Biochemical Verification of Tobacco Use and Cessation: An Update

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SRNT Webinar
October 13, 2017
Disclosures

• Consultant to pharmaceutical companies that market smoking cessation products, including Pfizer, GlaxoSmithKline and McNeil.

• Paid expert in litigation against tobacco companies.
Overview

• Major biomarkers: cotinine, CO, NNAL, minor tobacco alkaloids
• Optimal cut points
• Windows of detection
• Individual factors influencing biomarker levels
• Over the counter tests
• Distinguishing Vaping from Smoking
Constituents and Biomarkers of Tobacco Use
Chemical Constituents of Tobacco Smoke

Nicotine
Carbon Monoxide
Acrolein
Ammonia
Nitrogen Oxides
Benzene*
2-naphylamine*
4-aminobiphenyl*
Formaldehyde*
1,3-butadiene*
Benzo (a) pyrene*
Various nitrosamines*
Hydrazine*
Nickel*
Polonium-210*
Cadmium

* Known or suspected human carcinogen
Tobacco Smoke is a Mixture of Chemicals Including Gases and Particles

- Nitrogen Dioxide
- Carbon Monoxide
- Formaldehyde
- Benzene
- 1,3-Butadiene
- Acrolein
- Polycyclic Aromatic Hydrocarbons
- Solanesol
- Nicotine
- Benzo(a)pyrene
- PAH
- Metals
- Metals
- Nicotine

Gases

Particle-phase
Challenges in Assessing Exposure: Extent of Sorption and Stability Varies

Fraction in air at 2 h (furnished room)

Isoprene | Acrolein | Benzene | 2-Butanone | Toluene | Xylene | d-Limonene | TMBenzene | Pyridine | 3-EP | Naphthalene | Me-Naphth | Cresol | Phenol | Nicotine

Multiple tracers/biomarkers are desirable

Courtesy of Brett Singer, LBNL
Tobacco Specific Biomarkers

• Nicotine and metabolites
  – Cotinine
  – Urine total nicotine equivalents

• Minor tobacco alkaloids
  – Anabasine, anatabine
  – Nicotelline

• Tobacco specific nitrosamines
  – NNAL
Other Tobacco Smoke Biomarkers

• Carbon monoxide
• Thiocyanate (cyanide)
• Polycyclic aromatic hydrocarbons
  – 1 hydroxypyrene and others
• Volatile organic compounds
  – Acrylonitrile, acrolein, butadiene and others
Optimal Cotinine Levels to Distinguish Smoking and Nonsmoking
Plasma Cotinine Levels Throughout the Day

N = 31

Time

Plasma Cotinine ng/ml
SRNT Subcommittee on Biochemical Verification (NTR 2002)

Optimal cut-off points to distinguish tobacco use vs. no tobacco use.

• Plasma or serum cotinine  15 ng/mL
• Urinary cotinine  50 ng/mL
• Expired CO  8-10 ppm
• Plasma SCN  78-84 mmol/L
Optimal Serum Cotinine Levels for Distinguishing Cigarette Smokers and Nonsmokers Within Different Racial/Ethnic Groups in the United States Between 1999 and 2004

Neal L. Benowitz, John T. Bernert, Ralph S. Caraballo, David B. Holiday, and Jiantong Wang

Initially submitted April 17, 2008; accepted for publication September 3, 2008.

Cotinine, a metabolite of nicotine, is widely used to distinguish smokers from nonsmokers in epidemiologic studies and smoking-cessation clinical trials. As the magnitude of secondhand smoke exposure declines because of proportionally fewer smokers and more clean-indoor-air regulations, the optimal cotinine cutpoint with which to distinguish smokers from nonsmokers is expected to change. The authors analyzed data on 3,078 smokers and 13,078 nonsmokers from the National Health and Nutrition Examination Survey for 1999–2004. Optimal serum cotinine concentrations for discriminating smokers from nonsmokers were determined using receiver operator characteristic curve analysis. Optimal cotinine cutpoints were 3.08 ng/mL (sensitivity = 96.3%, specificity = 97.4%) and 2.99 ng/mL (sensitivity = 86.5%, specificity = 93.1%) for adults and adolescents, respectively.
NHANES Participants

• Nationally representative sample of U.S. civilian population
• Adolescents (12-19 y.o.) and adults (mean 45 y.o.)
• 13,078 nonsmokers and 3,078 smokers
• NHW (6,896); NHB (3,671); MA (4,472)
All Adults

Overall

Serum Cotinine, ng/mL

- Fixed: 14.0 ng/mL
  - Specificity = 98.5%
  - Sensitivity = 92.4%

- ROC: 3.08 ng/mL
  - Specificity = 97.4%
  - Sensitivity = 96.3%

Actual % = 57%
(cropped at 16%)
<table>
<thead>
<tr>
<th>Race/Ethnicity</th>
<th>Adults</th>
<th>Adolescents*</th>
</tr>
</thead>
<tbody>
<tr>
<td>NHW</td>
<td>5 ng/ml</td>
<td>3 ng/ml</td>
</tr>
<tr>
<td>NWB</td>
<td>6 ng/ml</td>
<td>3 ng/ml</td>
</tr>
<tr>
<td>MA</td>
<td>1 ng/ml</td>
<td>1 ng/ml</td>
</tr>
</tbody>
</table>

* sensitivity of cutpoint lower in adolescents – likely due to higher prevalence of occasional smoking and more under-reporting of smoking.
Caveats regarding cut points

• The optimal cut point is influenced by the prevalence and intensity of active tobacco use and secondhand smoke exposure.
• Higher prevalence and intensity of tobacco exposure will results in a higher optimal cut point
• Race- and physiology-related metabolic differences can influence cut points
Comparison of biofluids for cotinine measurement

• Blood (serum, plasma) is gold standard
• Saliva/plasma ratio = 1.04 (0.95 – 1.14)
  – Ratio influenced by saliva flow rate
• Urine/plasma ratio = 4.6 (4.0 – 5.3)
  – Ratio influenced by urine flow rate and pH

( ) – 95% C.I.
Window of cotinine detection

• Detection ~ 5 half-lives, depending on initial level and biofluid tested (longer detection in urine)
• Cotinine half-life range 10 to 30 hr
• Detection range 50 to 150 hours
Limitations of Cotinine as Biomarker of Nicotine and Tobacco Smoke Exposure
Nicotine Metabolic Profile

- **Nicotine**: 9.8%
- **Nicotine-1'-N-oxide**: 4.4%
- **Nor NICOTINE**: 0.4%
- **Nicotine glucuronide**: 4.2%
- **Cotinine**: 13.0%
- **Cotinine glucuronide**: 12.6%
- **Cotinine-N-oxide**: 2.4%
- **Nor cotinine**: 2%
- **Trans-3'-hydroxy cotinine**: 80%
- **Trans-3'-hydroxy cotinine glucuronide**: 7.4%
- **33.6%**
Cotinine as a Quantitative Measure of Nicotine Intake from Tobacco Use

Daily Generation of Cotinine =

Daily Nicotine Dose (D) \times \% \text{ Conversion of Nicotine to Cotinine (f)}

Elimination rate for Cotinine =

\text{Clearance of Cotinine (Cl}_{\text{COT}}) \times \text{Blood Cotinine Concentration (COT)}
Cotinine as a Quantitative Measure of Nicotine Intake from Tobacco Use

- The conversion factor $K = \frac{Cl_{COT}}{f}$

- $Cl_{cot}$ is determined by activity of CYP2A6, UGT2B10 and the renal clearance of cotinine

- $f$ is determined by activity of CYP2A6, UGT2B10, FM03 and the renal clearance of nicotine
Cotinine as a Quantitative Measure of Nicotine Intake from Tobacco Use

- Typical $K = 0.08 \text{ mg/24h/ng/ml}$

- A 20 cigarette per day smokers has a blood cotinine level of 300 ng/ml
  
  $D = (0.08) (300) = 24 \text{ mg/day} = 1.2 \text{ mg/cigarette}$
Factors Potentially Influencing Cotinine as a Biomarker of Nicotine Intake

Genetics
Sex
Race
Cigarette smoking
Kidney failure
Oral contraceptive/Hormone
replacement
Anticonvulsant drugs/rifampin
Menthol
Sex Differences in Rate of Nicotine Metabolism

Nicotine Clearance (ml/min/kg)

- Men: 15.6
- Women (no OCP): 17.6
- Women (+ OCP): 22.5
- Pregnant Women: 27.0

All Groups Significantly Different from One Another

JPET 2002; 301:594
CPT 2006; 79:480
K values in different populations of smokers

Units: mg nicotine/24 hr / ng/ml cotinine
Average K = 0.08
Lower in men
Higher in women
Very high in pregnancy
Lower in African Americans and Asians
Over the Counter Cotinine Tests
OTC Cotinine Tests

• Fastest, easiest and least expensive test
• Immunoanalysis using cotinine antibodies
• Urine and saliva tests
• Semi-quantitative with relatively high cotinine threshold – high specificity, low sensitivity
• Inadequate sensitivity for SHS and occasional tobacco/nicotine use
• Possible better classification when combined with expired CO
<table>
<thead>
<tr>
<th>Salimetrics ELISA Kit</th>
<th>iScreen OFD</th>
<th>SafeCare</th>
<th>NicAlert Strips</th>
<th>NicCheck I</th>
<th>COT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Specimen</td>
<td>Saliva</td>
<td>Saliva</td>
<td>Saliva (U)</td>
<td>Urine</td>
<td>Urine</td>
</tr>
<tr>
<td>Sensitivity</td>
<td>99%</td>
<td>99%</td>
<td>99%</td>
<td>98%</td>
<td>99%</td>
</tr>
<tr>
<td>Active Use Cutoff</td>
<td>4 ng/ml</td>
<td>30 ng/ml</td>
<td>20 ng/ml</td>
<td>10 ng/ml (S)</td>
<td>200 ng/ml</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>50 ng/ml</td>
<td>100 ng/ml (U)</td>
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<td></td>
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<td>200 ng/ml</td>
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</table>
Relative performance of common biochemical indicators in detecting cigarette smoking

Gina F. Marrone¹, Diaa M. Shakleya², Karl B. Scheidweiler², Edward G. Singleton³, Marilyn A. Huestis² & Stephen J. Heishman¹

Nicotine Psychopharmacology Section¹ and Chemistry and Drug Metabolism Section,³ Intramural Research Program, National Institute on Drug Abuse, National Institutes of Health, Baltimore, MD, USA and Department of Psychology, Stevenson University, Stevenson, MD, USA³
Urine and Saliva NicAlert and Smoking Status

<table>
<thead>
<tr>
<th>NicAlert Zones, Cotinine Concentrations (ng/ml)</th>
<th>Urine</th>
<th>Saliva</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heavy Smokers</td>
<td><img src="image1" alt="Urine Heavy Smokers" /></td>
<td><img src="image2" alt="Saliva Heavy Smokers" /></td>
</tr>
<tr>
<td>Light Smokers</td>
<td><img src="image3" alt="Urine Light Smokers" /></td>
<td><img src="image4" alt="Saliva Light Smokers" /></td>
</tr>
<tr>
<td>Exposed Nonsmokers</td>
<td><img src="image5" alt="Urine Exposed Nonsmokers" /></td>
<td><img src="image6" alt="Saliva Exposed Nonsmokers" /></td>
</tr>
<tr>
<td>Not Exposed Nonsmokers</td>
<td><img src="image7" alt="Urine Not Exposed Nonsmokers" /></td>
<td><img src="image8" alt="Saliva Not Exposed Nonsmokers" /></td>
</tr>
</tbody>
</table>

Marrone, Addiction 2011; 106:1325-1334
Optimal CO Levels to Distinguish Smoking and Nonsmoking
BLOOD NICOTINE AND CARBOXYHEMOGLOBIN CONCENTRATION WHILE SMOKING LOW (0.4 mg) AND HIGH (2.5 mg) NICOTINE CIGARETTES

Low Nicotine

High Nicotine

N=10

N=7

CLOCK TIME
Relative performance of common biochemical indicators in detecting cigarette smoking

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Breath CO and Smoking Status

![Graph showing Breath CO levels for different smoking statuses.]
## Expired CO and NicAlert Performance

<table>
<thead>
<tr>
<th>Measures</th>
<th>Cut Point</th>
<th>Sensitivity (%)</th>
<th>Specificity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Expired CO</td>
<td>≥ 5 ppm</td>
<td>94.4</td>
<td>97.6</td>
</tr>
<tr>
<td>Urine NicAlert</td>
<td>≥ 3</td>
<td>92.2</td>
<td>97.6</td>
</tr>
<tr>
<td>Saliva NicAlert</td>
<td>≥ 1</td>
<td>76.7</td>
<td>85.4</td>
</tr>
<tr>
<td>CO / Urine NicAlert</td>
<td>≥ 5 / ≥ 3</td>
<td>97.6</td>
<td>95.1</td>
</tr>
</tbody>
</table>
Window of CO detection

- Detection ~ 5 half-lives
- CO half-life:
  - Average 4 hr
  - Exercise 2 hr
  - Sleep 8 hr
Detection range 10 to 20 hr (CO levels can still be elevated after overnight abstinence)
Consider other sources of CO (vehicle exhaust; marijuana smoking)
Optimal NNAL Levels to Distinguish Smokers from Nonsmokers
Tobacco Specific Nitrosamines: NNK, NNN and NNAL

- NNK and NNN are potent lung, esophageal and oral cavity carcinogens that are derived by nicotine in the tobacco curing and combustion process.
- NNK is only found in tobacco products; its levels differ from product to product, depending on type of tobacco and nitrate content.
- NNK is metabolized in the body to NNAL, which can be measured in the urine of tobacco users.

Binnian Wei, Benjamin C. Blount, Baoyun Xia and Lanqing Wang
Urinary NNAL in Cigarette Smokers and Non-Smokers

NHANES 2011-2012
- 6705 Participants
- Ages ≥ 6 years old
- 62% Overall Detection Rate
- 95% percentile urine NNAL in nonsmokers = 11.4 pg/mg creat
Optimal urine cotinine and NNAL cut points to distinguish active vs passive smoking

Goniewicz et al. 2011: Cot 31.1 ng/ml; NNAL 47.3 pg/ml [337 smokers, 228 heavy SHS]

Benowitz et al. 2017: Cot 30 ng/ml; NNAL 14.4 pg/ml [54 smokers, 303 light SHS or non-exposed]
Urine NNAL with Use of Different Tobacco Products

Non-Tobacco use | Pipe or Cigar | Cigarette | Snuff or Chewing Tobacco

NNAL (pg/mg creatinine)
Among Non-Smokers
Urine NNAL Greatest in Children

Age

NNAL (pg/mg creatinine)

6-11
12-19
20-59
Window of NNAL detection

- Detection ~ 5 half-lives
- NNAL half-life range 10 to 16 days
- Detection range 6 to 12 weeks
Minor Tobacco Alkaloids as Biomarkers of Tobacco Use
Minor Nicotine Alkaloids

Nicotine

β-Nicotyrine

Cotinine

Nornicotine

Anabasine

Anatabine

Nicotelline

Anatalline
Anabasine and Anatabine are Biomarkers of Tobacco (as Opposed to Nicotine) Consumption

• Not present in NRT. None or minimal in E-cigarette liquid
• Urine concentrations of 2 ng/ml highly specific for tobacco use while using NRT
• Can estimate nicotine intake coming from tobacco even with concomitant NRT use
• Potential biomarker to assess tobacco vs e-cigarette vs dual use
Nicotelline: A Proposed Biomarker and Environmental Tracer for Particulate Matter Derived from Tobacco Smoke

Peyton Jacob, III,*,† Maciej L. Goniewicz,‡ Christopher M. Havel,† Suzaynn F. Schick,§ and Neal L. Benowitz†,‖

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‡Department of Health Behavior, Division of Cancer Prevention & Population Sciences, Roswell Park Cancer Institute, Buffalo, New York 14263, United States
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Nicotelline

• Because of stability and low volatility, nicotelline has been proposed as an environmental tracer and biomarker for particulate matter derived from tobacco smoke
Nicotelline Highly Correlated with PM in Cigarette Emissions

5 Brands of US Cigarettes

\[ y = 0.00064x + 0.048 \]

\[ r^2 = 0.95 \]
Electronic Cigarettes
EC Liquid and Aerosol

- Nicotine
- Propylene glycol
- Glycerin
- Particulates (including metals)
- Volatile organic chemicals (acrolein, formaldehyde)
- Flavorants
- Miscellaneous contaminants
Exposure to Nicotine and Selected Toxicants in Cigarette Smokers Who Switched to Electronic Cigarettes: A Longitudinal Within-Subjects Observational Study

Maciej L. Goniewicz PharmD, PhD\textsuperscript{1,2}, Michal Gawron PharmD\textsuperscript{2}, Danielle M. Smith MPH\textsuperscript{1}, Margaret Peng BSc\textsuperscript{3}, Peyton Jacob III PhD\textsuperscript{3}, Neal L. Benowitz MD\textsuperscript{3}
Reduced NNK exposure after Switching

- NNK

ng/g

BL | W1 | W2

* | *
E-Cig Study - Nicotelline in Urine

The graph shows the change in Nicotelline levels in urine from enrollment to follow-up (3-5 days). The y-axis represents Nicotelline levels in pg/mL, while the x-axis indicates time points: enrollment and follow-up. Multiple lines represent different participants, with a decline in Nicotelline levels observed from enrollment to follow-up for each participant.
Changes in Biomarkers after Switching from Cigarettes to E-Cigarettes

- TNE
- NNAL
- NICOTELLINE

Days: 0, 1, 2, 2 Months
Summary of Biomarkers to Distinguish E-cigarette from Tobacco Use

- Nicotine intake similar to cigarette smoking, so nicotine biomarkers are not useful
- Nicotelline is useful biomarker to distinguish smoking vs non-combustible tobacco use.
- Anabasine/abatabine potentially useful, but some E-liquids contain substantial amounts of these alkaloids
- For studies of E-cig derived toxicant biomarkers, to exclude dual tobacco use I recommend urine NNAL measurement (< 1 pg/mg creat)
Summary
Main points

• Cotinine, CO and NNAL all useful
• Optimal cut points depend on prevalence and heaviness of smoking and SHS exposure
• Differences in window of detection:
  – CO – hours
  – Cotinine – days
  – NNAL - weeks
Main points

• Cotinine levels influenced by individual metabolic differences
• CO influenced by environmental sources and smoking marijuana
• OTC cotinine tests easy and cheap, specific but lack sensitivity for light/occasional use
• Minor tobacco alkaloids most useful for distinguishing tobacco from nicotine or E-cigarette use
Optimal Biochemical Cut Points Separating Smokers and Nonsmokers

<table>
<thead>
<tr>
<th>Biomarker</th>
<th>Biospecimen</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cotinine</td>
<td>Blood/saliva</td>
<td>3-5 ng/ml</td>
</tr>
<tr>
<td>Cotinine</td>
<td>Urine</td>
<td>30 ng/ml</td>
</tr>
<tr>
<td>Carbon Monoxide</td>
<td>Breath</td>
<td>5-6 ppm</td>
</tr>
<tr>
<td>NNAL</td>
<td>Urine</td>
<td>10 pg/mg creat</td>
</tr>
<tr>
<td>Biomarker</td>
<td>Specimen</td>
<td>Method</td>
</tr>
<tr>
<td>-------------------------------</td>
<td>--------------</td>
<td>--------------</td>
</tr>
<tr>
<td>Exhaled CO</td>
<td>Breath</td>
<td>Portable Instrument</td>
</tr>
<tr>
<td>Cotinine</td>
<td>Urine</td>
<td>OTC Kit</td>
</tr>
<tr>
<td>Nicotine and Cotinine</td>
<td>Urine, Saliva Blood</td>
<td>GC-NPD</td>
</tr>
<tr>
<td>Nicotine</td>
<td>Urine, Saliva Blood</td>
<td>GC-MS/MS</td>
</tr>
<tr>
<td>Cotinine</td>
<td>Urine, Saliva Blood</td>
<td>GC-MS/MS</td>
</tr>
<tr>
<td>Cotinine</td>
<td>Urine</td>
<td>LC-MS/MS</td>
</tr>
<tr>
<td>Total Nicotine Equivalents</td>
<td>Urine</td>
<td>LC-MS/MS</td>
</tr>
<tr>
<td>NNAL</td>
<td>Urine</td>
<td>LC-MS/MS</td>
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