Biomarkers in Pediatric Environmental Health: A Cross-Cutting Issue

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It is not yet known the extent to which the environment adversely affects the health of the developing individual. Difficulties in this determination are the problems of a) the assessment of exposure, b) the long latency of many diseases induced by the environment, c) the number of confounding exposures, and d) the extrapolation of animal models to critical stages of human development. Biomarkers have the potential to be quantitative dosimeters of exposure and biologic effective dose, as well as early warning signals of biologic effect. Biomarkers may document individual susceptibilities, as well as defining critical windows of exposure. To be useful, biomarkers need to be validated in terms of their specificity and sensitivity. Biomarkers are useful across all disciplines including asthma and respiratory problems, developmental neurotoxicity, childhood cancer, and endocrine disruptors. Biomarkers have not been developed nor used widely in pediatric environmental health. Research by our group and others has documented the validity of biomarkers in pediatric environmental health. Advances in the field of biomarkers may have important implications for the detection, prevention, and treatment of environmentally induced diseases in children. Ongoing validation of promising biomarkers should be a research priority. — Environ Health Perspect 106(Suppl 3):813–816 (1998).

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Introduction

Developing individuals (embryos, fetuses, newborns, infants, children, and adolescents) are a uniquely susceptible population to insults from environmental hazards (1,2). Their increased susceptibility can arise from increased exposure to environmental toxins, increased exposure of individual organ systems from differences in distribution of toxins, immaturity of metabolic pathways, immaturity of excretory pathways, alterations in target organ susceptibility, and a longer life span in which to express illness. Although the enhanced susceptibility of infants and children to environmental toxicants has been shown in multiple studies, the nature and extent of pediatric illness secondary to environmental exposure has not been well characterized. There are several reasons for this deficiency. First, documentation of exposure is difficult in the fetal and pediatric population. Pregnant women and children do not wear personal monitoring devices as do workers in an occupational exposure setting. Modeling of exposure is difficult; there are few studies documenting where children spend their time. Even in situations with known exposures, the individual dose to a child is difficult to determine. The long latency of many environmentally induced diseases makes their etiology difficult to determine. Thus, retrospective studies are difficult to conduct. An individual is also exposed to more than one environmental toxicant and probably to other agents, which may confound the association of one toxicant to an illness. Extrapolation of animal models to human children is difficult. Many of the critical stages of development are not well characterized in animals. For example, an exposure that occurs during puberty in children may be difficult to model in an animal. Finally, classic epidemiology has limitations in sensitivity. For example, if thalidomide had caused mental retardation, the rarity of the exposure would never have significantly increased the rate of mental retardation above background rates, and hence, thalidomide would not have been recognized as a teratogen (3). Biomarkers have the potential to overcome many of these difficulties. They may be used to identify the early stages of health impairment and to understand basic mechanisms of exposure and response in research and medical practice. Because of this potential, in 1986 the National Academy of Sciences and the National Research Council created three subcommittees and an oversight committee to evaluate the state of knowledge of biomarkers in reproductive and developmental toxicology (4), pulmonary toxicology (5), and immunotoxicology (6).

What Are Biomarkers?

Biologic markers, or biomarkers, are indicators of variation in cellular or biochemical components or processes, structure, or function that are measurable in biologic systems or samples. Biomarkers are used widely throughout medicine. A well-known example is the measurement of creatinine in blood to assess kidney function. Biomarkers represent signals on a continuum between health and disease (Figure 1). A biomarker may not always function at the same point on this continuum, but may change roles as knowledge of the environment–organism interaction increases. A biomarker may also function at more than one place on this continuum. For example, blood lead concentration serves as both a marker of exposure (7) and a marker of neurotoxicity (8).

Three general categories of biomarkers have been defined: biomarkers of exposure to chemical or physical agents, biomarkers of effects of those exposures, and biomarkers of susceptibility. A biomarker of exposure is an exogenous chemical or its metabolite(s) or the product of an interaction between a xenobiotic agent and some target molecule or cell that is measured in a compartment within an organism. A biologic marker of effect is a measurable alteration of an endogenous component within an organism that, depending on magnitude, can be recognized as a potential or established health impairment or disease. A biomarker of susceptibility is an indicator of an inherent or acquired property of an
organism to increase the internal dose of a xenobiotic or to alter the response to the challenge of exposure to a specific xenobiotic substance. A biologic marker of susceptibility may also be a marker of resistance when the internal dose or the health effect is less than the general population in children with this marker.

**Biomarkers of Exposure**

Biomarkers of exposure can be divided into internal dosimeters or markers of biologically effective dose. An internal dosimeter measures the amount of a toxicant or its metabolite present in cells, tissues, or body fluids. Several studies in children have documented elevation in such biomarkers with several different types of exposures. For example, fetal exposure to some chemicals has been shown with cord blood measurements of polychlorinated biphenyls (9) and cotinine (a metabolite of nicotine) (10). We have used cotinine in cord blood as a biomarker for maternal environmental tobacco smoke exposure to show that this exposure may increase the risk of delivering an infant with persistent pulmonary hypertension of the newborn (10). Fetal exposure can also be documented with meconium analysis for drugs of abuse (11), ethanol (12), and lead (13). In children, blood lead concentration has been used as a marker for lead exposure, and urinary nitrophenol concentration has been used as a marker for methyl parathion exposure (14). Internal dosimeters take into account individual differences in absorption or bioaccumulation of the xenobiotic in question and are relatively easy to measure. However, they do not provide information about the interaction with critical cellular targets—the biologic effective dose. For example, a child 2 years of age and a newborn may have the same blood lead concentration, but the interaction of the lead with critical sites in the central nervous system may be greater for the newborn because of the lack of a blood–brain barrier.

The biologically effective dose is the amount of xenobiotic material that has interacted with a critical molecular site where the biologic effect is initiated. Frequently, surrogates are used because of lack of knowledge of the actual target molecule and/or inaccessibility of the target tissue, such as bone marrow or brain. Examples of this type of biomarker are carcinogen–DNA adducts with specific carcinogens that are measured in white blood cells (15). Because carcinogens are thought to act through effects on DNA expression, the measurement of carcinogen–DNA adducts in white blood cells is a surrogate for the measurement of the specific DNA–carcinogen interaction initiating the cancer in the target tissue. Carcinogen–protein adducts in blood have also been used as surrogates for the biologically effective dose in the target organ.

**Biomarkers of Effects**

Biomarkers of effects in an organism after exposure to an environmental agent can be categorized based on their relationship to health status—from normal health, to health impairment, to overt disease. Therefore, there can be a wide spectrum of measurable effects: a) an alteration in a tissue or organ; b) an early event in a biologic process that is predictive of development of a health impairment; c) a health impairment or clinically recognized disease; or d) a response peripheral or parallel to a disease process, but correlated with it and thus usable in predicting development of a health impairment (4). To summarize, a biomarker of an effect can be any qualitative or quantitative alteration that is predictive of health impairment resulting from exposure to an exogenous agent. A wide variety of biomarkers fall into this category. An example of a biomarker of effect is the alteration in pulmonary function tests in children after exposure to environmental tobacco smoke (16). Another biomarker of effect is hypoplasia of the corpus callosum in infants exposed to ethanol in utero (17). Biomarkers of effect are not chemical or agent-specific and can be affected by other exposures in the environment or by lifestyle. For example, nitrogen dioxide can also alter pulmonary function tests in children.

**Biomarkers of Susceptibility**

Biomarkers of susceptibility indicate individual factors that can affect response to environmental agents. These individual factors reflect variations between individuals in genetic structure. Typically, genetic variability refers to variations in gene structure that occur in more than 1% of the population (genetic polymorphisms) and to those that occur in less than 1% of the population (genetic mutations). For example, blue eye color is a genetic polymorphism and albinism is a genetic mutation (or inherited disease). Differences in gene expression with stage of development are not considered to be genetic variabilities but rather age-related variabilities. Some of these variations in genetic structure make the individual more susceptible to health effects from environmental exposures. These genetic variabilities occur in the absence of exposure, although exposure can increase or decrease susceptibility to the effects of later exposure. An example would be an exposure to a toxicant that induces the enzyme responsible for its excretion. A subsequent dose would be metabolized more readily and hence cause less harm. This is one explanation for the observed U-shaped dose–response curve for some toxicants that at low levels appear to have a beneficial effect.

How do genetic variabilities increase/decrease susceptibility to environmental toxicants? In general, to develop a disease from exposure to an environmental toxicant, the following must occur: exposure to the toxicant, absorption of the toxicant, distribution of the toxicant in the body, metabolism of the toxicant (activation, deactivation), excretion of the toxicant, interaction of the toxicant with the target
molecule, damage of the target molecule, and repair of the damaged molecule. Genetic variability may increase susceptibility to disease from an environmental toxicant if any of these processes are altered. Obvious examples are found in individuals with inherited diseases. Frequently the disease state presents following birth, when common environmental exposures occur. Such disease states are easily recognized because of their acuity and severity, for example, xeroderma pigmentosa and skin cancer from UV irradiation (inability to repair DNA damage), phenylketonuria and a diet with phenylalanine-containing proteins (inability to metabolize phenylalanine), or glucose 6-phosphate dehydrogenase deficiency and methemoglobinemia from exposure to naphthalene mothballs (inability to reduce the oxidized iron in hemoglobin). More difficult to detect are those genetic variations leading to genetic susceptibility in individuals with common genetic polymorphisms. They are common because either the increased susceptibility is to a rare environmental exposure, or the disease states are of a more chronic or subacute nature. For example, only newborns unable to metabolize dilantin delivered to mothers taking dilantin (a rare exposure) had features of phenytoin embryopathy. A biomarker of this susceptibility is the epoxide hydroxide activity of fetal amniocytes (18). Recent advances in determining genetic susceptibility are discussed by Suk and Collman (19) and Whyatt et al. (20) in this issue.

Validation of Biologic Markers

To validate the use of a biologic measurement as a biomarker, it is necessary to understand the relationship between the marker and the event or condition of interest. Determining the sensitivity and specificity are critical components of the validation process. Sensitivity refers to the ability of a test to correctly identify those with the condition or disease of interest. Specificity refers to the ability of a test to correctly identify those without the condition or disease of interest. Biomarkers of exposure or effect must be validated in terms of their ability to assess the true exposure or disease (sensitivity) and their ability to assess the lack of exposure or disease (specificity). One of the primary purposes of biomarkers in environmental health research is to identify exposed persons, so that risk can be predicted and disease prevented. Validation of biomarkers includes the backward process of associating a biomarker with exposure, and the forward process of linking a biomarker with effect. Appropriate validation for a biomarker depends on its anticipated use. A biomarker observed well before the onset of disease may have a low predictive value as a biomarker of effect, but be very useful as a biomarker of exposure, enabling long-term surveillance of an exposed population. In contrast, a biomarker of effect that is expressed long after exposure could be of relatively little use in exposure assessment, but be very useful in predicting progression of disease or in calculating risk. Animal models are useful for understanding the mechanistic bases of the expression of markers and relationships between exposure, early effects, and disease. The validity of a specific biomarker of effect depends on the reliability of studies that provide the background data, particularly on mechanisms. Estimates of the sensitivity of a biomarker must include its evaluation in an unexposed population or unexposed animals to determine a baseline value for the marker. This evaluation may be difficult in the pediatric population because of ethical issues involving invasive procedures with little benefit to the pediatric participant. Examples of two biomarkers that have been extensively validated for both exposure and effect are blood lead concentrations for both lead exposure and lead neurotoxicity, and cotinine in urine, serum, and saliva for both exposure to environmental tobacco smoke and for predicting reduction in birth weight. Examples of two biomarkers currently in the process of validation include dentin lead levels to predict elevated adult body burden of lead (biomarker of exposure) and reaction time on the Fagan Test of Infant Intelligence to predict future neurocognitive and behavioral outcome (biomarker of effect).

Implementation of Biomarkers in Population Studies

The identification of valid biomarkers that indicate exposure, effect, or susceptibility is a complicated process involving studies in animals, refinements in laboratory assays, and studies in special human populations. When validation has been completed in such studies, the application to larger populations is not straightforward. The Oversight Committee on Biologic Markers (4) suggests the following framework for implementing an identified, potentially informative biomarker in large populations:

- Establish normal baseline values and distribution for the marker in laboratory animals and humans.
- Evaluate the sensitivity and specificity of the marker in predicting a health outcome (e.g., asthma or genetic damage).
- Understand in detail the time course of response of the marker to a toxic chemical, with special attention to the recovery process.
- Develop a strategy for and a consensus on the use of multiple species in toxicologic studies.
- Develop human assays that use semen, saliva, or urine, rather than tissue or blood, whenever possible.
- Use noninvasive techniques such as ultrasound or magnetic resonance imaging whenever possible.
- Consider a battery of markers that reflect a wide array of physiologic functions and genetic damage and relate the marker in question to others in the battery.
- Identify populations at high risk for reproductive or developmental health impairment (perhaps populations exposed to drugs with reproductive or developmental toxicity, aging populations, or offspring of women exposed to diethylstilbestrol) to serve as test subjects for the initial assessment and validation of biologic markers.
- Include among high-exposure populations those with special or unique occupational exposures (e.g., children of agricultural workers).
- Encourage and support institutions in the development of sample banks, to speed the identification and validation of markers.
- Establish a task force to develop and coordinate strategy.

Biomarkers as a Cross-Cutting Issue

Work done by our laboratory and others has demonstrated the usefulness of biomarkers in pediatric environmental health. Validation and use of biomarkers of exposure, effect, and susceptibility in the areas of asthma/respiratory disease, cancer, and neurodevelopmental effects would hasten progress in understanding modes of exposure and risk assessment for children. Ongoing validation of promising biomarkers should be a research priority.
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