Identification of Needs in Biomarker Research

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Interest in the use of biological markers to evaluate future disease risk has increased greatly in recent years. Biomarkers are observable end points in a continuum of events leading from exposure to toxic agents to diseases that ultimately result from exposure. Because many significant diseases develop over long periods of time, methods for detecting early events that can predict risk are important for disease prevention. Biomarkers are generally categorized as detecting exposure, effects of exposure, or individual susceptibility to exposure. Although there has been significant progress in the technical development of biomarkers, implementation of their use in human populations has progressed much more slowly. We discuss four major needs in the development of biomarkers. First, new biomarkers need to be developed to fill gaps in our ability to observe steps in the continuum from exposure to disease. Second, the relationships between biomarker responses and disease pathology needs to be better understood. Third, the sensitivity, specificity, and variability of biomarkers need to be better characterized and they must be better validated as predictors of disease risk. Fourth, there are several societal impediments to the practical implementation of biomarker studies as public health tools. A common agreement among employers, employees, regulators, and the legal community must be established regarding appropriate and ethical uses and interpretation of biomarker data. — Environ Health Perspect 104(Suppl 5):895–900 (1996)

Key words: biomarkers, susceptibility, medical ethics, exposure, disease risk

Introduction

The use of biological markers in the evaluation of disease risk has increased markedly in the last decade. Biomarkers are observable end points that indicate events in the processes leading to disease. They are particularly useful in the evaluation of progressive diseases that manifest their symptoms long after exposure to initiating factors. In such cases, traditional early warning symptoms of developing disease may be lacking. At the same time, the disease, once clinically apparent, may be essentially irreversible. Diseases such as cancer and neurological disorders are examples.

The Committee on Biological Markers of the National Research Council (*1*) developed a conceptual framework for biological markers (Figure 1) describing a continuum of events from exposure to the development of disease. For a slowly developing disease, the events through early biological effects can be detected at about the time of exposure. Because the later stages of the process, in which the clinical symptoms of disease emerge, may not appear for years, detection of earlier events can provide a valuable early warning of risk. Interventions to reduce the long-term risk, such as control of exposure, may then be made possible.

Biomarkers are generally classified into three groups; biomarkers of exposure, effect, or susceptibility (*1*). A typical biomarker of exposure would be the level of a toxic substance, such as lead, in the blood or urine of an individual. An effect biomarker is the biological response to an exposure. Somatic cell mutation in response to a mutagen exposure (*2*), or reduced cholinesterase activity in response to an organophosphate insecticide exposure are examples (*3*). Biomarkers of susceptibility measure innate or induced capabilities of the individual for response to exposure to an environmental toxicant. These might include the activity of specific enzymes involved in activation or detoxification of a specific chemical (*4*) or DNA repair capacity for specific types of DNA damage (*5*). Some biomarkers fall at the boundaries between these classifications. For example, a DNA adduct may be associated with a specific chemical, making it characteristic of a biomarker of exposure, but its formation may result from the metabolism, distribution, and reaction of the chemical with DNA, which reflects cellular processes. Thus, to a degree, it is an effect biomarker as well. Typically, exposure biomarkers are specific to the chemical producing the exposure, while effect biomarkers may be responses to any of a class of chemicals. Studies of populations with exposures to mixtures of chemicals should use combinations of exposure and effect biomarkers. An exposure biomarker alone may document exposure but give little information about its biological significance, while an effect biomarker alone may reveal an increased incidence of an adverse effect without documenting a cause.

In addition to providing an early warning of potential future adverse effects of exposure, biomarkers may provide useful information about the mechanisms of toxicity in human subjects. Since most biomarkers that can be used in human studies can also be observed in laboratory animals, experimental studies in animals can be used to study the mechanisms leading to the expression of biomarkers in ways that are not appropriate in most human studies.

Although the last decade has seen rapid advances in the development of methods for biological monitoring, there has been less progress in their application to the assessment of human exposures, particularly in the United States. Reasons for this include limited federal funding for research in this area, lack of access to populations for study, and unresolved concerns about legal and ethical issues that might develop from human population studies.

Here we identify four major needs for the continued development and applications of biological markers to environmental health research. First, new biomarkers need to be developed, or existing ones refined to fill gaps in the continuum of

This paper was presented at the Conference on Air Toxics: Biomarkers in Environmental Applications held 27–28 April 1995 in Houston, Texas. Manuscript received 24 May 1996; manuscript accepted 5 June 1996.

The work of Dr. Ward was supported by grants from the U.S. EPA (R820108) and the National Institute of Environmental Health Sciences (R01 ES06015). The research of Dr. Henderson at the Inhalation Toxicology Research Institute is conducted under contract DE-AC04-76EV001013 with the U.S. Department of Energy.

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Abbreviation used: SCE, sister chromatid exchange.

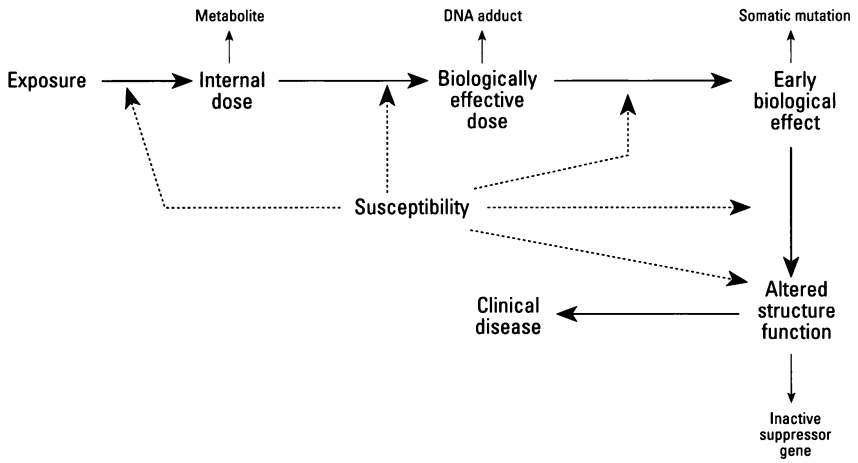

Figure 1. Biological markers and disease progression. A toxicant entering the body produces an internal dose as a consequence of its disposition and metabolism. Interactions of the agent or its metabolites with cells and macromolecules determine the biologically effective dose at a target tissue, which may result in biological effects. These may result in altered structure or function, which eventually leads to clinical disease. Events along the top row occur within a short time after exposure and may serve as useful biomarkers. Events along the bottom row may occur later and are usually not observable much before the occurrence of clinical disease. Examples of typical biomarkers are shown for specific events. Factors affecting individual susceptibility may impact any of the steps shown. From the National Research Council (*1*).

events from exposure to clinical disease expression. Second, the relationships of specific biomarkers to the pathophysiological mechanisms of disease must be better understood to more accurately estimate the risk of disease from evidence based on biomarkers. Third, biomarkers need to be better characterized with respect to their sensitivity, specificity, and variability, as well as validated as predictors of adverse health effects. Fourth, many issues related to the application of biomarker research to real-world situations need to be resolved.

Method Improvements

Biomarkers reflect discrete steps in the continuum of events leading from toxic chemical exposure to disease. To better understand the relevance of these events to disease risk, it would be desirable to define the continuum at a level of resolution as high as possible. There is a need for exposure biomarkers that identify a greater variety of chemicals and their metabolites. At this time such biomarkers exist for only a small number of chemicals. Better assessment of human exposures to toxicants will require measures of exposure to a greater variety of chemicals.

Biomarkers of effect are frequently surrogate measures for events on the exposure–disease continuum but are not on the continuum themselves. For example, somatic mutation biomarkers at the *HPRT* locus in lymphocytes (*6*) or the glycophorin *A* locus in erythrocytes (*7*) are used as early biological effect markers for risk of cancer. Although mutation is clearly a relevant event preceding cancer, neither of these genetic loci are mechanistically involved in the carcinogenesis process. In addition, the measurements are made in peripheral blood cells. While these cells may have some relevance for hematopoietic cancers, they may not be relevant for some of the other types of tumors. Ideally, mutations at genetic loci that are specifically related to neoplasia, such as suppressor genes, would be more relevant. In addition, it would be helpful if events in particular cell types that are relevant to malignancies of interest could be evaluated either directly or indirectly. These are difficult challenges, since no direct means for selecting or screening for rare mutants at such loci currently exist. In addition, relevant tissues are not easily obtained from human subjects in large numbers without unacceptably invasive techniques, such as biopsies. A potential approach to this problem would be to use the parallelogram approach described by Sobels (*8*). Accessible human biomarkers and their animal counterparts could be compared to determine the ratio of effects in the two species. A biological effect in the target tissue of interest in the experimental animal would be measured and used to estimate the effect in the human target tissue. Differences between humans and experimental animals in the disposition and metabolism of the chemical being studied would have to be taken into account. In many instances these differences may sharply limit the utility of the parallelogram approach. Research to develop biomarkers that are more relevant to specific diseases, and are observed in relevant target tissues, is a major need.

Existing techniques need to be refined and new ones developed to improve the accuracy, sensitivity, and efficiency of biomarkers. The volume of biological materials required by many current techniques limits their usefulness and restricts the numbers of tests that can be performed in combination on samples from a single specimen. Refinement of techniques to allow useful results to be obtained from a smaller sample would be helpful. In some cases, this might open additional tissues to observation by reducing the numbers of cells needed. Current tests are often complex and expensive to run. Changes that simplify and/or automate the more time-consuming aspects of these techniques would also be useful.

Application of Biomarkers to Risk Assessment

Because biomarkers represent steps on the exposure–disease continuum, they have potential applications in risk assessment. Biomarkers can facilitate the linking of exposure to given amounts of a chemical with the induction of specific health effects. Figure 2 illustrates some of the types of biomarkers that can help trace the steps from the initial exposure to the induction of an adverse health effect.

All of the chemical to which a person is exposed will not be absorbed; what is absorbed is the defined as the internal dose. Some of this dose may be excreted without resulting in health effects, some may end up in tissues that are not affected by the chemical, but some may reach a target tissue that is vulnerable to the chemical or its metabolites. Of the dose reaching the target tissue, some may not reach critical organelles, some may form noneffective macromolecular adducts, but some amount, the biologically effective dose, may reach a site that will result in biological alterations that may lead to disease.

The most useful markers of effect (Figure 2) are the earliest events on the pathway to the development of a disease state. Such markers may allow intervention in the disease process before it becomes irreversible. As in the markers of exposure, some of the early biological changes may

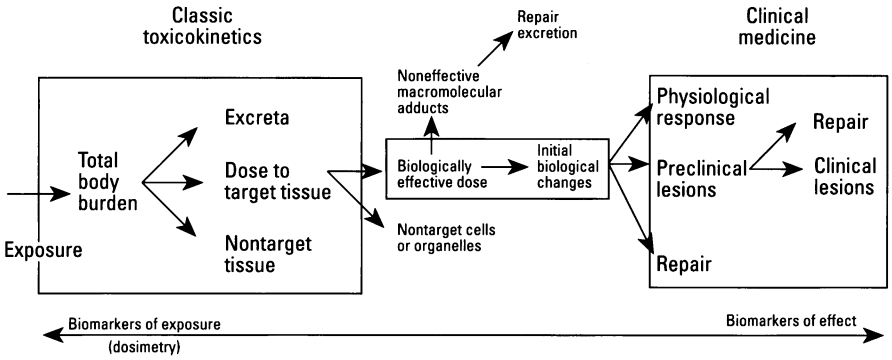

Figure 2. Application of biomarkers to risk assessment. The diagram represents the many paths a chemical may take after entering the body, leading to alternative fates including detoxification or injury and disease. The left box represents disposition of the chemical, the center box its metabolism and effects on target tissues, and the right box observable responses, some of which lead to clinical disease. Biomarkers of exposure predominantly detect events in the left box, while biomarkers of effect detect events in the center and right boxes. From Henderson (*20*), with permission.

not lead to disease, but instead are repaired. Some of the early changes induced by the chemical or its metabolites may not be on the pathway to disease, but are only physiological responses that are protective to the organism. Eventually, if there are enough changes that can result in disease and if the changes persist long enough without repair, a disease state may ensue.

Ideally, if one understood the quantitative, kinetic relationships between each of the markers shown in Figure 2, one could use the markers both to determine the degree of prior exposure and to predict the potential for adverse health effects from the exposure. Even markers that are not on the pathway leading to disease (such as hemoglobin adducts) can be useful if the quantitative relationship between these markers and the markers that are on the pathway is known. It is necessary to move past the stage of noting that markers such as hemoglobin and DNA adducts exist to determining the quantitative relationship between the amount of the adducts and the previous exposures as well as the probability of disease development. An example of the difference between a qualitative and a quantitative approach to biomarkers can be seen in the detection of ethanol on the breath of someone who has been drinking alcoholic beverages. If one is the parent of a teenager, one need only detect the smell of ethanol on the child's breath to take whatever action is required. If one is in the regulatory arena, however (i.e., a law enforcement officer) one must quantitate how much ethanol is in the person's breath and how that amount relates to inebriation before taking action.

The markers of exposure illustrated on the left side of Figure 2 are quantities that have long been studied in pharmacokinetics. Measures of the rate of formation of such markers following a given exposure and the rate at which they are cleared have led to the development of mathematical models that predict the level of the markers under different exposure situations. Figure 3 illustrates one approach to developing mathematical models for the disposition of chemicals in humans. First, the kinetic parameters are studied in animals and mathematical models are developed based on this kinetic information, plus physiological parameters such as blood flow rates, breathing rates, and other information as needed. Other important components of the model are the physicochemical properties of the chemical and its metabolites and how the agents partition between air and blood and between blood and tissues. When the animal model is complete, then the model can be modified based on human physiological parameters. Such models must then be validated with whatever data are available from humans accidentally or occupationally exposed to the chemical. Through such approaches, progress has been made in quantitating and modeling biomarkers of exposure.

The use of the biomarkers that are illustrated on the right side of the Figure 2 has been the purview of clinical medicine for centuries. Similar approaches to those used in the pharmacokinetic area could be used to determine the kinetics of disease processes to provide pharmacodynamic models of disease development. This type of research is only in its infancy and should be an area for future emphasis.

Perhaps the greatest need is for more mechanistic research on the early stages of disease development, as depicted in the center of Figure 2. One cannot have a marker of the biologically effective dose for a chemically induced disease if one does not know the mechanism of the induction. One cannot detect preclinical signs of disease development without knowledge of the early steps in that disease process. Thus, mechanistic research will undoubtedly be a major source of future biomarkers of the earliest stages of chemically induced adverse health effects.

At the current time, we often have more biomarkers than we can adequately interpret. For example, if we find chemical-specific DNA adducts in a person today we have no way of predicting what those adducts mean in terms of the probability of an impact on the future health of that individual. It is essential that we begin to fill in the blanks in the steps between exposure to chemicals and disease development so that biomarkers can become more useful in the risk assessment process.

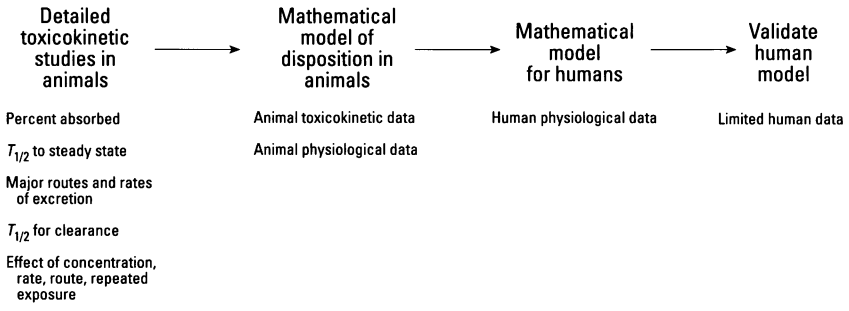

Figure 3. Use of animal toxicokinetic data in human risk assessment. This diagram depicts a scheme by which animal toxicokinetic data may be used in the development and interpretation of a human biomarker. The lower rows depict the types of data that may be generated at each step in the process. From Henderson et al. (*21*), with permission.

Characterization and Validation of Biomarkers

To be of practical utility in the evaluation of human population exposures to toxic agents, certain basic parameters of a biomarker must be known, and the value of the biomarker as a predictor of disease should be understood. In other words, to be useful a biomarker must be characterized and, to the extent possible, it must be validated.

The primary characteristics of concern for a biomarker are similar to those that are determined for most clinical laboratory procedures. Some adjustments must be made, however, to accommodate the fact that biomarkers are typically used to evaluate populations rather than individuals. Most biomarkers have a baseline response that is observed even in populations with no specific exposure to a toxic substance. Thus, DNA adducts are observed at some concentration in tissues from nonexposed subjects (*9*). The frequency of mutations at the *HPRT* locus in human lymphocytes has a baseline of one to two per million cells in the autoradiographic version of the assay (*10*) and a somewhat higher value in the clonal version of the assay (*11*). These "spontaneous" responses for biomarkers may result from the endogenous formation of chemicals detected by exposure biomarkers or the spontaneous occurrence of process such as mutation, which are detected as effect biomarkers. Alternatively, ambient exposure to low levels of chemicals that elicit biomarker responses may be responsible. In any case, the historical baseline response of a biomarker in a population with no unusual exposure to the toxic agent of interest should be known, and studies of populations potentially exposed to toxic agents should include an appropriate control or referent group.

As with clinical tests, the sensitivity and specificity of a biomarker assay are characteristics that must be known to determine appropriate circumstances for its use. Sensitivity is a fairly straightforward parameter to determine. Laboratory experiments with spiked samples and animal studies can be used to determine the analytical limits of detection of a biomarker. Finally, it is possible to obtain a reasonable estimate of the exposures of individuals or populations to the agent of interest by an independent method such as environmental sampling. The ability of a biomarker to respond to specific conditions of exposure to a particular agent can be easily established. To be of value, a biomarker must be sensitive enough to detect the exposures, or their effects, that are actually experienced by human populations.

The specificity of a biomarker is a more complex parameter to characterize and apply to hazard evaluation. By analogy with clinical laboratory tests, the specificity of a biomarker may be defined as the probability that it will not respond in a situation where a specific exposure does not occur. In general, exposure biomarkers are expected to be fairly specific. Since these biomarkers detect the actual internal exposure dose at a target tissue, they would not be expected to detect an exposure when none had occurred. On the other hand, effect biomarkers detect biological responses that occur after an exposure. In many cases these responses may occur after exposure to many different agents. For example, chromosome damage in lymphocytes is a well-documented response to many genotoxic agents including radiation, drugs, and environmental pollutants. Thus, as a biomarker, it has a rather low specificity. To be useful in assessing the hazards associated with a specific chemical or exposure scenario, an effect biomarker would not be useful unless the exposure experience of the study population was well characterized. The specificities of both exposure and effect biomarkers may be influenced by a variety of interfering factors. These might include lifestyle activities of the members of the study population, such as cigarette smoking or ethanol consumption, exposures to other agents in complex chemical mixtures that might occur in an occupational setting, or exposure to ambient pollutants in the environment. Lifestyle activities of pregnant women may even influence effect biomarkers in the fetus. For example, the cord blood lymphocytes of newborn infants of mother who smoked during pregnancy have elevated frequencies of *HPRT* mutant lymphocytes (*12*).

The consistency of response of a biomarker may be an important factor in its usefulness. If the normal variance of the biomarker response in a nonexposed population is small relative to the mean, significant increases in response will be easy to detect using small numbers of samples. A tight variance also suggests that the biomarker does not respond too strongly to unrecognized factors in typical populations.

The statistical power of a biomarker assay to detect reasonably sized changes in response is an important characteristic that must be known to design appropriate studies. Sensitivity, specificity, and consistency of response are important parameters in determining power. An assay that has a low and consistent baseline value and is sensitive enough to respond to an exposure with a change in response of an easily observed magnitude will be a useful biomarker. An assay that requires a large sample population or an unusually intense exposure will not be useful in evaluating exposures.

If a biomarker is to be considered valid, its ability to predict disease must be determined. Validation of biomarkers as predictors of health effects is a serious challenge. However, the question of real interest is, what is the relationship of exposure to disease risk? The diseases that biomarkers are intended to predict typically occur after latencies of many years. Effect biomarkers could be related to disease risk by carefully determining the response of the biomarker to exposure and then tracking the study participants over time to associate biomarker responses with subsequent health risk. This is a daunting task given the size of the population that would have to be evaluated and the time required to observe subsequent disease. Despite the difficulties, one such study has been conducted, the Nordic Collaborative Study (*13*). In this study chromosome aberration and sister chromatid exchange (SCE) frequencies were measured in workers in a variety of industries in the Scandinavian countries. The cohort has been followed for several years and cases of cancer identified. Using a nested case–control design, records of the earlier cytogenetic studies were compared in the cancer cases and suitable controls drawn from the cohort. A significant elevation in the odds of having increased chromosome damage but not SCEs was observed in the cases (*13*).

Dr. Richard Albertini has suggested a prospective study design that could efficiently validate biomarkers in a period of a few years. Cancer patients who receive alkylating agent therapy are at a significantly elevated risk of developing leukemias within 10 years or less following treatment (*14*). Blood samples could be obtained from patients before and soon after treatment, and the lymphocytes and other components could be cryopreserved for later analysis. As the cohort ages, patients who develop a second neoplasia could be identified and matched with patients who do not. Their cryopreserved samples could then be tested and the responses of the biomarkers determined for the case and control groups (*15*). This approach could be fairly efficient because exposures would be precisely documented,

and only a small percentage of the treated patients would be likely to develop a second neoplasia within 10 years. Effect biomarkers such as the assay for mutation at the *HPRT* gene could be evaluated. In addition, biomarkers of susceptibility could be characterized at the same time to determine their ability to predict risks from exposure to alkylating agents.

Implementation Issues

Two practical issues that must be addressed in order for the development of biomarkers to progress are reduction of cost and access to populations for research. Currently, biomarker development and characterization is a research activity. Typically, biomarker assays are time consuming and expensive. Analysis for DNA and protein adducts can cost more than $100 per sample, while tests requiring cell culture and subsequent analysis can cost several hundred dollars apiece. Since appropriate study designs may require combined assessment of two or more biomarkers, plus an exposure assessment, the direct costs of a study of a population of 50 subjects could cost $40,000 or more. At this price biomonitoring would not likely be attractive to industry as a routine monitoring tool. As biomarker techniques become more established, a significant research need will be to reduce their costs. This may be accomplished through modifications such as automation of assays or by reducing their complexity and the need for advanced technical skills to perform them.

The studies to develop, characterize, and validate biomarkers cannot be conducted unless populations are available to investigators. Much early work has been done with populations of patients exposed to toxic drugs or with volunteers who smoke cigarettes (*10,12*). Although such populations have been helpful, access to populations with well-documented exposures to toxic chemicals in the workplace are vitally needed to characterize biomarker assays. Many studies have been carried out in populations in developing nations or in Eastern European Bloc nations where occupational exposures to carcinogens are currently relatively high (*16,17*). Although studies of highly exposed populations in developing countries are useful in method development and mechanistic inquiries, the only way to address the issues of occupational health in typical United States workplaces is to conduct studies in this country. Studies in the United States require cooperation of both labor unions and employers. Without such cooperation, studies are hampered by limited access to facilities, restricted methods of exposure assessment, and potential misclassification of workers into inappropriate exposure groups. Excellent examples of such cooperation can be noted. One is the landmark study of the cytogenetic effects of ethylene oxide exposure conducted with the cooperation of Johnson and Johnson (*18*). A second is the participation of Texaco Chemical Company in studies of 1,3-butadiene exposure (*2,19,22*).

There are legitimate ethical and legal issues that inhibit employer participation in the development of biomonitoring. These issues, including how to explain results to participants and the legal ramifications that may result for employers, have been discussed from the beginning of the use of biomarkers in human studies. These issues are resolvable. As the value of biomarkers and the need for them become increasingly obvious, industry, labor, and the research community need to communicate to develop guidelines for biomarker use and to plan studies to bring biological monitoring into the modern American workplace.

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