PHYTANIC ACID ANALYSIS & INTERPRETATION – LABORATORY & CLINICAL CONSIDERATIONS IN DIAGNOSIS & FOLLOW-UP
Opening by Susan Kuranoff, Vice-President & Co-Founder of Global DARE Foundation

Phytanic Acid Analysis & Interpretation – Laboratory & Clinical Considerations by Professor Jean-Marc Nuoffer

Question & Answer Session
• All participants are in listen only mode
• How to ask a question during the Q&A:
  – Participants following on Zoom can type their questions in the Q&A box at any
time during the presentation or by raising their hand at the end to ask a question
  live
  – Participants joining by phone can press *9 on their phone to raise their hand.
• Questions will be answered in the following order:
  – Q&A box in Zoom
  – Dial in participants
  – Online participants
• Today’s session will be recorded for later viewing on Global DARE
  Foundation Website (www.defeatadultrefsumeverywhere.com)
DARE’S MISSION

Global DARE Foundation's mission is to promote world-wide awareness and better quality of life for all who are diagnosed with Adult Refsum Disease.
PHYTANIC ACID ANALYSIS & INTERPRETATION – LABORATORY & CLINICAL CONSIDERATIONS IN DIAGNOSIS & FOLLOW-UP

Professor Jean-Marc Nuoffer
University Hospital Bern, Switzerland
Phytanic acid analysis and interpretation: Laboratory and clinical considerations in Diagnosis and Follow-up

Jean-Marc Nuoffer, Prof. Dr med.
Swiss reference centre for inborn errors of metabolism Bern
Institute of Clinical Chemistry and Interdisciplinary Metabolic Team
University Hospital Bern, Switzerland
Ideally a laboratory test should

......for diagnosis:

- clearly separate healthy from diseased (sensitivity = true positive and specificity = true negative)
- be specific for a certain disease “group”

......for follow-up:

- allow precise evaluation of a therapeutic effect (critical difference)
- be comparable between different laboratories
Questions in phytanic acid (PA) analysis

……for diagnosis:

1. Do all patients with elevated PA have a peroxisomal disorder/ARD
   Do all patients with normal PA have no peroxisomal disorder/ARD

2. How specific is PA analysis for adult Refsum and what are other factors leading to elevated PA?

……for follow-up:

3. How precise are measurements of very high PA and how can the therapeutic effect be evaluated (critical difference)
   Are changes in PA 16-13 resp. 46 - 36, resp. 370- 250µM real?

4. How comparable is PA analysis between laboratories?
Essentials of PA Metabolism

Phytanic acid is a long-chain saturated, branched chain fatty acid.

1. **Phytanoyl CoA synthetase**
2. **Phytanoyl CoA hydroxylase**
3. **2-OH-phytanoyl CoA lyase**
4. **Pristanoyl CoA dehydrogenase**
5. **Pristanoyl CoA synthetase**

The process involves the following steps:

- **rER**: Rough Endoplasmic Reticulum
- **PEX**: Peroxisomes
- **CHROM**: Chromosome
- **PEX7**: Peroxisome biogenesis gene
- **PHTH**: Peroxisome biogenesis gene

Wanders et al. 2010 BBA
Peroxisomal metabolism and laboratory parameters

https://doi.org/10.1016/j.mcp.2018.02.001

Metabolic abnormalities in ZS
- VLCFA
- Bile acid intermediates
- Pristanic acid *
- Erythrocyte plasmalogen

Phytanic acid *

Urinary oxalate and glycolate #

L-Pipecolic acid

# Moderately elevated, AGT normal

* Maybe normal, dependant on diet

UNIQUE SET OF PEROXISOMAL BIOMARKERS

METABOLIC FUNCTIONS OF PEROXISOMES IN HUMANS

- Fatty acid β-oxidation
- Etherphospholipid biosynthesis
- Fatty acid alpha-oxidation
- Glyoxylate detoxification
- L-pipecolic acid oxidation
How is PA in Plasma and Serum measured in the lab?

Method: Gas-Chromatography-Mass Spectrometry analysis (GC/MS)

Preparation of fatty acids PA and VLCFA

We measure free FA and FA from triglycerides
1. Good diagnostic method with linear range up to 80-100uM
2. Coefficient of variation between 1.7 -10% (changes in repeated measurements)
Critical difference or are two measurements really different?

Depends on **intra-individual variation** and **measurement reproducibility** (CV)

Exp:

<table>
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</table>

Patient has **10 umol/L** and later **9 umol/L**

CV = 6 %

**Critical difference:** $2,8 \times \text{CV}\% = 2,8 \times 6.0\% = 16.8\%$

The critical difference at 10uM is $16.8\% = 10 \pm 1.68$

range 8.32-11.68

**This range can be explained by the analytical reproducibility**
Causes of PA elevation

- Zellweger Spectrum Disorders (PEX defects): (Zellweger neonatal ALD, infantile Refsum) PA normal to elevated
- Rhizomelic chondrodysplasia punctate I PA normal to elevated
- Alpha-methylacyl-CoA racemase deficiency PA mildly elevated
- Adult Refsums Disease 10-100 fold elevated
- Rare unexplained isolated PA elevation
Some peroxisomal disorder have normal PA! How about adult Refsum?

[Refsum's disease. Apropos of 2 cases disclosed by myocardiopathy]

[Article in French]
A Millaire, A Warembourg, D Leys, E Tison, S Tondeux, P De Groote, J Y Ketelers, F Fourrier, H Petit, G Ducloux

Case 1: 22y acute pulmonary edema revealing dilated, hypokinetic cardiomyopathy, with retinitis pigmentosa, ptosis, anosmia, myolysis. Repeatedly normal PA!!!! Conclusion of Kearns-Sayre syndrome.

5y later

older brother, 28y, dyspnea on effort due to hypokinetic, cardiomyopathy, arrhythmias, retinitis pigmentosa, anosmia and myolysis. Plasma phytanic acid was very high!!
Questions in phytanic acid (PA) analysis

......for diagnosis:

1. A) Do all patients with elevated PA have a peroxisomal disorder/ARD
   B) Does normal PA exclude a peroxisomal disorder/ARD

2. How specific is PA analysis for adult Refsum and what are other factors leading to elevated PA?

Answer:

1. A) No- In the general population isolated PA elevation may have other causes and some are still unclear.

   B) Normal PA does not exclude ARD. If clinical presentation is suggestive of ARD and retinopathy may be the only clue: enzymatic or genetic analysis should be done.

2. PA analysis is not specific for ARD (clinic and associated Lab!)
   PA levels in ARD dependent on mutation, diet & catabolism
What about phytanic acid (PA) in follow-up

…..for follow-up:

3. How precise are measurements of very high PA and how can the therapeutic effect be evaluated (critical difference)

   Are changes in PA 16-13 resp. 46 - 36, resp. 370- 250µM real?

4. How comparable are the PA analysis between laboratories?
What about very high PA in follow-up of ARD

Samples have to be diluted in the linear range.
**Effect with high PA concentration**

- **Example:**

  - Undiluted measurement
  - 1:10 diluted

  **Effect of dilution of PA concentration**

  PA over 100 uM should be diluted
Summary of performance of PA analysis in our Lab and effect on critical difference

How precise are measurements of very high PA and how can the therapeutic effect be evaluated (critical difference)

Intra-lab (Bern)

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<th>Sample</th>
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<th>High level</th>
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<th>ARD2</th>
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<tr>
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<td>15</td>
<td>300</td>
<td>900</td>
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<tr>
<td>SD</td>
<td>0.5</td>
<td>1.1</td>
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<tr>
<td>CV (%)</td>
<td>9</td>
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<tr>
<td>Critical difference</td>
<td>1.3</td>
<td>3</td>
<td>126</td>
<td>504</td>
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<td>Analyt. range</td>
<td>3.7-6.3</td>
<td>12-18</td>
<td>174-426</td>
<td>396-1404</td>
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The higher the PA concentration, the higher the imprecision will be!
Summary of performance of PA analysis in our Lab and effect on critical difference

Are changes in PA explained by CV of the method?
from 16 to 14 with CV 7%: yes (13-19)
from 46 to 33 with CV 10%: No (34.5-57.5)
resp. 370-250 with CV 15%: yes (215-525)

Intra-lab (Bern)

<table>
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<tr>
<th>Sample</th>
<th>PA (µM)</th>
<th>CV (%)</th>
<th>Critical difference</th>
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<tr>
<td>PA (µM)</td>
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<td>11.5</td>
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<tr>
<td>Critical difference</td>
<td>370</td>
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How comparable is PA analysis between laboratories?

**Analyte:** Phytanic acid

**Deadline:** 31/05/2019

**Unit:** μmol/L

**Your Method:** GC/MS

**Your Result:** 26.9

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**Analyte:** Phytanic acid

**Deadline:** 28/06/2019

**Unit:** μmol/L

**Your Method:** GC/MS

**Your Result:** 17.5

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[Graph showing data distribution]
How do different laboratories perform?

### Inter-lab (external quality control of PA, ERNDIM)

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<tr>
<td>CV (%)</td>
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<td>19</td>
<td>14</td>
<td>11</td>
<td>11</td>
<td>11</td>
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<td>8</td>
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→ Inter-lab not much higher than intra-lab

What is the CV (%) very far from the cut-off? Would be nice to have inter-lab comparisons for high PA
Summary

• PA analysis is a good screening test together with the other peroxisomal markers for peroxisomal disorders. Isolated high elevated PA is usually an indicator of ARD although there are some yet unknown pediatric cases of elevated PA

• But very rare ARD may be missed and therefore genetic exclusion may be necessary

• At concentrations near the cut-off (20 µM) the test performs well, with low CV but at very high levels the CV is higher

• The CV has an important impact on the interpretation

• There is no quality assurance and inter-laboratory comparison for high PA levels!
Thank you for your attention
Q&A

For more information contact:

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www.defeatadultrefsumeverywhere.org