Biomarkers of chemical exposure: state of the art.

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Biomarkers of Chemical Exposure: State of the Art

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Environmental toxicology has important implications for human health, and clinical chemistry is perhaps the medical discipline most closely related to this area. In June 1993, the International Federation of Clinical Chemistry organized, with the support and involvement of Beckman Instruments, Inc., the First Arnold O. Beckman/IFCC European Conference on Environmental Toxicology. The topic for this conference was biomarkers of chemical exposure, a burgeoning multidisciplinary research area. "Biomarker" is short for biological marker, used in environmental toxicology and related fields to denote a measurement that reflects an event in a biological system, such as the human body.

The meeting included 16 plenary presentations, all of which are published in this special issue of *Clinical Chemistry*. Sixty posters were selected for exhibition at the conference, and the organizing committee selected 10 of these for publication as extended abstracts. This overview is intended as an introduction to the field and as a summary of conclusions and controversies that emerged during discussions in plenary and at six round-table discussion sessions.

Types of Biomarkers

Usually, three specific types of biological markers are identified (*1*). The first is a biological marker of *exposure*, or exposure biomarker, that may be an exogenous compound (or a metabolite) within the body, an interactive product between the compound (or metabolite) and an endogenous component, or another event related to the exposure. The second, a marker of *effect*, may be an endogenous component, or a measure of the functional capacity, or some other indicator of the state or balance of the body or organ system, as affected by the exposure. Such effect markers are generally preclinical indicators of abnormalities. The third is a marker of *susceptibility*, whether inherited or induced, which serves as an indicator that the individual is particularly sensitive to the effect of a xenobiotic or to the effects of a group of such compounds. Although the three types of biomarkers may not always be easily separated, this overview will mainly address issues related to exposure biomarkers.

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The ideal exposure biomarker should have the following characteristics: (i) sample collection and analysis are simple and reliable; (ii) the biomarker is specific for a particular type of exposure with a clear-cut relation to the degree of exposure; (iii) the biomarker reflects a subclinical and reversible change only; (iv) relevant intervention or other preventive effort can be considered, if indicated by the biomarker result; and (v) use of the biomarker is regarded as ethically acceptable.

Research on biomarkers evolved mainly from studies in environmental and occupational toxicology. Exposure biomarkers first became useful tools in biological monitoring, especially in occupational health surveillance. Many biomarkers also became important predictors in epidemiological studies. Nowadays, biomarker studies reflect a burgeoning research area at the crossroads of several disciplines, including clinical chemistry and preventive medicine.

Biomarker research in general either attempts to provide further validation for a specific measurement or explores the potential fields for application of the biomarker.

Validity of Exposure Biomarkers

When assessing the usefulness of a particular exposure biomarker, one must consider three aspects of validity: analytical, toxicokinetic, and health risk.

Analytical

For optimal analytical quality, standardization is needed, but the specific requirements vary considerably between individual toxicants. Major areas of concern include: preparation of the individual; sampling procedure and sample handling; and measurement procedure that encompasses technical factors, such as calibration and quality assurance procedures, and individual-related factors, such as education and training of operators.

For documentation of analytical validity and traceability, there are global needs for certain matrix reference materials with concentrations of toxic substances or relevant metabolites at appropriate levels. For example, reference materials for measurements of solvents in blood or exhaled air are totally lacking. The importance of speciation has to be recognized in designing certain reference materials, e.g., the different forms of arsenic and the metabolites of xylene.

At the same time, the economics of characterizing and using reference materials to supplement quality-assurance procedures in general must be considered. Thus, the achievable quality of results, and the uses to which they are put, have to be balanced against the added costs of

quality assurance, including reference materials, manpower, and instrumentation. One possibility is to generate a short list of priority biomarker analyses for which recommendations for appropriate quality-assurance procedures and reference materials should be developed.

For biomarkers to be used in population studies or for diagnostic purposes, the participating laboratories must have well-documented analytical procedures with defined performance characteristics and accessible records to allow verification of the results. For this purpose, national and international organizations should generate and implement guidelines for internal and external quality-assurance procedures to be used by all laboratories generating data for assessing human health risks, including exposure biomarkers. Such guidelines could be based on documents already available from international associations, professional societies, and governmental agencies.

Modern methods of analysis may allow separation of isomers or congeners of organic compounds and determination of the speciation of metal compounds or isotopic ratios of certain elements. Such advanced techniques will no doubt gain importance for applications in biomarker studies, and the requirements for documentation of analytical validity are likely to be even more demanding than at present.

Scientific and clinical journals can accelerate the process of development and application of well characterized methods by accepting manuscripts only if they give sufficient and satisfactory data on the reliability of the biomarker measurements.

Toxicokinetic

Toxicokinetic principles must be applied to define the proper timeframe(s) represented by the biomarker, i.e., the extent to which the biomarker measurement reflects past exposure(s) and accumulated body burden. The degree to which the biomarker indicates retention in specific body compartments should also be considered. Peripheral blood is generally not regarded as a compartment as such, although it acts as a transport medium between compartments.

Life events, such as reproduction and senescence, may affect the distribution of a xenobiotic. Slow release from a "deep" compartment that has accumulated the chemical substance over a long time period may result in a protracted "endogenous" exposure of target organs. Studies in animals have been used to reveal toxicokinetic patterns, but extrapolation to humans may require confirmatory studies, e.g., in human volunteers.

Health Risk

For proper interpretation of the measurement result of an exposure biomarker, the diagnostic validity must be known, i.e., the translation of the biomarker value into the magnitude of possible health risks. In this area, metals serve as a paradigm for biomarker research. Recent research has demonstrated the complexity and subtlety of dose–response relationships, with considerable difficulty in identifying no-effect levels and therefore also in defining tolerable exposures. However, this kind of research has also illustrated the types of investigation and the refinements that are necessary to uncover the relevant information. For most organic compounds, quantitative associations between exposures and the corresponding adverse health effects are not yet available; in many cases, even the primary target organs are not known precisely.

Evaluation of toxicity data and biomarker concentrations in human studies is often complicated by exposure to mixtures of substances. Although studies in animal models may provide some guidance in this and other respects, extrapolation to human toxicity must take into account the species differences in kinetic parameters, metabolic pathways, and susceptibility.

Adduct formation between xenobiotics and macromolecules is a promising research area, but the analytical methods are new, expensive, and need further development and validation. They are useful for occupational monitoring but not yet ready for applications to environmental biomonitoring. Simpler analytical methods, e.g., based on immunological techniques, are needed so as to reserve current methods as reference procedures. Especially at low levels of exposure, concurrent exposure to tobacco smoke or other confounding factors may have a significant impact on the measurement results, causing severe difficulties in interpretation.

Application of Exposure Biomarkers

Given an acceptable degree of validity, exposure biomarkers may be used for several purposes. On an individual basis, a biomarker (usually an exposure biomarker) may be used to support or refute a diagnosis of a particular type of poisoning or other chemically induced adverse effect. In a healthy subject, an exposure biomarker may also serve as a basis for risk prediction and counseling. Other types of biomarkers may reflect individual hypersusceptibility to specific chemical exposures; such biomarkers should be used preventively rather than after the event. On a population basis, some biomarkers of exposure can be applied to assess the extent of compliance with pollution-abatement regulations or effectiveness of preventive efforts in general. The problems and research needs are well illustrated by some concerns specifically relating to the use of human milk for biomarker assays.

Contaminants in Human Milk

Among contaminants excreted in human milk, the persistent compounds have raised special concerns. However, although the concentrations may be higher than in other food items, the concentrations found in milk should be compared to the degree of transplacental transfer and to the total lifetime exposures from other sources. Nevertheless, the rapidly developing infant may be particularly vulnerable to toxic damage. Thus far, only limited information is available on metal concentrations in milk, particularly methylmercury. Many studies are of limited value because risk factors were not described, and the sampling and analytical protocols were not properly documented. For comparison purposes, infant formulas should be characterized with regard to their content of major chemical contaminants.

Human milk is not suitable for monitoring exposure levels or body burdens in the general population, but analyses of milk contaminants can be useful to determine the exposures incurred by nursing infants. Monitoring cannot be recommended as a basis for individual counseling about breastfeeding, but in some circumstances, women may be advised during pregnancy and lactation not to consume certain food items, such as fish from contaminated lakes.

The amounts of chemicals present in human milk are affected by many factors, including sampling techniques and maternal characteristics. To obtain the most reliable result, one should collect samples of mature milk 6–8 weeks post partum. Milk for analysis should be collected during the second half of a breast-feeding session to minimize the variability. Concentrations of lipid-soluble chemicals should be quantified on a lipid basis. The following information should be collected from each mother: age, parity, body mass and height, change in body mass during pregnancy and since parturition, life-style factors (diet, smoking, alcohol use), socioeconomic status, and frequency and duration of breastfeeding. Because human milk may contribute multiple chemical exposures, biomarkers of integrated effects would be of particular use to assess the total effect of exposure.

Priority Areas for Research

The foregoing considerations regarding validity suggest several areas of importance for biomarker research. While further documenting the advantages of exposure biomarkers, the limitations of this approach must also be kept in mind. Exposure biomarkers may not be at all useful for some chemicals that are short-lived in vivo; other means of exposure assessment should be developed. The route of exposure may also affect the biomarker measurement result and its interpretation. For example, direct exposure of the central nervous system via uptake by the olfactory nerve is likely to escape detection by measurement of exposure biomarkers in body fluids. These and other limitations need to be elaborated.

The need for standardization is obvious with regard to validation of biomarker analyses, but this need is much less clear-cut with epidemiological issues. Important concerns include whether the individuals examined are representative of the exposed population, and whether data on exposure predictors and health outcome measures are accurate. Other general standardization needs include protocols to interpret measurement results and guidelines for deciding subsequent action.

For the purposes of long-term epidemiological studies, tissue banks would be useful so that samples can be retrieved for analysis of exposure biomarkers at a time when health outcome measures are known and when more sensitive detection systems and methods may be available.

Application for Prevention

For preventive purposes, maximum allowable limits for exposure biomarkers may be useful. Such limits should be based on the best advice of clinicians and scientists from appropriate disciplines, and responsible administrators as "risk managers" should then take into account relevant ethical, social, cultural, and economic factors. The scientific basis should, if possible, include dose–response relationships supplemented by information on variations in susceptibility within the population at risk. In some countries, workers and members of the general public are involved in the standard-setting process and provide important input, particularly when scientific uncertainty is considerable. One of the major uncertainties is how to define an adverse health effect that should be prevented, e.g., whether adduct formation as an exposure biomarker represents by itself an adverse effect (i.e., effect biomarker) that should be prevented. Difficult questions are likely to arise when one is deciding whether it is ethically defensible, for the same compound, to have different limits for adventitious exposure on the one hand and for occupational exposure on the other.

Ethical Constraints

Our production of scientific data has greatly outdistanced our understanding of the needs to be satisfied when disseminating and utilizing this information in the framework of public health. This inconsistency relates in particular to the use of biomarkers in monitoring and surveillance studies.

The information generated by the use of biomarkers should generally be conveyed to the individuals examined within the physician–patient relationship. Ethical concerns in particular must be considered in connection with highly experimental biomarker analyses that cannot currently be interpreted in detail in terms of actual health risks; e.g., for the general population, little guidance can be given at present with regard to interpreting effects of exposure biomarkers other than the blood-lead concentration. Also of importance is the confidence in the data generated, i.e., whether appropriate sampling has been done, and whether sound quality-assurance procedures have been used in the laboratory involved. An additional area of concern relates to individual hypersusceptibility. These issues must be taken into account when one is providing feedback from the study.

All sectors of society affected by, or concerned with carrying out, a biomarker study need to be involved in the decisionmaking process on how to handle the information generated by the study. Specific procedures to prevent or overcome inevitable ethical conflicts should be developed within the legal and social frameworks of the region or country. However, each situation represents a different set of questions and pitfalls, and no single procedure for public involvement can be developed to cover all applications of exposure biomarkers.

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