Biomarkers in Public Health: Development and Applications

Irina Stepanov, Ph.D.
Assistant Professor
Division of Environmental Health Sciences and Masonic Cancer Center
University of Minnesota
Biomarker paradigm

Exposure → Clinical disease

1987, U.S. National Research Council
Biomarker paradigm

Exposure and Effect

Exposure

Internal dose

Biologically effective dose

Early biological effect

Altered structure/function

Clinical disease

Susceptibility

1987, U.S. National Research Council
What is a biomarker?

An indicator of exposure, effect, susceptibility, or clinical disease that can be measured in a biological system

<table>
<thead>
<tr>
<th>Category</th>
<th>Examples</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unchanged exogenous agents (exposure)</td>
<td>Asbestos fibers, metals</td>
</tr>
<tr>
<td>Metabolized exogenous agents (exposure, susceptibility)</td>
<td>Phenol for benzene, cotinine for nicotine, metabolite ratios</td>
</tr>
<tr>
<td>Endogenous molecules exposure effect susceptibility</td>
<td>Porphyrin ratios (metals, dioxins) DNA and protein adducts Gene polymorphisms</td>
</tr>
<tr>
<td>Cellular/tissue changes (effect, susceptibility)</td>
<td>Neutrophil to lymphocyte ratios, sperm counts</td>
</tr>
</tbody>
</table>
Research publications on biomarkers

Results of PubMed search

Number of publications

<table>
<thead>
<tr>
<th>Time Periods</th>
<th>Number of Publications</th>
</tr>
</thead>
<tbody>
<tr>
<td>1900 - 1950</td>
<td>17</td>
</tr>
<tr>
<td>1950 - 2000</td>
<td>170,000</td>
</tr>
<tr>
<td>2000 - present</td>
<td>500,000</td>
</tr>
</tbody>
</table>
Most diseases are believed to result from a complex interaction between an individual’s genetic make-up and environmental agents.

Exposure assessment approaches

- Questionnaires
- Interviews
- Diaries
- Biomarker measurements
Different biological sample types for the biomarker-based exposure assessment

- Teeth
- Hair
- RBC
- Serum/Plasma
- Urine
- Saliva/Sweat
- Exposure frequency questionnaire
- 24h recall
- Diary

Sample types:
- Early life
- Year
- Month
- Week
- Now
- Week
## Advantages and disadvantages of different biological sample types

<table>
<thead>
<tr>
<th>Type</th>
<th>Advantages</th>
<th>Disadvantages</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood</td>
<td>Easy to collect, regularly replenished, a way for chemicals to travel within the body</td>
<td>Invasive, cultural rejection, short lifespan</td>
</tr>
<tr>
<td>Urine</td>
<td>Non-invasive, large amounts</td>
<td>Metabolites are measured, more than a single time point or 24-h collections are often needed</td>
</tr>
<tr>
<td>Adipose tissue</td>
<td>Good matrix for analysis of non-polar chemicals</td>
<td>Invasive</td>
</tr>
<tr>
<td>Breast milk</td>
<td>Ease of collection, may reflect historical exposures to lipid-soluble chemicals</td>
<td>Limited application</td>
</tr>
<tr>
<td>Hair</td>
<td>Non-invasive, temporal distribution</td>
<td>Not many methods are validated, trace levels, potential of external incorporation</td>
</tr>
<tr>
<td>Nails</td>
<td>Non-invasive, reflect long-term cumulative exposure</td>
<td>Not many methods are validated, trace levels</td>
</tr>
<tr>
<td>Saliva</td>
<td>Non-invasive</td>
<td>Not widely used</td>
</tr>
<tr>
<td>Exhaled air</td>
<td>Non-invasive</td>
<td>Limited application (volatile compounds)</td>
</tr>
</tbody>
</table>
Characteristics of a good biomarker?

- Specificity (to exposure, disease)
- Sensitivity (in response to changes in exposure, disease)
- Technical feasibility (collection, measurement)
- Reproducibility (frame of reference)
- Biological relevance (timeframe, mechanism of effect and disease)
- Predictive value
Biomarker development
Biomarker development strategies

Hypothesis driven

- Food
- Food composition data; Bioavailability; Metabolism
- Identification of likely candidate markers
- Development of specific analytical method
- Dietary intervention with extremes (nil/high) of food
- Analysis of candidate biomarkers in collected specimens
  - Technical feasibility
  - Specificity
  - Sensitivity
- Confirmation of suitability of candidate biomarker
- Application in epidemiological or clinical settings
- Biomarker validation


1-methylhistidine

![Graph showing urinary 1-methylhistidine levels in different dietary conditions]
Biomarker development strategies

Hypothesis driven

Food composition data; Bioavailability; Metabolism

Identification of likely candidate markers

Development of specific analytical method

Dietary intervention with extremes (nil/high) of food

Analysis of candidate biomarkers in collected specimens

Technical feasibility
Specificity
Sensitivity

Confirmation of suitability of candidate biomarker

Application in epidemiological or clinical settings

Biomarker validation

Discovery driven

Food composition data; Bioavailability; Metabolism

Identification of likely candidate markers

Development of specific analytical method

Dietary intervention with extremes (nil/high) of food

Analysis of candidate biomarkers in collected specimens

Multivariate analysis to identify differences and candidate markers

Application in epidemiological or clinical settings

Biomarker validation

Examples of biomarker applications in Environmental Health
Lead (Pb)

- banned
- outlawed since 1978

banned, copper is used instead
however...
Temporal changes in blood Pb levels


* 95% confidence interval.
Aflatoxin exposure an hepatocellular carcinoma (HCC)

“… On the other hand, cohort analysis using all cases of HCC revealed no strong or statistically significant association between HCC risk and dietary aflatoxin consumption as determined from the in-person food frequency interview combined with the survey of market foods in the study region…”

Use of biomarkers in environmental health

- Identifying priority exposures
- Identifying at-risk populations
- Recognizing time-trends in population exposures
- Evaluation of exposure reduction and prevention activities
- Checking the validity of traditional exposure models
- Establishing reference ranges for comparison
Application of biomarkers in tobacco carcinogenesis studies
Stepanov Lab research focus:
Applying biomarker-based approach to characterize and quantify the links between exposures and health outcomes

- **Chemical toxicants and carcinogens in environmental sources**
- **Biomarkers:**
  - Exposure
  - Metabolism
  - Effect
- **Cancer**
  - Inflammation-driven diseases
  - Neurobehavioral abnormalities

Quantitative links
Biomarker expertise

**Tobacco exposures**
- Tobacco-specific N-nitrosamines
- Cotinine (nicotine)
- Polycyclic aromatic hydrocarbons (1-HOP)
- HPB-releasing DNA adducts

**Oxidative DNA damage**
- 8-oxo-dG
- 8-oxo-dA
- M$_1$dG

**Trace elements (sample collection and processing)**
- Manganese
- Iron
- Cadmium
Why do we study tobacco?

100,000,000
people died from tobacco use in the 20th century

1,000,000,000
people will die from tobacco use in the 21st century
Main health consequences of smoking

- Over 7,000 constituents
  - Nicotine
  - Numerous toxicants
  - More than 70 carcinogens

Addiction

Toxicity
- Respiratory
- Cardiovascular

Carcinogenicity
- 19 types of cancer
Why do we study tobacco?

Understand the diversity of tobacco products and related risks

Understand mechanisms of diseases caused by tobacco use
- Identify susceptible individuals
- Develop preventive measures

Build science base for tobacco product regulation by the FDA
Focus on NNN and NNK

- The most important carcinogens in smokeless tobacco
- NNN: oral and esophageal cancer
- NNK: lung and pancreatic cancer
Cancer deaths due to smoking (US)

Annual Number of Cancer Deaths Attributable to Smoking by Sex and Site, US, 2000-2004

Research approach
Exposure measurement

Product analysis
Biomarkers of NNK and NNN intake
Measuring the relationship between product content and constituent intake

Habitual users of various brands
Urinary total NNAL in US smokers of different cigarette brands

**NNK in cigarette smoke**

- Brand A: 80 ng/mg nicotine
- Brand B: 50 ng/mg nicotine

**Total NNAL in smokers’ urine**

- Brand A (48): 0.55 pmol/nmol cotinine
- Brand B (20): 0.32 pmol/nmol cotinine

$P = 0.04$
Relationship between constituent levels and biomarkers of intake in smokeless users

Smokeless tobacco users (343)
Products with wide range of nicotine and TSNA levels

Multiple regression analysis for biomarkers (P-values)

<table>
<thead>
<tr>
<th>Covariates</th>
<th>Total nicotine equivalents</th>
<th>Total NNN (NNN intake)</th>
<th>Total NNAL (NNK intake)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Constituent level in product</td>
<td>0.155</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Dip weight</td>
<td>0.086</td>
<td>0.068</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Total daily dip duration</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>
Measuring the relationship between product content and constituent intake

Switching studies
Total NNAL in urine of smokeless tobacco users assigned to low-TSNA products


Mean total NNAL (pmol/mg creatinine)

Visit

Baseline
Week 2
Week 4

Snus (low-TSNA product)
Nicotine patch
Research approach

Constituents → Intake → Risk

- Graph showing urinary biomarker levels and adjusted OR:
  - < 140 fmol/mg Cr
  - 140–290 fmol/mg Cr
  - > 290 fmol/mg Cr
Understanding individual susceptibility

DNA damage

metabolic activation

NNK

DNA damage

individual smokers
Linking exposure to cancer risk

Shanghai Prospective Cohort

18,244 men (45-64 years old) enrolled between 1986 and 1989

use of tobacco and alcohol, usual diet, and medical history

10-ml blood sample and a single spot urine sample

Yuan et al, Carcinogenesis (2011);32:1366-1371
Relationship between TSNA exposure and cancer risk in humans

J.-M. Yuan et al, Carcinogenesis 32(9): 1366-1371 (2011);
Ongoing non-tobacco studies
Oxidative stress and inflammation, induced by the dust from taconite operations and mediated by iron exposure, are important contributors to the development of cancer and cardiovascular disease in taconite workers.
Oxidative DNA damage in age-related macular degeneration (AMD)

- The leading cause of blindness among older adults in the developed world
- Approximately 30% of individuals over 75 years are affected
- Indications of increased oxidative mitochondrial DNA damage in the retinal pigment epithelium

11-55 ng mtDNA

Mitochondrial and nuclear pellet

DNA isolation

Internal standards + enzymatic hydrolysis (37°C, 2 h)

10K centrifugal filtration + solid phase extraction

Column purification

LC-NSI-MS/MS analysis

8-oxo-dG

8-oxo-dA