Fetal Alcohol Spectrum Disorder & Direct Alcohol Biomarkers

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October 6, 2017

How I Became Involved

CMH/NU Feinberg School of Medicine 1983
Before I Became Involved

How I Became Involved in This Journey

- CMH/NU Feinberg School of Medicine 1983
- Dr Ira Chasnoff/ Perinatal Center of Chemical Dependence

“Cocaine use is uniquely damaging to a fetus, far more damaging than other narcotics”
- Ira Chasnoff, MD 1985

How I Became Involved in This Journey

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So...What Was The Problem?

Cocaine was not the culprit, binge alcohol drinking was!

Then What Was The Solution?

A long-term marker for fetal binge alcohol exposure was desperately needed.
**Direct Alcohol Biomarkers**

Three non-oxidative EtOH metabolite(s) emerged

- Ethyl Glucuronide (EtG)
- Fatty Acid Ethyl Esters (FAEE)
- Phosphatidylethanol (PEth)

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**FASD & Direct Alcohol Biomarkers**

**Ethyl Glucuronide**

\[
\text{HO} \quad \text{O} \quad \text{OH} \\
\text{HO} \quad \text{O} \\
\text{HO} \quad \text{O} \\
\text{O} \\
\text{CH}_3
\]

**Ethyl Glucuronide** first to be characterized

- R.T. Williams identified and ran TLC 1953 – Rabbits
- Rec’d pure standard from Dr Artz, Corn Products, Argo, IL
- Derivatized as triacetyl b-ethyl-D-glucuronide methyl ester
- Williams had EtG ready for GC analysis, waited for instrumentation to arrive
- 1995 EtG GC analysis described
**Ethyl Glucuronide**

GC/MS EI of triacetyl b-ethyl-D-glucuronide methyl ester
Concentrated from urine of alcohol user

**Chromatographic Conditions:**

- HP 5971 GC with HP 5988 MS Detector
- Column: Chrompack CP Sil-5 (12 m x 0.25 mm)
- Injector Temp: 250 deg
- Column Program
  - Initial Temp: 140 deg x 1 min
  - Ramp 20 deg/min
  - Final Temp: 320 deg x 1 min
- In selected ion monitoring mode LOD – 100 ng/mL

**Method involved simple concentration of 1 mL urine**

- Needed dual derivatization to form methyl, acetyl derivative.
- A time consuming process, but a step forward
**FASD & Direct Alcohol Biomarkers**

Fatty Acid Ethyl Esters - Ethyl Stearate

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**Fatty Acid Ethyl Esters**

- Fatty acid ethyl esters described 1991
  - Lapasota and Lange
- USDTL began development in meconium 1992
  - Extraction proved difficult
  - Poly-unsaturated FAEE’s low recovery (<1%)
- NIAAA funded SBIR Phase I Grant 1996
- Breakthrough 1998 – Laposata serum method
  - Extraction with an aminopropyl SPE
  - High poly-unsaturates recoveries
  - First forensic test for fetal alcohol exposure

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**Fatty Acid Ethyl Esters**

*Clinical Chemistry 95*

Drug Monitoring and Toxicology

Prevalence of Fatty Acid Ethyl Esters in Meconium Specimens

Catherine Mcmen, 1 Joanne Jones, 1 Douglas Lives, 1 and Karen Bacon 1
Fatty Acid Ethyl Esters

- Chromatographic Conditions:
  - Agilent 5973 GC/MS System: Pos CI Methane
  - Column: SGE BPX-70 (30 m x 0.25 mm)
  - Injector Temp: 250 deg
  - Initial Temp: 100 deg x 1 min
  - Ramp 20 deg/min
  - Final Temp: 260 deg x 1 min
- In selected ion monitoring mode LOD – 50 ng/gram

- FAEE analysis extended to hair specimens
- European labs especially favored
- Over time, major issues arose:
  - FAEE’s contained in cosmetic hair products
  - EtOH on hair made FAEE’s in hair
  - EtOH wipes on meconium made FAEE’s in meconium

Phosphatidylethanol (PEth)

Phosphatidylethanol (PEth) discovered in 1983

Abnormal phospholipid that forms when:
- EtOH + Phosphatidylcholine + Phospholipase D all are present
- All human somatic cells contain Phosphatidylcholine + Phospholipase D
Phosphatidylethanol (PEth)

- PEth can form in red blood cells as a component of the cellular membrane.
- PEth is a direct alcohol biomarker, meaning that ethanol is incorporated into the final product.

Early PEth analyses used HPLC-ELSD
- HPLC-ELSD is relatively insensitive
- Assay could only detect HEAVY drinking
- Liquid/liquid extraction also a drawback
- Breakthroughs in LC/MS/MS changed PEth

In the early 2000’s LC/MS/MS sensitivity increased many fold.
- PEth became available at cutoffs 10 x lower than HPLC-ELSD
- Whole blood PEth now could detect a single binge drinking event for over a week.
Phosphatidylethanol (PEth)

With the arrival of ultra high sensitivity LC/MS/MS systems came:

- Sample size reduced to dried blood spots on filter paper – NO venipuncture!
- Newborn Specimens could be analyzed

Phosphatidylethanol (PEth) DBS vs. Whole Blood

- Easier Collection
- Shipped as non-biohazard
- Less room needed for storage
- Extraction quicker, safer and cleaner

Micro Liquid Chromatography (Micro-LC)

- Eksigent Micro-LC/AB SCIEX Triple Quad™ 5500
- Eksigent Halo
- 3.0 minute method
- 50 µL/min method
- Approximately 0 sq ft LC space
- 324 vial capacity ALS
- 3 110 outlets needed for LC
- LOQ 8ng/mL
- Analyst Software/Eksigent driver
- Operator friendly
### Micro-LC LC Program

- Micro LC - 50 μL/min
  
<table>
<thead>
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<th>Time</th>
<th>A%</th>
<th>B%</th>
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<tr>
<td>3.50</td>
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- A = 50% 2mM Ammonium Acetate/25% Acetonitrile/25% Isopropanol
- B = 60% Acetonitrile/40% Isopropanol

### Micro LC-MSMS LOQ 8ng/mL

![LC-MSMS LOQ 8ng/mL graphs]

### FASD & Direct Alcohol Biomarkers

The reduction of specimen size to DBS also led to:

- Newborn Heel Stick specimens
- Newborn umbilical cord blood sampling
  - The problems of FAEE analysis in meconium are gone
  - The sensitivity as a evidentiary test is superior
  - The nursing staff are far more compliant
  - We still can’t believe how well it works!
- A definitive study is currently funded by NIAAA and is ongoing
Fingernail Testing for Alcohol

With the arrival of ultra high sensitivity LC/MS/MS systems also came:

- EtG in fingernails

Why fingernails?

- It is another keratin matrix, like hair
- Unlike hair, it grows in two dimensions, length and thickness

Anatomy of Fingernail

FASD & Direct Alcohol Biomarkers

- We obtained 606 paired hair and fingernail specimens from college age subjects
- EtG in fingernails averaged 2.5 x the hair concentrations
- EtG in fingernails showed no gender bias, hair did.
- May be due to female use of cosmetic products on hair.
- No difference in compliance.
FASD & Direct Alcohol Biomarkers Summary

- We have come a very long way since 1953
- We now have validated forensic tests that provide:
  - 3 Day window – Urine EtG (and EtS)
  - 3 Week window – PEth
  - 3 Month window – Fingernail/Hair EtG

All of these advancements are the results of:
- Instrument manufacturer’s constant improvements in LC/MS/MS
- Academic researchers exploring new compounds and pathways
- Providers who take chances and implement these new ideas
FASD & Direct Alcohol Biomarkers

Where do the Direct Alcohol Biomarkers fit into identifying FASD?

FASD Diagnostic Algorithm.


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Moms self report alcohol use in pregnancy rarely.

When Moms report alcohol use, they almost always underreport usage.

PEth does not confabulate.

PEth has yet to have a documented False Positive – 100% Specificity.

The PEth result for newborns is a voice for a voiceless.
FASD & Direct Alcohol Biomarkers

Why use PEth?

- Extremely Sensitive
- Already DBS specimens are collected
- PEth in DBS are highly stable and easily stored

FASD & Direct Alcohol Biomarkers

- PEth analysis positivity has varied by regions
  - Midwest state snapshot found 4% Positive
  - Southeast State snapshot found 20+% Positive
  - Montevideo, Uruguay hospital random study found 85+% Positive
  - Drinking cultures vary, so do newborn results

FASD & Direct Alcohol Biomarkers

- NIAAA funded U01 study in Montevideo, Uruguay and Sao Paulo, Brazil will allow longitudinal study of relationship of PEth and FASD
  - First study of its kind to provide this kind of data
  - Dr. Philip May’s Active Surveillance Method will be used to assess FASD.
FASD & Direct Alcohol Biomarkers

- We’ve come a long way from the “Cocaine Babies” of the 1980’s
- It took over 30 years to find a biomarker and the analytical system to reliably use it to identify fetal alcohol exposure.
- This next step of studying the relationship of exposure to disease is the most exciting of all.

Q&A Time

Thank You