Biomarkers in environmental toxicology: state of the art.

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Biomarkers in Environmental Toxicology: State of the Art

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Together with many other research-based disciplines, environmental toxicology has profoundly benefited from recent advances in molecular biology and analytical chemistry. As a result of these accomplishments, clinical and experimental studies of biomarkers have developed into a multidisciplinary research area. The IFCC has convened two conferences: to review biomarkers of chemical exposure, in the first conference, and biomarkers of effects and of susceptibility, in the second. As in the first conference, the second meeting included 16 plenary presentations, all of which are published in this special issue of *Clinical Chemistry*. Ninety posters were selected for display at the conference and, on the basis of the quality of the poster abstracts, the Organizing Committee chose 13 of these for publication as extended abstracts in this issue. The present overview is a summary of controversies and conclusions that emerged during discussions at the roundtable discussion sessions.

Biomarkers of Complex Effects

Environmental toxicology deals with complex interactions between thousands of environmental chemicals and a multitude of potential adverse effects. When a single xenobiotic has different targets, a biomarker may exist that reflects the integrated response of the organism. However, a particular marker is likely to represent only one individual toxic pathway. Nonetheless, the expression of early changes in one organ system could well correlate with changes in other systems. Thus, the accumulation of zinc protoporphyrin in erythrocytes during increased lead exposure is due to toxic effects on heme biosynthesis, but it may also represent a useful biomarker of overall toxicity caused by the exposure. Unfortunately, examples of such biomarkers are few, and it is not likely that research in this field will be as productive as efforts to obtain an improved understanding of mechanisms of toxicity.

Different chemicals may have the same target, the best known example from environmental toxicology probably being acetylcholine esterase. In this case, changes in the biomarker will represent the integrated response to a possible array of exposures that act on the same target. In a more general sense, actions caused by individual xenobiotics at the molecular level may trigger a cascade of biochemical and physiological effects, and some of the reactions may be common to different etiological agents. Biomarkers of the integrated exposure or integrated response may then be developed. Relevant examples include carboxyhemoglobin in blood as a biomarker of exposure to carbon monoxide or methylene chloride, urinary dialkylphosphate excretion after exposure to common organophosphorus pesticides, and urinary mercapturates as a marker of exposure to certain electrophilic compounds. However, the biochemical mechanisms must be understood to allow proper interpretation of this type of information. Some such integrated biomarkers may be too far down the toxic chain reaction to be useful for preventive purposes. For example, a general toxic effect is the intracellular accumulation of calcium that precedes cell death. Although probably a common event in many toxic reactions, its usefulness as a biomarker is limited by serious cellular damage having already occurred by the time a significant change can be detected. Research in this area needs to be closely linked to studies in mechanisms of action so as to explore more effectively the potentials for development of new biomarkers.

The hypothesis regarding estrogenic and other hormonal effects of environmental chemicals has attracted much interest recently. Although such effects have been demonstrated in experimental studies and in some wildlife studies, their significance for human health is still a matter of controversy. Nonetheless, this research area is very promising and has stimulated the development of new techniques. Serum hormone concentrations, e.g., of testosterone, estradiol, and prolactin, may be useful as biomarkers. Other hormonal effects may also occur because of interaction with hormone receptors or effects on the metabolism of individual hormones.

Genetic Biomarkers

Significant advances have been made in the development of sensitive and specific DNA-based and other genetic assays, as detailed in papers presented at this conference. Extremely sensitive assays of DNA and protein adducts are now available as biochemical markers. Progress has also been made toward the development of biological marker assays of accumulated gene-specific and cell-specific adducts. Some of these assays seem to have reached the stage of applicability in human biomonitoring studies. Further development is needed with regard to assays for nonphenotypically selectable mutations.

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This new technology allows determination of extremely small alterations at the cellular level, but the significance of most of these changes is unknown. Although such changes may be unwanted, they are not necessarily hazardous. The range of normal variation is often incompletely known, and the validity of a reference interval, if at all available, must be carefully considered. Timing or duration of exposure and timing of sampling may affect the biomarker result. Inter- and intraindividual variations depend on a spectrum of determinants such as other exposures, circadian rhythm, ethnicity, and constitutional factors including age and sex. Hence, macromolecular adducts are a good measure of exposure at the level of the individual, but their utilization in the context of determination of risk is problematic.

A major problem to be considered is that between DNA damage and carcinogenesis lie important steps that can significantly affect the dose–response relations, e.g., cell proliferation (mitogenesis) and "promotion." Better conceptual tools are needed for the incorporation of biomonitoring data in the risk assessment process as well as for the development of methods to observe and quantify markers of the different stages of carcinogenesis.

Accordingly, we now have at our disposal powerful methodologies for measuring biomarkers that reflect the biologically active dose in humans. Such adduct and mutation methodologies should be applied in the context of well-designed population studies that must (*a*) fulfill strict epidemiological criteria for quality and efficiency and (*b*) include multiple endpoints, as well as a range of additional biomarkers, such as measures of the internal dose (e.g., urinary metabolites), and genetic variations of metabolic capacity. In this way, applications of currently available methodologies in well-designed studies will shed important light on the needs for future technological developments and should be regarded as a high research priority.

In addition, with better knowledge concerning genetic heterogeneities, biomarkers of genetic hypersusceptibility are being developed. Much of the research in this area has focused on classical phase I or phase II metabolic enzymes, and a considerable number of polymorphisms have been already characterized. These genotypes affect the metabolism of xenobiotics and thereby the associated risk of adverse effects. Additional heterogeneities are likely to be discovered during the next few years, and simple assays may well be developed to determine individual sensitivity to many specific environmental exposures.

Application for Prevention

New biomarkers are urgently needed as an integral tool in preventive medicine and environmental health. Biomarkers can range from the very simple ones, such as a blood hemoglobin measurement, to very complex and expensive determinations, e.g., of DNA adducts. The human genome project is likely to lead to the development of simple and practical tests that will be useful, especially for monitoring exposures to carcinogens and for assessing the carcinogenic and other toxic risks to individuals. This technology should allow the healthcare system to become more involved in disease prevention.

For the assessment of individual risk from biomarker results, the positive predictive value of the test needs to be determined. An acceptable level for the positive predictive value of a test will depend on the action that would follow from the test result. For example, if a positive test result would lead to an intervention to prevent an outcome that is relatively low-risk, then, relatively speaking, a lower positive predictive value for the test may be acceptable. Conversely, if a positive test result would lead to an intervention to prevent an outcome that is high-risk, the test would need a high positive predictive value to be acceptable. The negative predictive value of the test should also be considered, because if it is high (regardless of the positive predictive value), it can be reassuring for an individual with a negative test result. Unfortunately, while carcinogenic risk is of great clinical importance, no single marker among the many assays available is likely to be of general value. Nonetheless, biomarkers with limited usefulness in the prediction of individual risk may still be useful for population studies.

The question of reference data must also be considered. As most biomarkers have been developed only recently, data on unexposed populations are rarely available for comparison. An individual may be his or her own reference, if comparable data are available both before and after the exposure occurred. In a monitoring program, the frequency with which a biomarker is to be determined must be considered also from an economic viewpoint, as the cost of primary prevention may be less than an extensive surveillance program.

Biomarkers are routinely used to monitor exposures in occupationally exposed populations. Administrative limits, sometimes called biological exposure limits, have been determined for the interpretation of such measurements. These limits are based on accumulated knowledge and obviously cannot take into account risks yet unknown. Not surprisingly, the limits used in different countries, e.g., for exposures to lead and certain solvents, have generally been lowered during recent years, usually because of better documentation of toxic effects. Hypersusceptible subgroups may exist, and their need for special protection ought to be taken into account. If they cannot be safeguarded by the designated exposure limit, then other protective means must be adopted, while avoiding discrimination and stigmatization. Unfortunately, occupational exposure limits are of limited use with regard to environmental exposures, which are likely to be longer term and may affect large subgroups with decreased resistance against toxicity. Moreover, the occupational exposure limits are of little use for diagnostic purposes or inter-

pretation of new or unsolved problems such as multiple chemical sensitivities.

Biomarkers may also help in improving regulatory risk assessment by validating presumptions of acceptable (or unacceptable) allowable levels of exposure. For example, the finding of a biomarker with a high positive predictive value for the outcome of concern in a population with exposures below the allowable level (as well as in populations with exposures above the allowable level) would indicate that the allowable exposure level is not as protective as assumed.

In applying biomarkers in environmental health and preventive medicine, ethical issues must be addressed. The sampling procedure, the storage and analysis of the sample, and the reporting and interpretation of the result all raise potential ethical problems. The outcomes of the genome project will emphasize these issues, especially with regard to privacy and protection against discrimination. The practices seem to vary between occupational health and environmental health, from country to country, and between different cultures. Although no definite answers can be provided, a heightened awareness is needed, and solutions must be found on the basis of cooperation between responsible scientists and the stakeholders involved.

Research Recommendations

Although not necessarily agreed by all participants in the meeting, the following conclusions derived from the discussions at the Cannes conference:

1) Basic research into mechanisms of action, such as interaction of environmental chemicals with hormone receptors, and progression from mutation to cancer, is needed to allow development of biomarkers and appropriate interpretation of the results.

2) Biomarker research in laboratory models is needed to illuminate findings in humans. Extrapolation across species should be done with caution. For example, the caffeine test for CYP1A1 expression is a good marker for enzyme induction in rats, but not in humans, and the Ah receptor from various species shows different affinity characteristics.

3) Given the large number of chemicals in environmental exposures, biomarkers that reflect an integrated effect on the body or an organ system would be useful, but this research approach may not be very fruitful because of the intrinsic complexities.

4) Although DNA-based biomarkers have been developed and refined considerably during recent years, methodological development is especially needed with regard to accumulated gene-specific and cell-specific adducts and nonphenotypically selectable mutations.

5) Most of the currently available biomarkers have not been evaluated in detail with regard to their validity for practical applications in exposed human populations, and appropriate reference intervals are generally not available. Special efforts in this area are therefore urgently needed to allow interpretation of the data.

6) Appropriate human biological samples from well-defined populations should be systematically stored for future studies by analyses not necessarily developed as yet and to enable studies of the relation to disease outcomes in the future. National and international agencies should cooperate to facilitate such work.

7) Applications of biomarkers are likely to be useful in risk assessment efforts, and a strengthened research program on the development and application of biomarkers is therefore appropriate.

8) While we recognize that the solutions may be complex, we must address ethical issues in all biomarker research, and we must resolve potential conflicts related to application of biomarkers in an open forum.

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