Application of Biomarkers in Cancer Epidemiology
Toniolo, P., Boffetta, P., Shuker, D.E.G., Rothman, N., Hulka, B. and Pearce, N., eds
IARC Scientific Publications No. 142
International Agency for Research on Cancer, Lyon, 1997

Transitional studies

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Transitional studies are studies using biological markers that bridge the gap between laboratory experiments and population-based epidemiology. The goal of these studies is to characterize and validate biomarkers and to assess the following: intra- and inter-subject variability; the feasibility of marker use in field conditions; confounding and effect-modifying factors for the marker; and mechanisms reflected by the biomarker. Another goal is to optimize the conditions for the use of biomarkers. Transitional studies involving biomarkers of exposure or effect are distinguished from etiological studies because the biomarker is generally the outcome or dependent variable. Despite this difference, transitional studies can be epidemiological studies, but they may also include laboratory studies to assess reliability (and accuracy) and to identify parameters for collecting, processing and storing biological specimens prior to assay. Generally, transitional studies involve healthy people, patients or workers with specific exposures. At some point in the validation of a biomarker the line between transitional and etiological studies becomes blurred. None the less, it is useful to identify transitional studies as a distinct set of efforts to validate and characterize biomarkers. Transitional studies can be divided into three functional categories: developmental, characterization and applied studies.

Transitional studies have been described as studies using biological markers that bridge the gap between laboratory experiments and population-based epidemiology (Hulka, 1991; Schulte, 1992; Schulte *et al.*, 1993; Rothman *et al.*, 1995). The goal of these studies is to characterize and validate biomarkers and to assess the following: intra- and inter-subject variability; the feasibility of marker use in field conditions; confounding and effect-modifying factors for the marker; and mechanisms reflected by the biomarker. Another goal is to optimize the conditions for use of biomarkers. Transitional studies involving biomarkers of exposure or effect are distinguished from etiological studies because the biomarker is generally the outcome or dependent variable. Despite this difference, transitional studies can be epidemiological studies, but they may also include laboratory studies to assess reliability (and accuracy, if possible) and to identify parameters for collecting, processing and storing biological specimens prior to assay. Generally, transitional studies involve healthy people, patients or workers with specific exposures. At some point in the validation of a biomarker the line between transitional and etiological studies becomes blurred. None the less, it is useful to identify transitional studies as a distinct set of efforts to validate and characterize biomarkers.

Transitional studies can be divided into three functional categories (Table 1): developmental, characterization and applied studies (Schulte *et al.*, 1993; Rothman *et al.*, 1995). There is no clear demarcation between the types of transitional study, but the categories are useful in identifying the clustering of preparatory research that is needed before a biomarker is ready for population research. All three types of transitional study are efforts to determine aspects of the validity of a biomarker.

Transitional studies use epidemiological methods in the development, testing and validation of biomarkers. These studies represent preparatory efforts to determine the parameters, limitations or characteristics of a biomarker prior to its use in etiological, prevention or other kinds of intervention efforts. Transitional studies build on scientific knowledge from various types of laboratory studies, including tissue culture and animal studies. The conduct of transitional studies is not a one-time step in the development of biomarkers but can represent steps in an iterative process. Thus a biomarker may be developed in the laboratory, validated in a transitional study using epidemiological methods, and then applied in an etiological study that raises new questions that stimulate new laboratory and transitional studies.

Table 1. Types of transitional studies

Type	Description
Developmental	Builds on laboratory and *in vitro* animal studies and involves the test of assay in humans
Characterization	Examines the variability of biomarkers in population subgroups and factors that could confound associations between markers and what they represent
Applied	Assesses the relationship between a marker and an underlying event such as an exposure, a biological effect or susceptibility

Overview of the validation process

Validation of biomarkers involves the clarification of factors that influence the ability of the marker to predict exposure or outcome in a test population. The issues covered here are discussed by Schulte & Perera (1993) and Perera & Mooney (1993). During the validation process, evidence is weighed to determine whether a biomarker measures what it claims to measure, be it exposure, disease or susceptibility. A more epidemiological definition is that a biomarker is valid to the extent that it measures the true marker populations, that is, with no measurement error (see White, this volume).

As shown in Table 2, validation can be considered to be a two-stage process, although many of the steps require iteration as new information becomes available. The ultimate goal of the validation effort is to allow biomarkers to be used in appropriate epidemiological and clinical applications, which will require a selection of the best biomarkers based on the criteria described below.

The first step is laboratory validation, in which the shape of the dose–response curve, low-dose sensitivity (the ability to detect the exposure at levels low enough to be of biological interest), exposure specificity, and reproducibility of the assay are tested. In the second step, known as epidemiological validation, the population sensitivity and specificity, intra-individual and interindividual variation in response and persistence, predictive value, and biological relevance and feasibility are evaluated.

In this validation process, pilot studies in high-dose groups are useful. Molecular epidemiological studies to monitor the carcinogenic potential of

Table 2. Criteria for molecular epidemiological validation of biomarkers

Laboratory	Epidemiological
Dose–response curve	Population sensitivity and specificity
Detection limit or low-dose sensitivity	Intra-individual variation over time:
Exposure specificity	a. without altering exposure
Reliability of the assay:	b. when exposure is removed (persistence/half-life) or changed
a. from run to run	Inter-individual variation:
b. from day to day	a. response to a given exposure
c. from one laboratory to another	b. persistence of biomarker
Optimal conditions for sample collection, processing and storage	Half-life in surrogate tissues
	Positive predictive value (yield of high-risk people)
	Feasibility:
	a. amount and availability of tissue
	b. cost
	c. time required for each assay
	Biological relevance to disease

Source: Perera & Mooney, 1993

industrial chemicals in workers have been a useful approach to validation because occupational exposures tend to be higher and more controlled than ambient exposures (Perera & Weinstein, 1982; Perera & Santella, 1993). Initial research linked a number of occupational exposures to increases in adducts, mutation and other biomarkers in workers. State-of-the-art studies have broadened to include assessment of smoking and dietary factors (e.g. vitamins, fat, oxidative damage), inherent factors such as genetic predisposition to biotransformation and repair capacity, and endogenous levels of enzymes and promoters.

Interdisciplinary collaboration

Effective collaboration between laboratory scientists and epidemiologists is critical in conducting transitional studies. These studies involve the first field testing of the markers and they require both laboratory and epidemiological expertise. The interaction of different disciplines requires that attention be paid to the underlying assumptions, paradigms and language of the various disciplines. Epidemiologists generally speak in terms of groups and risk to groups. Laboratory scientists tend to focus on individuals or components of an individual. Epidemiology is an observational science whereas laboratory disciplines use controlled experimental designs. Epidemiologists and laboratory researchers use the same word to mean different things. For example, when laboratory researchers speak of a valid marker, they are referring to the characteristics of the assay for the marker, whereas when epidemiologists speak of a valid marker, they are generally referring to one with a high predictive value or correlation with exposure or disease.

The scale of transitional studies may be a hindrance to effective collaboration. Laboratory research generally takes place on a small scale, whereas epidemiological studies can produce vast quantities of specimens. Even in a study that is small by epidemiological standards, e.g. of 30 people, the volume of specimens is often more than the research laboratory is used to processing. The laboratory workers can easily feel that they are not engaged in true scientific research but are merely providing a service. Meanwhile, the epidemiologist wonders why the laboratory is not able to handle the volume of specimens required for the study. (Wilcox, 1995).

Another hindrance to good interdisciplinary collaboration is the tension between assay consistency and assay improvement (Rothman, 1993; Wilcox, 1995). The relatively long term for epidemiological studies may span a period when improvements are made in the assay. The laboratory collaborators will naturally want to take advantage of the improved assays and incorporate them in the study without consulting the epidemiologist. This can be disastrous from the epidemiological point of view. The use of two different assay methods can ruin an epidemiological study. As new and more reliable assays are developed, scientists will be persuaded to replace previous essays. When a marker assay is new, measurements may differ from those in previous assays for the same marker. Although it may be reasonable to wait until laboratory techniques and estimates of variability display consistency, it is not reasonable or feasible to wait until a technique is so standard that no refinement is likely to occur. However, before modifying a technique during the course of a study, the old versus new results must be carefully evaluated for overall comparability.

Developmental studies

Reliability

When a candidate biomarker is identified in the laboratory, some very basic issues need to be solved before it can be considered for use in population studies. The first priority in evaluating a marker for use in population studies is to determine its reliability or reproducibility. As long as the assay is reliable, the ordering of subjects by the measure is preserved (Rothman *et al.*, 1995). Hence, there will be consistency within a study. Since this is all that is required for studying a marker–disease relationship, reliability and not accuracy is of primary importance (Rothman *et al.*, 1995). Reliability can be assessed optimally through analyses of blind replicate human samples that are representative of a range of values likely to be found in human populations.

Reliability encompasses both unsystematic random laboratory variation observed in repeated measurements and bias caused by non-random variation (Vineis *et al.*, 1993). To assess random error, multiple measurements are needed. Obviously, the random error in the arithmetic mean of several measurements is smaller than the random error of

an individual measurement. Quantitative indices of the extent of random variation of a biological marker can be used to determine whether the reliability of a given measure is sufficient for the purpose being considered. The two most common indices are the standard error of the measurement and the reliability coefficient (Massey, 1986).

Analysis of random laboratory variation involves several steps. First, multiple analytical measurements of the same biological specimen (or several biological specimens from the same individual at the same point in time) must be made to estimate the variability due to random analytical errors. This fraction of the total random variability of a biological marker is usually minor. Second, multiple measurements of a marker must be made for one individual over time to estimate the intra-individual temporal variability. Third, multiple measurements across different individuals must be made to estimate interindividual variability in the value of the marker. In the second and third cases, random error is a component of the variability but systematic errors may also contribute (e.g. circadian cycles in the value of a marker or differences among individuals due to genotype). Most molecular epidemiological research using biological markers seldom requires large numbers of individual measurements. Thus, a small number of individuals can be used as a sample of the infinitely larger population to which the distribution refers. The standard error indicates how the mean of that sample is distributed around the mean of the larger population. Hence, the standard error of the mean reflects the reliability of the sample mean as an indicator of the population mean (Massey, 1986). This value may not be as informative as the reliability coefficient for evaluating markers to be used in epidemiological studies.

The reliability coefficient is technically known as the intraclass coefficient of reliability (Shrout & Fleiss, 1979; Fleiss, 1986) and ranges from 0 to 1. If each measurement is identical, the intraclass coefficient is 1.0. The greater the variation among measurements, the lower is the reliability. Fleiss (1986) has evaluated the impact of unsystematic bias variation in measurement, described the problematic consequences of unreliability, and recommended how unreliability can be controlled. The consequences described by Fleiss (1986) include the need to increase sample size to reduce unreliability; the high rates of misclassification in studies of the association between exposure and disease; and the consequent underestimation of the association between a health measure and the measured extent of exposure to an environmental risk factor. All these factors pertain to studies using biological markers of exposure or effect. Fleiss (1986) recommends that unreliability be controlled by conducting pilot studies and replicating measurement procedures on each study subject.

Another aspect of developmental transitional studies is to define the optimal conditions for collecting, processing and storing biological specimens. The issues mentioned here are discussed by Winn & Gunter (1993) and in other chapters in this volume. The major lessons are that, at all stages of specimen handling, variation and/or error can be introduced, and careful attention is required to prevent these untoward aspects. In addition to the reliability of biomarkers, it is important to understand the biokinetics and stability aspects before application in population studies. These issues have been addressed in previous publications (Droz, 1993; Bernard, 1995). As discussed, valid biomarkers, particularly of exposure, will be those that have biological relevance, defined pharmacokinetics and temporal relevance. For markers of exposure, the parameters that best summarize the behaviour of a chemical in biological systems is the elimination half-life, which reflects both the affinity of the chemical for the biological matrix and the efficiency of excretory or metabolic processes of elimination. Bernard (1995) has suggested four categories for biomarkers of exposure: half-life less than 12 hours; half-life between 12 and 100 hours; half-life between 100 hours and 6 months; and half-life greater than 6 months. Generally, for etiological epidemiological studies, biomarkers in the latter two categories will be most useful.

Biological relevance

The biological relevance of a biomarker is of prime importance in the selection and validation of a marker. The validation of a specific biomarker must include consideration of its biological relevance to the disease or exposure under study and the position in the continuum between exposure and disease. Given the fact that many exposures cause multiple diseases, the validation of a biomarker's

relationship to a disease first requires a clear definition of the disease end-point. A well developed hypothesis of the relationship between the biomarker and other events in the exposure–disease continuum is necessary.

While knowledge of the stability and natural history are important before population application of biomarkers of effect, it is important to assess the attributable risk or proportion (Schatzkin *et al.*, 1990; Benichou, 1991; Trock, 1995). The attributable proportion associated with a particular biomarker is an estimate of the proportion of cancer cases that must progress through the biomarker. This is not simply the proportion of all cases that are positive for the biomarker, because the biomarker will occur in some cases ('background cases') even when the exposure of interest or the biological events associated with the biomarker are not etiological events for those cases (Trock, 1995). This assessment will help to identify the possible mechanism by which the biomarker is related to the causal pathway for the cancer and to determine the extent to which the biomarker truly represents a biological event intermediate between exposure and cancer.

Shatzkin *et al.* (1990) have developed a framework for considering exposure–marker–disease relationships:

- A single marker is known to be linked causally and, hence, is necessary and sufficient for disease.
- Multiple pathways and multiple biomarkers are identified and any marker is sufficient but not necessary for disease.
- An exposure leading to disease operates through an unobservable event which in turn lends to an intermediate marker that is not directly biologically related to the disease but which may correlate with its occurrence.

It is the role of developmental and applied transitional studies to refine the view of these various mechanisms and to attempt to confirm which mechanism describes the type of marker being considered. It is important to understand underlying mechanisms in order to distinguish measurement error problems from multiple pathways.

Determining the number of subjects

The number of subjects needed in transitional studies is a matter of judgment. If the objective is to determine simply whether an assay 'works', it is appropriate to test the assay on a small number of available subjects without regard for sample size or representativeness. However, in transitional studies to determine the validity of a marker and its variability, it is important that subjects are selected through defensible sampling designs. Critical in this regard will be the need to minimize selection bias and to generate adequate statistical power when null hypotheses of no difference among groups or perhaps no association among biomarkers are to be tested.

For biomarkers of exposure, it has been shown to be useful to select subjects from groups known to have high exposures (such as chemotherapy patients or workers in specific occupational exposure groups) to maximize exposure differences between groups and to obtain an indication of whether an assay will identify a potentially large 'signal', and ultimately a dose–response relationship. For biomarkers of effect, studies involving histologically defined subsets of patients are useful in determining the association of biomarkers and a particular cancer. The calculation of sample size for transitional studies is the same as for etiological studies. For a situation in which two groups (e.g. cases and controls) are compared on the basis of presence or absence of some biomarkers, the formulation for the calculation of sample size for a given combination of significance level, power and size of expected difference as measured in terms of relative risk is well known (Schesslman, 1982; Hertzberg & Russek-Cohen, 1993).

When groups of subjects are being compared for shifts in central tendency (such as mean values), the sample size is calculated according to a well known formula described in elementary textbooks that gives the sample size as a function of alpha, power, the size difference to be detected and the variance of marker levels within groups. When the investigator is interested in inferring the relative risk of a disease as a function of incremental change in the level of a given biomarker, the sample size is determined by a formulation involving type I and II error rates, mean levels in cases and controls, and variance in cases and controls, respectively (Hertzberg & Russeck-Cohen, 1993).

The determination of sample size reflects a tension between feasibility and cost issues on the one

hand and confounding on the other. What are the best design and analysis strategies to address these problems? For biomarkers of exposure it is essential to have an unexposed comparison group or subgroups with a range of exposures. Matching exposed and unexposed subjects on potential confounding factors can increase the precision of the measure of association between exposures and outcome, but this may decrease the study's power while controlling for confounding on the matching variables (Hulka, 1990). Alternatively, a study design could involve restriction of suspected confounders, i.e. limiting the subjects eligible to those with or without a particular potentially confounding factor. This may minimize confounding but will not allow for analysis of effect modification by the restriction variables.

In practice, the number of subjects will depend on both the statistical power and the cost of obtaining subjects, specimens and assays. Unlike more traditional epidemiological studies, in which unexposed persons may be more accessible and cheaper to recruit than exposed persons, the opposite situation may occur in transitional studies where there is little incentive for non-exposed or non-diagnosed persons to provide biological samples (Hulka, 1990).

Characterization studies

Once a marker has been sufficiently developed in terms of the reliability of the assay and optimal conditions for handling, it is necessary to assess its characteristics in human populations (Schulte *et al.*, 1993; Rothman *et al.*, 1995). The objective of these studies is to identify factors that are confounders or effect modifiers which should be taken into account in etiological or public health studies.

One type of characterization study involves the assessment of the frequency of a marker in various population subgroups, determined by characteristics such as age, race, sex, medical condition, behaviour, etc. These types of study should be conducted on a marker-by-marker basis depending on the underlying biology, mechanism and relation to various host factors.

The ultimate goal of characterization-type transitional studies is to assess interindividual variation and the genetic and acquired factors that influence the variation. Large interindividual variation will make it more difficult to predict the risk of disease if the responses of exposed and unexposed people overlap. Interindividual variation in response is thought to result from differences in a variety of factors, including personal exposure, ability to metabolize carcinogens, and differences in DNA repair, immune surveillance and nutritional status. Therefore, people with the same apparent exposure may vary widely in their response to carcinogens. For example, interindividual variation in the level of xenobiotics adducted to DNA results from processes that vary between people, affecting the concentration at the target tissue after distribution *in vivo*, metabolic activation and detoxification, capacity of repair, and persistence of the xenobiotic and cellular target *in vivo*.

A goal of molecular epidemiology is to elucidate the mechanisms that explain why people vary in their risk of disease. To do this, we must understand which part of the biological or biomarker measurement is due to laboratory variability or to intra-individual variation and which part reflects true interindividual variation. The mechanisms underlying interindividual differences in biomarker response are important because they are the same mechanisms that the body uses to mitigate a disease outcome. This means that biomarkers have potential as targets of interventions (Perera & Mooney, 1993).

Within-person biological variability may or may not be time-dependent (Hulka & Margolin, 1992). Biological specimens collected from the same person at different times might show changes because the person has aged, has been exposed to mutagenic substances or has been exposed to a medical procedure. The actual biological matrix, e.g. white blood cells, will have changed in the intervening time. Some cells will have died, while others may have been generated.

Time-independent biological variability will also occur because the distribution of matter (e.g. xenobiotics) can vary across organs and cells. Each biological sample will not capture the same biological material (Hulka & Margolin, 1992).

Intraperson variability has important implications for sample size and power in transitional studies (Hulka & Margolin, 1992). This has been demonstrated in cytogenetic studies, such as that by Hirsch *et al.* (1984) who found that cell-to-cell variability in the frequency of sister chromatid exchanges from an individual person was greater

than the variability in the mean frequency of sister chromatid exchanges from person to person. To compensate for this intra-individual variability, the number of cells scored per person was increased. Interperson variability in biomarker measures is generally addressed in the same way as other types of variable measures in epidemiological studies. The exception may be that the variability of biomarkers between persons may reflect acquired or inherited susceptibility which can serve as an effect modifier in relation to exogenous exposures of interest (Hulka & Margolin, 1992). Susceptibility to environmental agents is likely to arise from complex interaction between activating and inactivating chemical metabolizing enzymes. For example, Tang *et al.* (1995) have estimated that the combination of an inherited deletion of a gene for the detoxifying enzyme glutathione-S-transferase (GSTM1) and high PAH-DNA adduct levels confers a 12-fold risk of lung cancer.

The ability to measure biomarkers at the molecular and genetic levels has resulted in the identification of a degree of interperson variability not previously imagined. The major sources of this variability need to be accounted for prior to the use of biomarkers in etiological or public health applications. Characterization-type transitional studies may involve determining the prevalence of particular alleles in specific racial subgroups, evaluating the correlation between genotypic and phenotypic assays, estimating the likelihood and impact of allele misclassification and evaluating potential induction effects. Additionally, transitional studies can evaluate the biological plausibility of gene–environment interactions observed in etiological studies (Rothman *et al.*, 1995).

Applied transitional studies

Applied transitional studies are those that assess the relationship between a marker and the event that it marks, namely exposure, disease or susceptibility. These studies are often conducted on healthy subjects, and generally, in most cases involving biomarkers of exposure or effect, the biomarker is treated as the outcome variable. However, with susceptibility biomarkers and in certain study designs, a biomarker of exposure or effect may be used as an independent variable (e.g. serum organochlorines and breast cancer; carcinogen DNA adducts and lung cancer). Applied transitional studies are generally cross-sectional or short-term longitudinal designs and are not capable, in and of themselves, of establishing or refuting a causal relationship between a given exposure and disease. They do, however, provide mechanistic insight and may yield useful information on relationships between biomarkers and the events they represent (Rothman *et al.*, 1995). Where case–control studies that link a biomarker with disease are conducted, inferences about causality can sometimes be made. Another type of applied transitional study that has been reported involves the use of biomarkers in intervention studies. These include interventions with smokers, workers and the treatment of high-risk populations (e.g. in treatment with antioxidants, biomarkers are being used to monitor the efficacy of the intervention) (Perera & Mooney, 1993). The studies are designed to test not only the presence of the biomarkers, but also the level of change with alteration of the risk factor. These studies are transitional in that they establish the ability of the marker to serve as an early or intermediate 'end-point' with which to monitor efficacy or compliance.

The objective of the studies is to determine one of the following relationships:

- exposure/marker
- marker/disease
- exposure/susceptibility marker (high disease risk or low disease risk).

Applied transitional studies can be used to assess the attributable proportion of a particular biomarker. Hence, they build on the mechanistic knowledge obtained in characterization-type transitional studies.

Exposure/marker

The assessment of the relationship between an exposure and a marker represents much of the previous effort termed molecular epidemiology. Exposures to carcinogens have been evaluated to determine changes in DNA (DNA adducts) or proteins (e.g. haemoglobin or albumin adducts), or in some cases cytogenetic changes (e.g. chromosomal aberrations, micronuclei). These have generally been cross-sectional in nature. It is critical in such studies to be rigorous not only in the assessment of the marker but also in the assessment of exposure and

control for confounding. This is illustrated in the work of Mayer *et al.* (1991) and Schulte *et al.* (1992), who demonstrated the value of putting appropriate resources into the exposure characterizations when validating the relationship between exposure to ethylene oxide and formation of haemoglobin adducts. Too often, emphasis is placed only on the assay and not on the measure of exposure.

In characterizing the relationship between a marker and exposure, it is important that all sources and rates of exposure are considered, since a marker generally represents the integration of these. Attention should also be given to the toxicokinetics and natural history of the marker in order to understand the appropriate sampling and specimen collection times. A marker with a short half-life will not be detectable in samples a long time after exposure has ended. Moreover, there is a need to assess the relationship between the marker and different regimens of exposure (continuous or intermittent).

Marker/disease

The relationship between a marker and disease is often the most frequently considered issue in assessing whether a marker is ready for use in an epidemiological study. Of interest is how well the marker predicts or represents disease. These types of validation studies are difficult to accomplish because of the temporal factor. To identify an early change—i.e. a change in pathogenesis or a change predictive of disease—generally requires a prospective study, although cross-sectional clinical studies of heavily exposed individuals and case–control studies can be used to great advantage. However, when not using a prospective design, care must be taken to avoid biased associations. This is often difficult, and hence prospective studies are the best approach for validation. However, prospective studies are expensive and time-consuming, and few are conducted. For example, despite the large number of studies on cytogenetic markers, there is still little consensus on their predictive value, since most of the studies have been cross-sectional and suffer from temporal ambiguity. Specifically, in epidemiological terms, predictive value means the percentage of those who test positive for a marker who actually develop the disease. To perform the appropriate prospective studies of sister chromatid exchanges would take a large population and a relatively long time. The best and possibly only example of such a study is the Nordic prospective study on the relationship between peripheral lymphocyte chromosome damage and cancer morbidity in occupational groups (Brøgger *et al.*, 1990). Ten laboratories in four Nordic countries participated in a study of a combined cohort of persons (mostly from occupational groups) who had been cytogenetically tested. The cohort will be followed prospectively for cancer morbidity. The cohort comprises 3190 subjects, of whom 1986 (62%) have been scored for chromosome aberrations and 2024 (63%) have been scored for sister chromatid exchanges. Preliminary analysis indicates that chromosomal aberrations are associated with cancer.

Exposure/susceptibility to disease

Validated biological markers of susceptibility can serve as effect modifiers in epidemiological studies. Effect modification is a term with statistical and biological aspects. Statistically, effect modification is analysed by examining the joint effects of two or more factors. The interpretation of effect modification depends on the statistical method (e.g. multiplicative or additive) used to model interaction. From the biological perspective, effect modification can explain why two similarly exposed individuals do not develop a disease. The answer, in part, is individual variability in metabolic, detoxification and repair capabilities.

To validate a susceptibility marker, it is important to minimize misclassification, which can occur as a result of laboratory or epidemiological factors that affect phenotyping or genotyping (Rothman *et al.*, 1993). Next, it is necessary to demonstrate that the susceptibility marker either increases the biologically effective dose or elevates the risk of disease.

Genetic susceptibility markers can be characterized in terms of the six possible patterns of gene–environment interaction identified by Khoury *et al.* (1988). These are shown in Table 3. Khoury *et al.* (1988) recommend that an epidemiological approach be used to evaluate genetic marker–disease associations and their interaction with specific environmental risk factors. However, prior to conducting such studies, a major challenge is to determine which environmental factors might be involved in the etiology of a specific disease.

Table 3. Patterns of genotype–environment interactions observed in ecogenetic studies

Pattern	Effect of genotype in the absence of the environment	Specificity of environmental effect *vis-à-vis* genotype	Notations
1	Innocuous	Specific	$R_g = 1$, $R_e = 1$
2	Innocuous	Non-specific	$R_g = 1$, $R_e > 1$
3	Risk factor	Specific	$R_g > 1$, $R_e = 1$
4	Risk factor	Non-specific	$R_g > 1$, $R_e > 1$
5	Protective	Specific	$R_g < 1$, $R_e = 1$
6	Protective	Non-specific	$R_g < 1$, $R_e > 1$

Source: Khoury *et al.*, 1988.

In cancer epidemiology, interest has been focused on susceptibility genes that are common in the population and are generally considered to be polymorphisms (i.e. with a minor allele frequency of more than 1%), that are probably associated with relative risks under 10 (and as such do not exhibit familial patterns of inheritance) and that may interact with a particular exposure (Rothman *et al.*, 1995). The objective of applied transitional studies with regard to susceptibility markers is to determine whether the markers are effect modifiers. As mentioned earlier, the distinction between a transitional study and an etiological study becomes slightly blurred in this effort, so that such distinctions are arbitrary. The emphasis in a transitional study is to determine whether the marker can be used to investigate populations in terms of risk (i.e. serve as an outcome variable or as an independent variable).

Preparing for transitional studies

Selecting candidate markers

Many more markers are identified in laboratories than could reasonably be tested and used in the field. For this reason, there is a need to select among candidates which markers are beneficial for field testing and use. To gauge utility, it is helpful to envision a framework for candidate markers such as the continuum between exposure and disease. A potentially useful candidate marker will be one that can be related to some heuristic continuum and for which successful field testing will add relevant information to various etiological or public health questions. In some cases, a transitional study may only provide mechanistic information. This can be very useful but should be oriented towards confirming a hypothesized link in a continuum.

It is of great importance in identifying a continuum of events between exposures and disease that the marker represents a 'critical effect'. (Schulte, 1989; Borm, 1994). A critical effect is the biological marker that is deemed most representative of a particular component in the continuum and is ultimately most pathognomonic. This requires a series of independent studies, primarily toxicological, but also clinical and epidemiological. It is necessary to develop a hypothesis concerning the role of the marker in the development of the disease. As more causal components are identified, it becomes necessary to elucidate quantitative relationships of the kinetics, natural history and rates of transition along the continuum.

Determining level of effort

After a candidate marker has been identified, it is useful to determine what field testing is required. This is a useful exercise because it allows for research planning and funding and assures that a comprehensive approach is considered. The alternative is that a marker becomes labelled as validated and ready for field use when it is not ready or eligible, and this can lead to flawed and costly studies. An example of a check list for what needs to be considered is shown in Table 2.

Selecting candidate populations

A key factor in transitional studies is selecting and accessing populations with the kind of characteristics thought to be important to the testing of a

marker. These may be populations with contrasting levels of exposure, demographic or behavioural factors, or ethnicity; or they may be populations homogeneous for such factors. The similarity between these populations and those in whom the marker will ultimately be used for etiological or applied purposes is important.

Reporting results of transitional studies

Transitional studies can be highly informative regardless of their outcome. Those that show a positive relationship between a marker and exposure, disease or susceptibility are obviously important. However, those that show negative relationships between a biomarker and a particular event, or have small biomarker frequencies in a population subgroup, provide useful information about the utility, generalizability or limitations of a marker. Such negative results in well conducted studies should be published in the peer-reviewed scientific literature. Clearly, statistical power considerations should be discussed in such studies.

There appears to be a wide variation in the approaches used in reporting test and study results to participants in the research. Some investigators and their supporting organizations require all test and subject results to be communicated to participants regardless of clinical relevance and with the most truthful interpretation possible, while others limit reporting to those results that are clinically relevant. This difference hinges on the tension between beneficence and autonomy (see Schulte *et al.*, this volume, for a discussion of the ethical issues). Should participants be told only when something can be done and not told when there might be anxiety without benefit, or should participants have a right to information that is held about them regardless of whether it is considered by the holder to be of benefit? These questions need further consideration.

Support for transitional research

Transitional studies are important for the successful and effective use of biomarkers in cancer epidemiology. However, they are perceived as having neither the excitement nor the appeal of basic laboratory or etiological research or public health application. Thus, they are not widely and intensively supported by funding agencies. Since their outcome is the characterization of biomarkers and their limits, they are intermediate rather than end results about cancer causation or controls; yet without this effort, the end results may not be obtained. Currently, much of the work of transitional studies is subsumed in pilot or feasibility studies conducted prior to a larger study. This may not be the most effective way of conducting transitional research because researchers may be forced to trade off those funds available for conducting an etiological study against those available for the assessment of the utility and limits of a marker. More funding agencies should designate and support transitional biomarker studies if a wide range of useful tools are to become available.

References

Benichou, J. (1991) Methods of adjustment for estimating the attributable risk in case–control studies: a review. *Stat. Med.*, 10, 1753–1773

Bernard, A.M. (1995) Biokinetics and stability aspects of biomarkers: recommendations for application in population studies. *Toxicology*, 101, 65–71

Borm, P.J.A. (1994) Biological markers and occupational lung disease: mineral dust-induced respiratory disorders. *Exp. Lung Res.*, 20, 457–470

Brogger, A., Hagmar, L., Hansteen, I.L., Heim, S., Hogstedt, B., Knudsen, L., Lambert, B., Linnainmaa, K., Mitelman, F., Nordenson, I., Reuterwall, C., Salomaa, S., Skerfving, S. & Sorsa, M. (1990) An inter-Nordic prospective study on cytogenetic endpoints and cancer risk. *Cancer Gene Cytogenet.*, 45, 85–92

Droz, P.O. (1993) Biological monitoring and pharmacokinetic modeling for the assessment of exposure. In: Schulte, P.A. & Perera, F.P., eds, *Molecular Epidemiology: Principles and Practices*, San Diego, Academic Press, pp. 137–157

Fleiss, J.L. (1986) Statistical factors in early detection of health effects. In: Underhill, D.M. & Radford, E.D., eds, *New and Sensitive Indicators of Health Impacts of Environmental Agents*, Pittsburgh, PA, University of Pittsburgh Press, pp. 9–16

Hertzberg, V.S. & Russek-Cohen, E. (1993) Statistical methods in molecular epidemiology. In: Schulte, P.A. & Perera, F.P. eds, *Molecular Epidemiology: Principles and Practices*, San Diego, Academic Press, pp. 199–216

Hirsch, B., McGue, M. & Cervenka, J. (1984) Characterization of the distribution of sister chromatid exchange frequencies: implications for research design. *Hum. Genetics*, 65, 280–286

Hulka, B.S. (1990) Methodologic issues in molecular epi-

demiology. In: Hulka, B.S. Wilcoskey, T.C. & Griffith, J.D., eds, *Biological Markers in Epidemiology*, New York, Oxford University Press, pp. 214–226

Hulka, B.S. (1991) Epidemiologic studies using biological markers: issues for epidemiologists. *Cancer Epidemiol. Biomarkers Prev.*, 1, 13–19

Hulka, B.S. & Margolin, B.H. (1992) Methodological issues in epidemiologic studies using biologic markers. *Am. J. Epidemiol.*, 135, 200–209

Khoury, M.S., Adams, M.J., Jr & Flanders, W.D (1988) An epidemiologic approach to ecogenetics. *Am. J. Hum. Genetics*, 42, 89–95

Massey, B.S. (1986) *Measures in Science and Engineering* ,Chichester, Ellis Horwood

Mayer, J., Warburton, D., Jeffrey, A., Pero, R., Andrews, L., Walles, B., Toor, M., Latriano, L., Tang, D., Tsai, W.Y., Kuroda, M. & Perera, F.P. (1991) Biologic markers in ethylene oxide-exposed workers and controls. *Mutat. Res.*, 248, 163–176

Perera, F.P. & Weinstein, I.B. (1982) Molecular epidemiology and carcinogen-DNA adduct detection: new approaches to studies of human cancer causation. *J. Chronic Dis.*, 35, 581–600

Perera, F.P. & Mooney, L.A. (1993) The role of molecular epidemiology in cancer prevention. In: DeVita, V.T., Hellman, S. & Rosenberg, S.A., eds, *Cancer Prevention*, Philadelphia, J.B. Lippincott, pp 1–15

Perera, F. & Santella, R. (1993) *Carcinogenesis*. In: Schulte, P.A. & Perera F., eds, *Molecular Epidemiology: Principles and Practices*, San Diego, Academic Press, pp. 277–300

Rothman, N. (1993) Epilogue. In: Schulte, P.A. & Perera, F.P., eds, *Molecular Epidemiology: Principles and Practices*, San Diego, Academic Press, pp 199–216

Rothman, N., Stewart, W.F., Caporaso, N.E. & Hayes, R.B. (1993) Misclassification of genetic susceptibility biomarkers: implications for case-control studies and cross-population comparisons. *Cancer Epidemiol. Biomarkers Prev.*, 2, 299–303

Rothman, N., Stewart, W.F. & Schulte, P.A. (1995) Incorporating biomarkers into cancer epidemiology: a matrix of biomarker and study design categories. *Cancer Epidemiol. Biomarkers Prev.*, 4, 301–311

Schatzkin, A., Freedman, L.S., Schiffman, M.H. & Dawsey, S.M. (1990) Commentary: validation of intermediate end points in cancer research. *J. Natl Cancer Inst.*, 82, 1746–1752

Schesselman, J.J. (1982) *Case-control Studies*, New York, Oxford University Press

Schulte, P.A. (1989) A conceptual framework for the validation and use of biological markers. *Env. Res.*, 48, 129–144

Schulte, P.A. (1992) The use of biological markers in occupational health research and practice. *J. Toxicol. Env. Health*, 40, 359–366

Schulte, P.A., Boeniger, M., Walker, J.T., Schober, S.E., Pereira, M.A., Gulati, D.K., Wojciechowski, J.P., Garza, A., Froelich, R., Strauss, G. et al. (1992) Biologic markers in hospital workers exposed to low levels of ethylene oxide. *Mutat. Res.* 278, 237–251

Schulte, P.A. & Perera, F.P. (1993) Validation. In: Schulte, P.A. & Perera, F.P., eds, *Molecular Epidemiology: Principles and Practices*, San Diego, Academic Press, pp. 81–109

Schulte, P.A., Rothman, N. & Schottenfeld, D. (1993) Design consideration in molecular epidemiology. In: Schulte, P.A. & Perera, F.P., eds, *Molecular Epidemiology: Principles and Practices*, San Diego, Academic Press, pp. 159–198

Shrout, P.E. & Fleiss, J.L. (1979) Intraclass correlations: uses in assessing rator reliability. *Psychol. Bull.*, 86, 420–428

Tang, D.L., Chiamprasert, S., Santella, R.M. & Perera, F.P. (1995) Molecular epidemiology of lung cancer: carcinogen-DNA adducts, GSTM1 and risk. *Proc. Am. Assoc. Cancer Res.*, 36, 284

Trock, B.J. (1995) Application of biological markers in cancer environmental epidemiology. *Toxicology*, 101, 93–98

Vineis, P., Schulte, P.A. & Vogt, R.F., Jr (1993) Technical variability in laboratory data. In: Schulte, P.A. & Perera, F.P., eds, *Molecular Epidemiology: Principles and Practices*, San Diego, Academic Press pp. 109–135

Wilcox, A.J. (1992) Molecular epidemiology: collision of two cultures. *Epidemiology*, 6, 561–562

Winn, D.M. & Gunter, E.W. (1993) Biologic specimen banks: a resource for molecular epidemiologic studies. In: Schulte, P.A. & Perera, F.P., eds, *Molecular Epidemiology: Principles and Practices*, San Diego, Academic Press, pp. 217–235

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Application of Biomarkers in Cancer Epidemiology

Edited by P. Toniolo, P. Boffetta, D.E.G. Shuker,
N. Rothman, B. Hulka and N. Pearce

IARC Scientific Publications No. 142

International Agency for Research on Cancer, Lyon, 1997

Published by the International Agency for Research on Cancer,
150 cours Albert Thomas, F-69372 Lyon cedex 08, France

Distributed by Oxford University Press, Walton Street, Oxford, UK OX2 6DP (Fax: +44 1865 267782) and in the USA by Oxford University Press, 2001 Evans Road, Carey, NC 27513, USA (Fax: +1 919 677 1303).
All IARC publications can also be ordered directly from IARC*Press*
(Fax: +33 4 72 73 83 02; E-mail: press@iarc.fr).

IARC Library Cataloguing in Publication Data

Application of biomarkers in cancer epidemiology/
editors, P. Toniolo ... [et al.]
(IARC scientific publication ; 142)

1. Genetic Markers
2. Neoplasms - epidemiology
3. Neoplasms - prevention & control I. Toniolo, P. II. Title III. Series

ISBN 92 832 2142 7 (NLM Classification: W1)
ISSN 0300–5085