Methods and Issues in Using Biological Measurements in Epidemiologic Research

EPI/ENVH 573

Spring 2003
Course Orientation

Course Goals

- identify biomarker strengths and limitations, characterization
- develop familiarity with basic concepts of molecular biology
- identify goals and strategies in conducting transitional studies
- identify sources and impact of biomarker measurement error
- identify strengths/limitations of epidemiologic study designs for incorporating biomarker measurements;
- develop skills for critiquing scientific reports from human populations that involve biologic measures.
Course Orientation (cont.)

Course Format

• methodologic lectures
• biomarker-specific lectures
• article discussions
• assignment discussions
• field trips

Assignments/Grading

• 3 interim assignments (15% each)
• 1 final paper & presentation (35%)
• participation (20%)
Course Orientation (cont.)

Readings

• texts

• journal articles, etc

Website  http://courses.washington.edu/epi573/

About the Instructor
## Schedule

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| March 31     | Introduction (Schwartz)  
  Epidemiologic Criteria for Selection and Evaluation of Biomarkers (Schwartz) |
<p>| April 2 &amp; 7  | Reliability Studies for Biological Marker Data (White) |
| April 9 &amp; 14 | Overview of Molecular Biology (Nickerson) |
| April 16 &amp; 21| Transitional Studies (Schwartz) |
| April 23 &amp; 28| Study Design and Implementation (Schwartz) |
| April 30     | Laboratory Quality Control (Bigler) |
| May 5        | Cardiovascular Disease (Austin) |</p>
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<td>Application: Biomarker Research <em>In Silico</em> (Ulrich/Sparks)</td>
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<td>May 12</td>
<td>Application: Nutritional Biomarkers and Disease Mechanisms (Lampe)</td>
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<td>May 14</td>
<td>Application: Biomarkers and Early Detection of Cancer (McIntosh)</td>
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<td>May 19</td>
<td>Application: Cervical Carcinoma (Schwartz)</td>
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<td>Application: Studies of Protein Expression in Tissue (Porter)</td>
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<td>May 28</td>
<td>Ethical Issues in Biomarker Studies (Schwartz/Mastroianni)</td>
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<td>Student Presentations</td>
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EPI/ENVH 573

Session 1

Introduction to Biomarkers in Epidemiologic Research
1. Definition of “Biomarker”

A measure of a chemical, cellular, molecular, immunologic, genetic, or physiologic signal, biologic event, or biologic state, measured in biologic material.

• particular interest is in biomarkers of abnormal signals, events, or states.

• studies of human populations that use biomarkers often called “molecular epidemiologic” studies

• is it possible to measure biologic signals, events, or states without obtaining biologic material?
2. Legacy of Biomarkers in Epidemiologic Research

a. Syphilis

Wasserman test (1906)

Positive Complement Fixation Test:

1. Complement inactivated by heating to 60°C
2. Guinea pig serum added to provide a known amount of complement

Specific antigen added

Sheep red blood cells added as an indicator

Tube incubated to allow antigens and antibody to react

Complement is fixed

Red cells do not lyse

Negative Complement Fixation Test:

1. Complement inactivated by heating to 60°C
2. Guinea pig serum added to provide a known amount of complement

Specific antigen added

Sheep red blood cells added as an indicator

Tube incubated to allow antigens and antibody to react

Complement is not fixed

Red cells lyse

Complement causes lysis of the red blood cells. The cells do not lyse in the positive test because complement has been bound (fixed) by the reaction between antigen and antibody. In the negative test, no antibody was present to bind with the antigen. Complement was therefore not fixed and was available to lyse the red blood cells.
2. Legacy of Biomarkers in Epidemiologic Research

a. Syphilis

Wasserman test (1906)
1st serologic test for infectious agent
use in epidemiologic seroprevalence surveys in 1920s

b. Polio and diphtheria

Skin and serologic markers (1920s)
descriptive epidemiologic studies
identified urban-rural differences
2. Legacy of Biomarkers in Epidemiologic Research

c. General markers of infection

Leukocyte counts

d. Cardiovascular disease

blood lipid levels (1940s, 1950s)
Framingham study
risk profile development

e. Cancer

pap smear (1940s)
natural history studies
3. Qualities of Epidemiologic Studies Involving Biomarkers

a. Interdisciplinary

   heavy collaboration with laboratory-based disciplines
   (or with population disciplines, if you are lab-based)

   varying views on disease etiology, scientific methods &
   paradigms, career goals

b. Terminology: expanded and confusing

   e.g., meaning of “controls”
3. Qualities of Epidemiologic Studies Involving Biomarkers

c. Closer to cutting edge of technologic and biologic knowledge

via interactions with laboratory colleagues

new technology expands feasibility and scope of questions to be asked

time frame of research shortened

d. New study design and expanded analytic strategies

   case series

   continuous data

   repeated measures
3. Qualities of Epidemiologic Studies Involving Biomarkers

e. Demonstrating feasibility and capability particularly important

- laboratory work viewed as more complex than fieldwork
- specimen collection techniques can be very complex
- new biomarkers need preliminary data to support large and expensive research effort
4. Goals of Epidemiologic Studies Involving Biomarkers

a. Enhance our understanding of:
   → the natural history of disease
   → distribution and determinants of disease occurrence
   → distribution and determinants of disease outcome;

b. Identify approaches to the prevention of disease and its adverse sequelae
5. Biomarker Types, by Epidemiologic Design Feature

a. Exposures (predictors)
   
   E.g. Internal or biologically effective dose

b. Disease (outcomes)
   
   E.g. pre-disease, early stage disease, pre-clinical disease

c. Effect modifier (susceptibility)
   
   E.g. inherited genetic mutation
5. Biomarker Types, by Epidemiologic Design Feature

Cigarette Smoking → Mutated p53 gene → Cancer

Exposure biomarker

Susceptibility biomarker

Disease biomarker
6. Biomarker Types, by Characteristic

a. Exogenous compounds (xenobiotics)
   
   i. Minimal or no metabolism
      
      polychlorinated biphenyls (PCBs)
   
   ii. Extensive metabolism
      
      polycyclic aromatic hydrocarbons (PAHs)
6. Biomarker Types, by Characteristic

b. Endogenous compounds
   
   hormones (insulin), cytokines (IL-6)

c. Molecular characteristics
   
   DNA sequence variation

d. Cellular characteristics
   
   Morphologic changes (e.g., sperm motility)
Criteria for Selection and Evaluation of Biomarkers
1. Biomarkers vs. Assays

a. Biomarkers represent biologic events or states

   e.g., insufficient p53 function is the “state” and mutated p53 is the “biomarker”

b. Assays are used to measure biomarkers

   single-strand conformation polymorphism analysis
   DNA sequencing
   immunohistochemistry
1. Biomarkers vs. Assays

Biological event/state: **Net effect of DNA damage and repair**

Biomarker: *Chromosome breaks following clastogen*

Assay: *Bleomycin sensitivity assay*
2. What features of a biomarker should be evaluated?

a. Are there potential advantages compared to not using a biomarker?

   strengths vs. limitations

b. How well is the natural history of a biomarker known and thus amenable to use in an epidemiologic study?

   Want to know when, where, and why

c. What assays are available for use in an epidemiologic study?

   Want to measure biomarker accurately in many samples
3. Possible Benefits to Using Biomarkers?

A. Exposure, Disease, Susceptibility Biomarkers
   
   i. Biologic phenomena not otherwise measurable
      
      • genetic damage/alterations
         
         E.g., gene “silencing” via promoter methylation

      • cell kinetics
         
         E.g., abnormal levels of cell proliferation or programmed cell death (apoptosis)
3. Possible Benefits to Using Biomarkers?

ii. Reduce impact of differential exposure misclassification

E.g., reduce recall bias of smoking using cotinine

iii. Turn some “noise” (non-differential misclassification) into knowledge
3. Possible Benefits to Using Biomarkers?

iv. Identify individual etiologic agents

- dietary constituents
  - fruits
  - &
  - vegetables
  - vitamins
  - fiber
  - flavonoids

- carcinogens
  - cigarette smoke
  - polyaromatic hydrocarbons
  - heterocyclic amines
  - nitrosamines
3. Possible Benefits to Using Biomarkers?

iv. Identify individual etiologic agents (cont.)

• genetic factors

family history of venous thrombosis ➔ mutation in factor V gene (G1691A)
3. Possible Benefits to Using Biomarkers?

B. Disease biomarkers

   i. Predisease events/states

      earliest molecular changes
3.B.i. Example of Biomarkers in Pre-Disease States

Oral Cancer (Zhang et al., 1997)

• To what extent is oral lichen planus (OLP) a precursor to oral neoplasia? Can biomarkers help discern when OLP is more or less likely to progress to malignancy?

• Measured loss of heterozygosity (LOH) at several points (loci) in DNA in tissue from a spectrum of OLP, oral pre-cancer, and oral cancer.
Overview of Theory Behind LOH

LOH in Knudson’s “Double-Hit” Tumorigenesis Model

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STEP 1
Pro-oncogenic Point Mutation

STEP 2
Loss of Heterozygosity

Li-Fraumeni Syndrome:
germline p53 mutation
3.B.i. Example of Biomarkers in Pre-Disease States

Oral Cancer (Zhang et al., 1997)

• Findings

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>% LOH</th>
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<tbody>
<tr>
<td>OLP</td>
<td>6%</td>
</tr>
<tr>
<td>Mild/moderate dysplasia</td>
<td>40-46%</td>
</tr>
<tr>
<td>Severe dysplasia/CIS</td>
<td>81%</td>
</tr>
<tr>
<td>Invasive cancer</td>
<td>91%</td>
</tr>
</tbody>
</table>
3. Possible Benefits to Using Biomarkers?

B. Disease biomarkers

   i. Predisease events/states

      *earliest molecular changes*

   ii. Preclinical disease

      *earliest molecular and pathologic changes*
3. Possible Benefits to Using Biomarkers?

B. Disease biomarkers

iii. Link exposure to natural history

*Identify at which points exposures may be important*

iv. Increase disease homogeneity (etiologic or prognostic)

\[\uparrow \text{Disease homogeneity} \rightarrow \uparrow \text{detection of associations}\]
\[\downarrow \text{smaller sample sizes}\]
3.B.iv. Example of Biomarkers to ID Homogeneity
Lung Cancer In Uranium Miners (Vahakangas et al., 1992)

• Uranium miners exposed to high levels of radon, which have been associated with elevated lung cancer rates.

• Miners also smoke cigarettes. Question as to whether lung cancers in miners are due to cigarette smoking or radon exposure (or both!).

• p53 inactivation (via mutation) is important biological state leading to lung cancer, but type of mutation expected to depend on exposure (G:C to T:A for tobacco)
3.B.iv. Example of Biomarkers to ID Homogeneity
Lung Cancer In Uranium Miners (Vahakangas et al., 1992)

• Studied 20 lung ca in uranium miners. Used direct sequencing of p53 sequence using DNA from paraffin-embedded tissue

• Findings:

<table>
<thead>
<tr>
<th>Type of p53 “lesion”</th>
<th># Tumors</th>
</tr>
</thead>
<tbody>
<tr>
<td>G:C to T:A</td>
<td>0</td>
</tr>
<tr>
<td>Other amino acid change</td>
<td>5</td>
</tr>
<tr>
<td>Deleted base pair or amino acid</td>
<td>2</td>
</tr>
<tr>
<td>Premature stop codon</td>
<td>1</td>
</tr>
<tr>
<td>None</td>
<td>12</td>
</tr>
</tbody>
</table>
3. Possible Benefits to Using Biomarkers?

B. Disease biomarkers

v. Shorter studies

Pre-disease events and pre-clinical disease usually are more common than clinical disease
3. Possible Benefits to Using Biomarkers?

C. Potential Overall Benefit

i. Make better inferences re: disease mechanisms

\[\text{data derived from studies in humans rather than animals or cell systems}\]

ii. Improve process of causal inference

“Biologic plausibility”
3. Possible Benefits to Using Biomarkers?

C. Potential Overall Benefit

iii. Improve risk assessment

more accurate exposure and susceptibility classification in individuals

iv. Improve potential for developing effective prevention strategies

Knowledge of mechanism theoretically leads to more efficient design and testing of candidate drugs or compounds
3. Possible Benefits to Using Biomarkers?

C. Potential Overall Benefit
   v. Improve potential for developing effective monitoring and management of patients

   markers that are predictive of outcome could be used to stratify patients to different therapies

D. Downside to biomarkers?
   i. Costs
   ii. Often not what policy is based on
   iii. Can’t measure every phenomena with them
4. Knowledge of Natural History

5. Assays Available for Use

To be covered in future sessions!
End of Session 3