Serological Markers of Cancer and Their Applications in Clinical Trials

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Abstract

Strategies for the prevention of cancer include those aimed at reducing the incidence of cancer (primary prevention) and cancer mortality through early detection of tumors (secondary prevention). The efficacy of prevention interventions is evaluated by clinical trials. The conduct of clinical trials is aided by the use of serological markers of the carcinogenic process measured using plasma or red or white blood cells. The accessibility and acceptability of obtaining blood samples for the measurement of serological markers of carcinogenesis permit widespread applications in the conduct of clinical trials. Serological markers must be shown to be valid and reliable before their use. Serological markers identify a variety of stages in the process of carcinogenesis such as inherited or acquired susceptibility to cancer, environmental exposures to carcinogens, biological effects of exposures, and the presence of preinvasive or invasive cancer. Serological markers may be used in clinical trials to select high risk but disease-free individuals for participation in clinical trials based on susceptibility factors or carcinogenic exposures. Other uses of serological markers include monitoring adherence to interventions and establishing trial outcomes of intermediate cancer endpoints or incident invasive disease. Examples of these applications are discussed.

Serological markers of carcinogenesis have widespread applications in clinical research and potentially for clinical practice. Currently, the only limitation to their widespread use is the availability of validated serological markers. Because of the ease and acceptability of their use, research into the development of serological markers should continue. Methods for quickly validating serological markers should be developed in order to aid the transition to clinical applications.

Introduction

Strategies for the prevention of cancer include those aimed at reducing the incidence of cancer (primary prevention) and cancer mortality through early detection of tumors (secondary prevention). Evaluation of the safety and efficacy of proposed primary and secondary cancer prevention interventions is necessary before the widespread application of these interventions. Several steps are involved in the evaluation of interventions usually culminating in clinical trials to establish the efficacy of the procedure or administered agent in reducing the mortality and/or incidence of cancer. The challenge facing the clinical researcher is to conduct the evaluation in a safe and timely manner. The incorporation of serological markers into several aspects of clinical trials of prevention interventions may aid in reducing the size and duration of trials, thus reducing costs and time for the conduct of the trial.

The process of carcinogenesis involves inherited and acquired susceptibility factors, exposures to exogenous and endogenous carcinogens, and promotion and protection factors (Fig. 1). Interventions may be conducted at a variety of time points along this process in order to reduce the occurrence of proliferative, in situ, or invasive cancers (primary prevention applied in earlier stages in the process) or cancer morbidity and/or mortality (secondary prevention applied in later stages of the process) (Fig. 2). Serum or plasma, RBC and WBC cells, may contain markers of these processes of carcinogenesis.

For example, exposures may result in the formation of albumin, hemoglobin, or DNA adducts that can be measured in plasma or RBC or the DNA of WBC. Tumor-associated antigens may be detected in serum or plasma indicating the presence of an early stage of cancer. DNA from WBC may be examined for the presence of inherited genetic alterations associated with an increased risk of cancer. These serological markers of carcinogenesis have widespread applications in clinical research, particularly in the conduct of clinical trials to measure the efficacy of primary prevention or screening interventions. This paper examines the use of serological markers in clinical trials of cancer prevention interventions.

Characteristics of Serological Markers

Accessibility and Acceptability. The accessibility and acceptability of obtaining blood samples are key practical advantages to using serological markers in clinical research. Because of the ease and acceptability of blood sampling, serological markers may be used in large-scale prevention trials with minimum risk to participants. Blood levels of tumor-associated antigens as markers of early stage disease would present a great advantage in acceptability over other early detection methods such as digital rectal examination or sigmoidoscopy that have low acceptance rates (1). At present, genetic or proliferative changes that occur in the early stages of carcinogenesis may be detected only by obtaining tissue samples. If serological markers of these changes, such as altered gene products, could be detected, noninvasive monitoring of the process could be accomplished.

Quality of Serological Markers. Serological markers of carcinogenesis should be valid and reliable. Validation studies should be carefully conducted to ensure that the marker is accurately measuring the specific stages in the disease process. The sensitivity and specificity of the tests are measures of its validity (2). Sensitivity measures the ability of a positive test for a marker to correctly identify the disease process, whereas specificity measures the ability of a negative test for a marker to correctly identify those who do not have the disease process. The goal is to maximize sensitivity and specificity in order to minimize false positive and false negative tests that would lead to misclassification. For example, CA-125 is an ovarian cancer tumor-associated antigen. Most studies in the literature use a CA-125 levels of 35 units/ml to designate a positive test (3). In two studies of prediagnostic levels of CA-125, the sensitivity of a CA-125 of 35 units/ml in detecting the presence of ovarian cancer was only 20–57%; within 3 years of the blood test, the specificity was over 95% (3, 4). False negative tests may result from low tumor burden or histological type. CA-125 levels can be elevated in benign conditions such as endometriosis or pregnancy, resulting in false positive tests. The magnitude of the potential misclassification of the presence of ovarian cancer by CA-125 levels limits the ability of this marker to function as a screening test for the early detection of ovarian cancer (3).

A valid test must also be shown to be reliable. Repeatability of the test is critical for both clinical interpretations and research applications. It has been proposed that the rate of change in prostate-specific antigen levels is a better marker of the presence of prostate cancer than a single prostate-specific antigen level reference value (5). This type of application depends on assay reliability on repeated measures to ensure that the rate of change is due to biological processes and not...
Serological markers have widespread applications in clinical trials, such as in selecting the appropriate study population, monitoring adherence to interventions, and determining trial end points such as intermediate cancer end points or incident invasive cancer. Examples of each of these applications are presented.

Selection of Study Population

Serological markers have the potential for several applications in selecting appropriate participants for a cancer prevention trial. Markers of exposure may be used to select only individuals with known carcinogenic exposures who would benefit from interventions aimed at preventing the effects of specific exposures. Levels of carcinogens may provide a more precise indication of exposure level than can be obtained through a questionnaire. For example, serum cotinine levels may provide a better indicator of exposure to sidestream smoke than can be obtained from an exposure assessment questionnaire. Markers that measure a biological effect of the exposure would have the advantage of selecting individuals who were exposed and suffered an effect of that exposure, providing a population at higher risk than those exposed but whose own defenses protected any adverse affect of the exposure. Adduct levels provide evidence of a biological effect of an exposure. An example of a trial design incorporating serological markers of biological effects of an exposure is shown in Fig. 3. Aflatoxin increases the risk of primary hepatocellular cancer, particularly in the presence of the hepatitis B virus (6). A liver cancer prevention trial may then be conducted among high risk population living in high aflatoxin areas, e.g., in regions of China or Africa (7). Since not all individuals in the area may be exposed, individuals may be further selected for eligibility based on the presence of albumin aflatoxin adducts, documenting the exposures. In this example, the intervention agent is proposed to augment the detoxification of aflatoxin. Thus, adduct formation may also be used as a trial end point. Not all individuals exposed to carcinogens are susceptible to the effects of that exposure. Markers of inherited or acquired susceptibility factors may be used to select individuals for trial participation who are not only exposed but also susceptible to the carcinogenic effects of the exposure. Cytochrome P-450 enzymes are involved in the metabolism of many carcinogens. Metabolic phenotypes or gen-

Table 1 Sample size of selected cancer prevention trials

<table>
<thead>
<tr>
<th>Trials with cancer incidence or mortality as end point</th>
<th>Sample size</th>
<th>Trials with intermediate end points</th>
<th>Intervention</th>
<th>Sample size</th>
</tr>
</thead>
<tbody>
<tr>
<td>Trial (Ref.)</td>
<td>Intervention</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tamoxifen Prevention Trial (13)</td>
<td>Tamoxifen</td>
<td>16,000</td>
<td>Prevention of adenomatous polyps (19)</td>
<td>Low fat, high fiber, high fruit and vegetable</td>
</tr>
<tr>
<td>Physicians Health Study (14)</td>
<td>β-Carotene</td>
<td>22,071</td>
<td>Prevention of oral carcinogenesis (20)</td>
<td>Isotretinoin</td>
</tr>
<tr>
<td>Nurses Health Study (15)</td>
<td>ASA, β-carotene</td>
<td>44,600</td>
<td>Prevention of premalignant cervical cancer (21)</td>
<td>β-Carotene</td>
</tr>
<tr>
<td>CARET (16)</td>
<td>Vitamin E</td>
<td>17,700</td>
<td></td>
<td></td>
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<tr>
<td>Women’s Health Initiative (17)</td>
<td>β-Carotene</td>
<td>70,000</td>
<td></td>
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<tr>
<td>PLCO Screening Trial (18)</td>
<td>Low fat diet</td>
<td>74,000</td>
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<tr>
<td></td>
<td>Prostate-specific antigen</td>
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<td></td>
<td>CA-125 and ultrasound</td>
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<td></td>
<td>Chest X-ray</td>
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<td></td>
<td>Sigmoidoscopy</td>
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</table>

Fig. 3. Hypothetical scheme of an intervention trial using serological markers of exposure in the selection process and as a study outcome.
otypes of these and other enzymes (Phase I and II) involved in the metabolic pathways of carcinogen activation or detoxification may be used to select susceptible individuals into cancer prevention trials. Since many interventions have risks as well as benefits, markers of susceptibility would ensure that appropriate populations are targeted; therefore only those at risk and who are likely to benefit from an intervention are exposed to potential adverse effects. Limiting participation to susceptible individuals in a population also improves the efficiency of the study by reducing required sample sizes.

Serological markers that aid in the detection of early stage cancer may be applied in the recruitment process of cancer prevention trials to select individuals who are disease free at study entry. Sample size calculations and statistical analyses of clinical trials are based on the assumption that any event occurring after randomization is new and is included in the data analysis. This assumption is unlikely to be true for events such as the development of an invasive cancer. The process of carcinogenesis is long and an invasive cancer may be present years before it is clinically detected. Incident cancer cases diagnosed even up to 1 year after randomization to an intervention were likely to have been present but undiagnosed at the time of entry into the trial. The cases occurring within a short time of enrollment into the trial are unlikely to be affected by the applied intervention, resulting in a diminished statistical power of the study to detect an effect of the intervention (8). Approaches to this problem include arbitrarily setting a time after entry to begin “counting” cases as end points of the trial and base sample size on the delayed projected incidence of cancer, including a lag time to intervention efficacy, or to count all cases and to increase sample size to adjust for preexisting cases. The first approach may introduce biases into the study and requires knowledge of the natural history of cancer, which varies considerably and is often unknown. All approaches increase the required sample size of the study, thereby increasing costs. Screening for cancer at time of trial entry to select disease-free individuals for participation has been suggested as alternative cost-effective approach (8). A test with a sensitivity as low as 65% may reduce sample size and costs of the trial despite the additional expense of applying the screening test during the recruitment process (8). For example, a serological marker such as prostate-specific antigen with a sensitivity for the detection of prostate cancer estimated to be 75% (8) may provide a useful serological screening test to apply in a prostate cancer prevention trial to select disease-free individuals for trial participation.

**Monitor Trial Adherence**

Monitoring adherence to a trial protocol is necessary for the interpretation of trial results. One of the criticisms of the Women’s Health Trial, an intervention trial of a low fat diet for the prevention of breast cancer (10), was the inability to adequately monitor adherence to a low fat diet over the 10-year duration of the trial (11). Serological markers reflecting changes in lipid levels and lipid composition of cell membranes may provide a feasible and acceptable method of monitoring compliance. Adherence to chemoprevention trials with drugs or nutrients may be monitored by assays of drug levels or serum nutrient levels. Beta-carotene levels, in conjunction with pill counts, were used to monitor adherence to skin cancer intervention trial with beta-carotene (12). Monitoring for “drop-in” from the comparison group to the intervention arm is as important as monitoring adherence to the active intervention.

**Serological Markers of Trial End Points**

**Intermediate End Points**

The use of intermediate markers in the carcinogenic process as surrogate end points in cancer prevention trials aids the conduct of the trials by reducing sample size and shortening trial duration. Intermediate markers may also serve to identify populations at high risk of developing invasive cancers. Sample sizes of several primary and cancer prevention trials are displayed in Table 1. Trials in which cancer incidence or mortality is the primary trial end point are associated with much larger sample size compared to trials using intermediate markers of cancer as the end point. The magnitude of the effect of the proposed intervention also influences sample size as well as when multiple end points are involved, as is the case with the Women’s Health Initiative and the Nurses Health Study.

The validity of markers as intermediate end points of cancer must be validated before their use in clinical trials. The markers should be part of the causal pathway of the process of carcinogenesis (22). Colon adenomatous polyps, oral leukoplakia, and cervical dysplasia are examples of accepted intermediate cancer end points. Few serological markers of intermediate carcinogenic processes are available and/or have been validated as intermediate markers. Serological markers such as aflatoxin B1 N7-guanine adducts or aflatoxin albumin adducts may be used as surrogate end points in chemoprotection trials using agents aimed at detoxifying specific carcinogenic exposures (7). The increasing knowledge of the genetic alterations associated with cancer progression may provide additional intermediate markers to use as trial end points.

**SEROLOGICAL MARKERS OF CANCER**

The identification of tumor-associated antigens has the potential for screening for the presence of early stages of cancer. Tumor-associated antigens such as prostate-specific antigen and CA-125 are under evaluation for their efficacy in the early detection and prevention of prostate and ovarian cancer mortality (18). These same markers also have the potential to be used in primary cancer prevention trials to screen for incident cancers as the primary trial end point.

**Summary**

Serological markers of carcinogenesis have widespread applications in clinical research and potentially for clinical practice. Potential applications in cancer prevention trials include selecting susceptible high risk populations, screening for incidence disease at baseline, monitoring adherence to interventions, and determining outcome events such as the occurrence of intermediate cancer end points or incident invasive cancer. Currently, the only limitation to their widespread use is the availability of validated and reliable serological markers. Because of the ease and acceptability of their use, research into the development of serological markers should continue. Methods for quickly validating serological markers should be developed in order to aid the transition from laboratory to clinical applications.

**References**

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