C
CHP-D
NON-CHP

CONCORDANT MTG x FELA
79.7 % (173/217) SUBJECTS

PATHOLOGICAL

0,0
-50
30,0

100
200

150
350
550
750

FELA $\mu$g/g

STATIC & DYNAMIC ASPECTS

100
200

$\mu$g cut-off FELA

30 % cut-off MTG

CONCORDANT MTG x FELA
79.7 % (173/217) SUBJECTS

PATHOLOGICAL

0,0
-50
30,0

100
200

150
350
550
750

FELA $\mu$g/g
214 patients susp. for chronic pancreatitis,
144 CHP confirmed by imaging methods
70 subjects - CHP excluded
Discrepant results in 25.7% of CHP
Discrepant results > 40% - groups B and D

Discrepant results: FELA < 100 mg/g & $^{13}$C-MTG normal, cPDR (6hr) > 30%
Discrepant results: FELA < 100 mg/g & $^{13}$C-MTG normal, cPDR (6hr) > 30%

CHP - A
High/normal FELA
High/normal MTG
92.0%

CHP - B
Low/patological FELA
High/normal MTG
42.3%

CHP - C
High/normal FELA
High/normal MTG
60.7%

CHP - D
Low/patological FELA
High/normal MTG
37.5%

High/normal FELA
Low/patological MTG
3.6%

Low/patological FELA
High/normal MTG
47.5%
CONCLUSIONS

$^{13}$C-mixed triglyceride test (MTG) for exocrine pancreatic function was performed in 225 patients suspected of chronic pancreatitis using 250mg of Glyceryl-1,3-dioctadecanoate-2-octanoate-1-$^{13}$C. Cumulative recovery < 30 % was interpreted as pancreatic insufficiency. When compared with fecal elastase level we found 79.7% consensual results, in 217 subjects.

Five different human pancreatic elastases (PA I, IIA, IIB, IIIA, IIIB) are known, of which human pancreatic Elastase I is not expressed in the adult pancreas. All antibodies that are commonly used in a polyclonal ELISA BioServ preferentially detect human Elastase Isoforms IIIA and IIIB. Clinical literature that describes human elastase-1 activity in the pancreas is actually referring to elastase IIA (ELISA ScheBo).

277 fecal samples were analysed using both methods ScheBo and BioServ ELISA. Consistent results in 0 - 100 - 200 scale were found in 225/277 = 81.2 %.

Discrepant results: FELA < 100 mg/g & 13C-MTG normal, cPDR (6hr) > 30 % we found mainly in groups CHP-B and CHP-D, 42.3% and 40.0% of CHP confirmed patients.

Measurement of fecal elastase is simple, non-invasive, robust test, which well correlates with morphological, static, extent of tissue damage. $^{13}$C - MTG-BT is better in evaluation of dynamic and kinetic aspects. Performance of both tests could improve clinical values.

This poster could be on-line downloaded as PDF file (Adobe Acrobat) on my web-page: http://www1.lf1.cuni.cz/~kocna/uegw_2008_mtg.pdf