

An epidemiologic perspective on biomarkers

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Abstract. Schatzkin A, Freedman L, Schiffman M (National Cancer Institute, Bethesda, Maryland, USA). An epidemiological perspective on biomarkers. *Journal of Internal Medicine* 1993; 233: 75–79.

The authors discuss biological markers from an epidemiologic perspective, emphasizing the importance of integrating biomarkers into large-scale observational and intervention studies. Whereas any biologic phenomenon can be considered a biomarker, an intermediate end-point is defined as being on the causal pathway between exposure and disease. An intermediate end-point is a valid surrogate for a disease in relation to a given exposure if, and only if, that exposure causes a similar change in the occurrence of both the intermediate end-point and the disease. Cancer studies using surrogate end-points may be shorter, smaller and cheaper than those using malignancy *per se* as an outcome. Three types of studies may be carried out to determine whether a given biomarker is an intermediate end-point and whether it can serve as a surrogate: (i) exposure–marker studies, (ii) marker–disease studies, and (iii) studies comprising all three elements, exposure, marker, and disease. The authors discuss statistical aspects of these three types of studies and provide examples from investigations of alcohol–hormones–breast cancer, diet–epithelial proliferation markers–large bowel adenomatous polyps, and reproductive risk factors–human papillomavirus infection–cervical cancer.

Keywords: Biomarker, cervix dysplasia, colonic polyps, diet, epidemiology, malignant neoplasm.

Introduction

Advances in cellular and molecular biology have spawned what one conference participant called ‘an overwhelming abundance of suggested mechanisms’ for cancer and other chronic diseases. In this paper we suggest an approach to sorting through this abundance by integrating biological markers into large-scale epidemiologic studies.

Biomarkers and intermediate end-points

A biomarker can be defined as a measured structure or process at any level of biologic reality (molecule, cell, tissue, organ, organ system, or whole organism). In other words, any biologic phenomenon can be considered a biomarker.

Intermediate endpoints represent a more restricted subset of biomarkers. A biomarker (M) is an intermediate endpoint if, and only if, it is on the causal pathway between exposure (E) and disease (D) such that $E \rightarrow M \rightarrow D$ [1]. ‘Exposure’ may be viewed quite broadly, encompassing both exogenous

(environmental) as well as endogenous (either genetic or acquired) factors. Intermediate endpoints may serve several functions. These are listed below with examples.

(1) Exposure assessment. Aflatoxin–DNA adducts have been detected in human urine [1].

(2) Elucidation of pathogenic microprocesses (‘mechanisms’). The extremely strong association of human papillomavirus (HPV) with both cervical neoplasia and its reproductive risk factors (e.g., number of lifetime sexual partners) suggests a causal role for this infectious agent [2].

(3) Surrogate end-points for disease. Mucosal hyperproliferation has been suggested to be a necessary precursor in the formation of large bowel neoplasms [3]. Mucosal proliferation status has been used as the primary outcome in a number of studies of putative modifiers of the large bowel neoplastic process [4, 5].

(4) Early detection. Blood-borne markers such as prostate specific antigen [6] and CA125 [7] may prove useful in the early detection of prostate and ovarian cancer, respectively.

Although there is considerable overlap in these functions, one can distinguish roughly between 'early' markers, those lying more to the left on the E---->M---->D continuum, and 'late' markers, found more to the right on the continuum. Thus, DNA adducts could be considered early markers (reflecting both exposure and possible early mutational events) relative to late markers in the neoplastic process like cervical dysplasia or microadenomas [8].

Identification and validation of surrogate end-points

Cancer studies that use surrogate end-points may be shorter and smaller (and, therefore, cheaper) than investigations using cancer *per se* as the outcome. We now suggest some guidelines for deciding whether a marker can be properly used as a cancer surrogate.

First, a definition. An intermediate end-point is a valid *surrogate* (S) for cancer in relation to a given exposure if, and only if, that exposure causes a similar change in the occurrence of both the intermediate end-point and cancer. These requirements can be met within two causal contexts: (i) the surrogate lies directly on the causal pathway,

$$E\text{----}>S\text{----}>CANCER$$

or, (ii) the surrogate is tightly linked to some unobserved intermediate end-point (UIE),

$$\begin{array}{c} S \\ E\text{----}>UIE\text{----}>CANCER \end{array}$$

An example of such a tightly linked potential surrogate might be micronucleated cells that generally do not replicate but do indicate prior geneotoxic events [9].

Potential surrogate markers are often first identified in (i) case series, (ii) ecologic studies, and (iii) animal studies. We give an example of each. The presence of HPV in cervical neoplasms has been observed repeatedly over the last decade [10]. The mucosal proliferation labelling index has been found to be higher in population groups at increased risk of large bowel cancer compared to groups at lower risk [11]. Aberrant crypt foci (microadenomas) have been reported in laboratory animals administered potent large bowel chemical carcinogens [8].

To determine more definitively whether a specific biomarker is truly an intermediate end-point, and,

therefore, to evaluate whether that marker can serve as a surrogate, three types of study may be carried out: (i) exposure-marker studies, (ii) marker-disease studies, and (iii) studies comprising all three elements of our continuum, exposure, marker, and disease. We now discuss each of these in greater detail.

Exposure-marker studies (E---->M)

These investigations are designed to answer the following question: in observational epidemiologic studies, is a risk factor associated with the marker, or, in intervention studies, does the intervention affect the marker?

Epidemiologists have identified a number of life-style and occupational factors associated with increased risk of cancer at various sites. The question here is whether these risk factors are associated with the marker of interest. For example, in a case-control study of cervical dysplasia, Manos *et al.* have shown that the odds ratio for HPV infection is over 10 for women reporting six or more lifetime sexual partners relative to women reporting one such partner [12].

Small intervention (metabolic) studies provide another forum for examining the exposure-marker connection. Stadler *et al.* showed that a bolus of corn oil increased the large bowel labelling index in a group of normal male volunteers [5]. In a controlled feeding study involving consumption of the equivalent of two alcohol drinks per day, Reichman *et al.* observed increased oestrogen levels in the young women in the alcohol-consuming group [13].

Sample size requirements tend to be rather modest for these exposure-marker studies. For an intervention study, fewer than 100 participants are generally necessary to demonstrate a modest exposure effect (say, a 30% reduction) on a marker. (For more detail on these sample size calculations, see [14].)

Marker-disease studies

The question pertinent to this type of investigation is: is the marker causally related to cancer? This question can be adequately addressed in case-control and cohort epidemiologic studies. In case-control studies, one ascertains marker status in those with the disease and in an appropriately selected control group. Reverse causation is a concern here, since in some instances a marker may be influenced by the

Table 1.

	Cancer	
	Yes	No
Intermediate endpoint	+ A - C	B D

disease itself. In cohort studies, marker status is ascertained well before the development of disease so that reverse causation is not likely to be an issue.

The epidemiologic concept of attributable proportion (AP) is useful for quantifying the relation between a marker and disease [15]. AP in this context indicates the proportion of disease that is accounted for by a given marker. The formula for AP, based on Table 1, is

$$AP = S(1 - 1/R)$$

where $S = \text{sensitivity} = A/(A + C)$ and $R = \text{relative risk} = [A/(A + B)]/[C/(C + D)]$. An intermediate endpoint that is necessary for the development of disease—implying that all causal pathways must converge on this marker—has an AP of 1.0. (Error in marker measurement, however, might reduce the observed AP to less than 1.0, even if the marker is truly a necessary precursor to disease.) Conversely, a marker with an AP near 0 is not on the causal pathway to disease, or is at best a minor causal component.

Schiffman *et al.* have provided an example of the case-control marker-disease study in their recent investigation of HPV infection and cervical dysplasia (Schiffman MH, personal communication).

They found an odds ratio over 20 for the association between HPV-positivity and cervical dysplasia. (Cervical dysplasia, particularly high grade dysplasia, is considered a necessary precursor for cervical cancer. Thus, although dysplasia could be considered an intermediate end-point in its own right, because of its strong causal connection to cancer it can be considered 'disease' in M----> D studies.) The AP for HPV infection and cervical dysplasia was approximately 0.9. These data clearly indicate a very strong relation between HPV infection and neoplastic disease of the cervix.

Wargovich *et al.* as an example of the cohort approach to this question, are examining whether the large bowel mucosal labelling index predicts subsequent recurrence of adenomatous polyps (War-

govich M, personal communication). A similar study is nested within the Polyp Prevention Trial, a multi-institutional intervention study of the effect of a low-fat, high-fibre, high-vegetable and fruit eating pattern on large bowel adenomatous polyp recurrence [16]. Studies of hormone levels and subsequent risk of breast cancer in women represent another example of this type of study.

Sample size requirements for marker-disease studies, which have relatively infrequent occurrence of neoplasms as primary end-points, are larger than those for exposure-marker studies. Prospective studies of, for example, large bowel polyp recurrence in relation to mucosal proliferative indexes require a few hundred participants for adequate statistical power [14].

Exposure-marker-disease studies (E----> M----> D)

The underlying question here is: does the marker *mediate* the relation between exposure and cancer, that is, does the marker account for risk factor associations (in observational epidemiologic studies) or intervention effects (in intervention studies)?

The strategy in these type of studies is to *adjust* risk factor associations or intervention effects for the marker of interest [15]. If the marker fully mediates risk factor-disease associations, then relative risks adjusted for the marker should be reduced to 1.0. In a similar vein, if the marker fully mediates the intervention-disease relation, then the marker-adjusted intervention effect should become 0. (Some allowances, of course, should be made for biologic variability and measurement error in marker assessment. Even with full mediation, observed marker-adjusted relative risks may not decline all the way to 1.0, nor will observed marker-adjusted intervention effects likely fall completely to 0.)

We can examine the effect of marker adjustment on risk factor-disease associations in both case-control and cohort studies. Temporal sequence is again an issue, particularly for case-control investigations. The case-control approach is appropriate only if we can be reasonably certain that the marker is unaffected by disease *and* risk factors reflect exposure prior to the marker as well as disease. In cohort studies, if we ascertain marker status only at baseline then we cannot be certain that the risk factors precede the marker. This concern is alleviated somewhat if marker status is assessed during follow-

up. The investigator in an intervention study can be reasonably confident of the correct exposure-marker-disease temporal sequence if marker status is ascertained after baseline but prior to disease occurrence.

To continue with our HPV-cervical dysplasia example, Schiffman *et al.* found that the HPV infection-adjusted relative odds of cervical dysplasia for 6+ lifetime sexual partners reduced to above 1.5 (the unadjusted value was approximately 4.5). Thus, HPV infection can be said to account for most of the relation between the classical sexual risk factor and cervical dysplasia. A few large cohort studies in the world, including the Malmö study, can examine the extent to which hormone levels mediate the relationships of reproductive risk factors, and possibly diet, to breast and other cancers. The Polyp Prevention Trial will examine the extent to which mucosal hyperproliferation mediates the relation between dietary pattern and adenomatous polyp recurrence.

Sample size requirements for exposure-marker-disease studies are relatively great. In the PPT, the full sample size of 2000 is required to show with statistical power of 0.85 that cell proliferation explains at least one half of the intervention effect (a 22% reduction in polyp recurrence over 3 years) [14]. Sample size requirements for observational epidemiologic studies depend on the relative risks for various exposure categories (which tend to be larger than the intervention effects anticipated in trials) as well as on the disease incidence rate (which may be substantially smaller than, say, the annual polyp recurrence rate).

Additional issues

First, there may be considerable biologic variation in markers. Hormone levels, for example, may vary with time of day, day of the menstrual cycle in women, and so on. This variability needs to be considered in designing studies (e.g., standardizing blood drawing times), calculating sample size, and analysing results.

Second, marker ascertainment techniques are evolving rapidly. Also evolving is the realization among epidemiologists that some of these techniques are sufficiently error prone to attenuate biologic relations between observability. In an earlier study of lifetime sexual partners, HPV infection, and cervical dysplasia that used a less accurate test of HPV status, Schiffman *et al.* found that the HPV-infection-

adjusted relative risk for 6+ lifetime sexual partners did not approach 1.0 as it did in the more recent study with a more accurate HPV determination.

Third, for large observational and intervention studies, especially those involving multiple institutions and field sites, careful attention must be paid to standardization of marker ascertainment techniques and the appropriate training required by field personnel to provide adequate biologic specimens. This is not likely to be much of a problem in blood drawing but it is a critical issue in, for example, obtaining rectal biopsies for mucosal proliferation assays.

Conclusion

Three points can be made in summary:

- (1) A valid surrogate must be on (or tightly linked to) the causal pathway to cancer.
- (2) Markers should be integrated in observational epidemiologic and intervention studies.
- (3) For inclusion in large, particularly multi-institutional, field studies, markers must be able to be easily and accurately measured.

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Received 13 May 1992, accepted 3 July 1992

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